African Journal of Rural Development, Vol. 7 (2): May-August, 2022: pp.176-180. ISSN 2415-2838

Evaluation of tropical maize inbred lines for resistance to Fusarium Ear Rot in Uganda

S.B. AYESIGA,[1,2] P. RUBAIHAYO, [1] I.O. DRAMADRI,[1 2] R. EDEMA[1,2] and J.P. SSERUMAGA[3]*
[1]Department of Agricultural Production, College of Agriculture and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda
[2]Makerere University Regional Center for Crop Improvement (MaRCCI), College of Agriculture and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda
[3]National Livestock Resources Research Institute, National Agricultural Research Organization, P.O. Box 5704, Kampala, Uganda
***Corresponding Author:** j.serumaga@gmail.com

ABSTRACT
Fusarium Ear rot (FER) caused by *Fusarium verticilliodes* is a worldwide threat to maize production causing both yield losses as well as producing a mycotoxin that is harmful to the safety of both human food and animal health. Although host resistance is the most suitable strategy for disease management, no resistant varieties have been identified, and the first step in introducing resistance into maize germplasm would be to find sources of genetic resistance. This study evaluated a genetically diverse collection of maize inbred lines as potential sources of resistance to Fusarium Ear Rot caused by *Fusarium verticilliodes* by artificial inoculation using the toothpick method in the field for two seasons. At harvest, the ears were scored and assessed for FER development. Twelve inbred lines were found to be moderately resistant. The inbred lines CKL150038, CKL150105, CKL150109, CKL150105, JPS25-11, JPS26-4, JPS25-40, JPS25-36, JPS25-14, JPS25-11, DL141392 and WL429-24 had consistently low disease severity across the two seasons. These lines could be potential sources of resistance in breeding programs against *F. verticilliodes*.

Keywords: *Fusarium verticilliodes*, host-plant resistance, mycotoxin, *Zea mays*

RÉSUMÉ
La pourriture de l'épi (FER) causée par *Fusarium verticilliodes* est une menace mondiale pour la production de maïs, entraînant à la fois des pertes de rendement et la production d'une mycotoxine nocive pour la sécurité de l'alimentation humaine et de la santé animale. Bien que la résistance de l'hôte soit la stratégie la plus appropriée pour la gestion de la maladie, aucune variété résistante n'a été identifiée, et la première étape de l'introduction de la résistance dans le matériel génétique du maïs serait de trouver des sources de la résistance génétique. Cette étude avait évalué une collection génétiquement diversifiée de lignées consanguines de maïs comme sources potentielles de résistance à la pourriture de l'épi causée par *Fusarium verticilliodes* par inoculation artificielle en utilisant la méthode du cure-dent dans le champ pendant deux saisons. À la récolte, les épis étaient évalués pour le développement du FER. Douze lignées consanguines se sont avérées modérément résistantes. Les lignées consanguines CKL150038, CKL150105, CKL150109, CKL150105, JPS25-11, JPS26-4, JPS25-40, JPS25-36, JPS25-14, JPS25-11, DL141392 et WL429-24 présentaient constamment des faibles intensités de la maladie au cours des deux saisons. Ces lignées pourraient constituer des sources potentielles de résistance dans les programmes de sélection contre le F. verticilliodes.

Mots-clés : *Fusarium verticilliodes*, résistance de la plante-hôte, mycotoxine, *Zea mays*

Cite as: Ayesiga, S. B., Rubaihayo, P., Dramadri, I.O., Edema, R. and Sserumaga, J.P. 2022. Evaluation of tropical maize inbred lines for resistance to Fusarium Ear Rot in Uganda. *African Journal of Rural Development* 7 (2): 176-180.

INTRODUCTION

Maize is the world's third most important cereal crop after wheat and rice (Lobulu *et al.*, 2019). In Sub-Saharan Africa (SSA), maize is the most important cereal crop as food, feed, and industrial crop, grown on over 40 million hectares of land (Cairns *et al.* 2021), and it accounts for 30-50% of low-income household expenditures in Eastern and Southern Africa (Chemiat and Makone, 2015). Despite its importance in the world as whole, its production is constrained by many factors, both biotic and biotic stresses that lead to lower yields than expected, one of them being Fusarium Ear Rot caused by *Fusarium verticilliodes*. Fusarium ear rot (FER) is one of the most prevalent fungal diseases in maize worldwide, affecting grain yield and quality, causing yield reduction estimated between 10 and 30% (Lanubile *et al*., 2017) with losses reaching 50% in severe conditions (Ding *et al.,* 2008; Yao *et al.,* 2020). Infected kernels are covered with white or pink mold (Lanubile *et al.,* 2017). This fungus can cause disease at all developmental stages of the plant. In addition, *Fusarium vericilliodes* produces a mycotoxin, primarily Fumonisin B1 (FB1), (Balconi *et al.,* 2014) which affects the quality and marketability of grains. Another increasing concern about Fumonisins is the mounting evidence of their involvement in a number of human and animal diseases including esophageal cancer and neural tube birth defects (Robertson *et al.,* 2006) in humans, equine leukoencephalomalacia (Ross *et al.,* 1992) and porcine pulmonary oedema (Robertson *et al.,* 2006) in animals. Contaminated feeds have been reported to cause leukoencephalomacia in horses as well as pulmonary oedema and hepatic syndrome in swine (Ross *et al.,* 1990; Lanubile *et al.,* 2017). This situation is further complicated by the common occurrence of fumonisins in symptomless infected kernels (Munkvold *et al.,* 1997; Afolabi *et al.,* 2007). Unfortunately, high levels of Fumonisin contamination in maize have been reported in a number of African countries including Algeria, Ghana, Kenya, Ethiopia, and Uganda, among others (Afolabi *et al.,* 2007; Yli-Mattila and Sundheim, 2022). This implies that a significant portion of the maize crop in several parts of Africa could be affected when environmental conditions are suitable for fumonisin accumulation in grain. Therefore, there is a great need to control FER in maize so as to obtain good quality grain and thus reduce the potential health effects associated with the consumption of fumonisin-contaminated grain.

Breeding for disease resistance is the best strategy to control FER because it is efficient, economically sound and environmentally safe (Chen *et al.*, 2016), and many studies have focused on the search for resistance (Clements *et al.,* 2004; Lanubile *et al*., 2011; Lanubile *et al.,* 2017; Maschietto *et al.,* 2017), but unfortunately, there is no evidence of complete resistance to the pathogen. Despite the advantage of using resistant genotypes, there are a few resistant genotypes on the market and this is attributed to the complexity of the genetic architecture of resistance to FER (De Jong *et al.,* 2017). The problems associated with Fusarium ear rot of maize can be prevented by proactively screening maize germplasm for resistance to *F. verticillioides*. In addition, sources of resistance should be identified preferably in locally adapted inbred lines for use in breeding programs (Tembo *et al.,* 2022). Existing maize cultivars in Uganda are not known to have resistance to Fusarium ear rot or fumonisin contamination, and limited information is available on the inbred lines. Therefore, this study evaluated maize lines adapted to tropical conditions for resistance to Fusarium Ear Rot. The Inbred lines possessing resistance to FER would provide a valuable breeding stock for use as parents of hybrids and synthetics that can be grown by farmers.

MATERIALS AND METHODS

Genetic materials. A total 151 diverse maize inbred lines collected from the National Agricultural Research Organization (NARO) in Uganda, and CIMMYT maize breeding programs were used in this study. The lines were screened for FER resistance for two seasons at the National Crops Resources Research Institute (NaCRRI), Namulonge, Uganda, which falls in the mid-altitude agro-ecological zone, located at 0º 32'N and 32º 35´E, at 1150 meters above sea level. Alpha lattice experimental design was used, with two replications. The plots consisted of two 5m long rows, with spacing of 0.75m by 0.5m between rows and plants, respectively. Two seeds per hill were planted and later thinned to one plant per hill four weeks after emergence. The recommended agronomic and cultural practices were followed, including weeding and fertilizer application at a rate of 77 kg N and 27 kg/ha^{-1} split over two applications at planting and for topdressing four weeks after planting, respectively. Planting was done under favorable conditions; supplemental irrigation was also provided as necessary for optimal disease development.

Pathogen culture, inoculation, and disease assessment. We isolated the pathogen from infected ears got from the Institute fields. Infected grains were sterilized for three minutes in 10% commercial bleach of the JIK brand which contains 0.39% sodium hypochlorite (NaClO) solution (Langa *et al*., 2012), rinsed three times using distilled water, and then 2 – 3 seeds were plated on 3% potato dextrose (Becton Dickinson, Sparks, MD, USA) agar plates and incubated at 28 – 30 C. Sub-culturing was done after four days and the fungal cultures were ready for transfer to disinfected toothpicks after 5 – 7 days. Fungal plugs from pure cultures were then placed in a flask together with toothpicks. Before use, the toothpicks were first autoclaved. The flask containing the fungal plugs and the toothpicks was then sealed and left for three weeks for the fungus to grow throughout the toothpicks (Chambers, 1988).

After the toothpicks were fully colonized, we used them to inoculate the maize cobs approximately seven days after flowering. Inoculation was done by piercing through the middle of the primary ear, using the colonized toothpicks and these remained until harvest. Inoculation was done on 10 plants of each inbred line, and paper bags were used to cover the ears to avoid allo-infection.

At maturity, when the moisture content was approximately less than 20% (Balconi *et al*., 2014), the ears were harvested by hand, de-husked, and individually scored for Fusarium Ear Rot symptoms (Fig. 1) using a nine-point scale; FER scores 1=0%, 2=1%, 3=2-5%, 4=6-10%, 5=11-20%, 6=21-40%, 7=41-60%, 8=61-80%, 9=81-100% (Guo *et al*., 2020) of kernels exhibiting visual symptoms of infection, such as brown, pink, or reddish discoloration of kernels and pinkish or white mycelial growth (Clements, 2003).

Statistical analysis. Effects of seasons and selected inbred lines on the severity of Fusarium ear rot were analyzed using R software (R Core Team 2021). Analysis of Variance was performed where the inbred lines were considered fixed effects, whereas replications and seasons were considered random effects. Fisher's protected least significant difference test at p≤0.05 was used to determine significant differences between the inbred lines.

Score 1

Score 9

Figure 1. Maize cobs showing the FER scoring done at harvest

RESULTS

Fusarium ear rot severity for all 151 inbred lines across the two seasons ranged from 2 (1% ear rot symptom) to 9 (i.e., 81 to 100% ear rot symptom). The average visual rating of Fusarium ear rot infection of maize ears was 4 for season one and 2.8 for season 2. Analysis of variance showed that there was significant variation ($P < 0.05$) among the inbred lines used in this study, and there was significant variation across seasons as well as for the genotype by season interaction (Table 1). The significant difference observed across seasons and genotype X season interaction could be due to seasonal factors. In the combined analyses of variance, differences among environments and inbred lines were significant for FER. Twelve inbred lines showed the lowest scores for FER across the two seasons and these were CKL150038, CKL150105, CKL150109, CKL150105, JPS25-11, JPS26-4, JPS25-40, JPS25-36, JPS25-14, JPS25-11, DL141392 and WL429-24.

DISCUSSION

Developing adapted maize germplasm with resistance to Fusarium Ear Rot is an important breeding objective. Ear rot caused by *Fusarium* spp. affects maize production and grain quality. The use of resistant germplasm is the most cost-effective and durable means of reducing the damage from ear rot (Chen *et al.,* 2012).

This study assessed the response of 151 maize inbred lines to FER under artificial inoculation for two seasons. Results revealed significant differences among lines for FER, indicating the presence of sufficient genetic variation that can be utilized in breeding for resistance to Fusarium Ear Rot. Similar results were reported by Afolabi *et al*. (2007) and Balconi *et al*. (2014). In this study, the mean of disease severity was affected by the season and genotype-by-season interaction effects. Thus, evaluations for selecting resistant lines should be conducted across several seasons to expose the genotypes to a wide range of environmental conditions (Tembo *et al.,* 2022).

A number of inbred lines with low or high disease severity in season 1 did not have a correspondingly similar level of disease severity when re-evaluated in season 2, implying that the interaction between genotype and environmental factors plays an important role in determining Fusarium ear rot symptoms (Parsons and Munkvold, 2012; Balconi *et al.,* 2014). Maize inbred lines with potential resistance to Fusarium ear rot caused by *F. verticillioides* were identified though no lines were found to be completely resistant and this is in agreement with a previous study by Small *et al*. (2012). According to Chen *et al*. (2016), although genetic variation for resistance to FER exists among maize inbred lines and hybrids, there is no evidence of complete resistance to either FER or fumonisin contamination in maize.

Table 1. Combined ANOVA of 151 maize inbred lines evaluated under artificial *F. verticilliodes* inoculation at Namulonge across two seasons

SOV	Df	Sum Sq	Pr(>F)
Rep	1	4.86	0.02459
Season	1	276.08***	< 2.2e-16
Genotype	150	331.02 ***	2.273e-08
Rep:Block	109	120.36	0.17699
Season:Genotype	149	374.67 ***	1.232e-10
Residuals	192	181.78	
CV	34.49		

*, **, ***Significant at the .05, .01, and .001 probability levels, respectively
SOV: Source of Variation, Df: Degrees of freedom

Finally, while many plant breeders may rely on natural infection when assessing maize germplasm for resistance to FER, there are a few locations that offer sufficient uniformity to make efficient and successful selections (Mesterházy *et al.,* 2012). In addition, there is likely to be season-by-season variation in the degree of fungal attack and to overcome this disadvantage, artificial infection is a suitable approach for testing genotypes for resistance to the fungus (Balconi *et al.,* 2014).

CONCLUSION

This study identified 12 inbred lines that showed considerable resistance to FER and these will be important in the development of hybrids with good levels of resistance to Fusarium Ear Rot. The resistant lines could also be used to develop mapping populations to fine-map QTLs for FER resistance. Furthermore, since the inbred lines were derived from different crosses, they can be used to widen genetic diversity in other tropical maize breeding programs.

ACKNOWLEDGMENT

The authors thank the International Maize and Wheat Improvement Center (CIMMYT) and National Agricultural Research Organization (NARO)-Uganda for making their germplasm available for the study.

STATEMENT OF NO CONFLICT OF INTEREST

The authors declare that there are no competing interests in this publication.

REFERENCES

Balconi, C., Berardo, N., Locatelli, S., Lanzanova, C., Torri, A. and Redaelli, R. 2014. Evaluation of ear rot (*Fusarium verticillioides*) resistance and fumonisin accumulation in Italian maize inbred lines. *Phytopathologia Mediterranea* 53 (1):14-26.

Chen, J., Ding, J., Li, H., Li, Z., Sun, X., Li, J. and Wu, J. 2012. Detection and verification of quantitative trait loci for resistance to Fusarium ear rot in maize. *Molecular Breeding* 30: 1649-1656.

Chen, J., Shrestha, R., Ding, J., Zheng, H., Mu, C., Wu, J. and Mahuku, G. 2016. Genome-wide association study and QTL mapping reveal genomic loci associated with Fusarium Ear Rot resistance in tropical maize germplasm. *G3: Genes, Genomes, Genetics* 6 (12): 3803-3815.

Clements, M. K. 2003. Evaluation of inoculation techniques for Fusarium ear rot and fumonisin contamination of corn. *Plant Disease* 87 (2):147-153.

De Jong, G., Pamplona, A. K., Pinho, R. G. and Balestre, M. 2017. Genome-wide association analysis of ear rot resistance caused by *Fusarium verticillioides* in maize. *Genomics* 110 (5): 291-303.

Lanubile, A., Maschietto, V., Borrelli, V. M., Stagnati, L., Logrieco, A. F. and Marocco, A. 2017. Molecular basis of resistance to fusarium ear rot in maize. *Frontiers in Plant Science* 8: 1774.

Small, I. M., Flett, B. C., Marasas, W. F., McLeod, A., Stander, M. A. and Viljoen, A. 2012. Resistance in maize inbred lines to *Fusarium verticillioides* and fumonisin accumulation in South Africa. *Plant Disease* 96 (6): 881-888.

Tembo, E., Minnaar-Ontong, A., Menkir, A., Marais, G., Magorokosho, C. and Labuschagne, M. T. 2022. Inheritance of resistance to *Fusarium verticillioides* ear rot in maize inbred lines of Southern, West and Central Africa origin. *Crop Science* 62(5): 1818-1833.

Yli-Mattila, T. and Sundheim, L. 2022. Fumonisins in African countries. *Toxins* 14 (6): 419 doi:https://doi.org/ 10.3390/toxins14060419