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SHORT NOTE [NOTA CORTA]

FIELD EVALUATION OF ANTHRACNOSE DISEASE RESPONSE FOR THE SORGHUM GERMPLASM COLLECTION FROM THE KAYES REGION OF MALI

[EVALUACIÓN DE CAMPO DE LA RESPUESTA A LA ANTRACNOSIS POR GERMOPLASMA DE SORGO DE LA REGIÓN KAYES DE MALI]

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SUMMARY

Sorghum germplasm from the Kayes region of Mali maintained by the United States of America Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA, ARS, NPGS) was inoculated with *Colletotrichum sublineolum* and evaluated for foliar anthracnose resistance in 2003 and 2004 at the USDA, ARS, Tropical Agriculture Research Station in Isabela, Puerto Rico. From the 277 accessions evaluated, 119 showed a resistant response over multiple growing seasons to anthracnose pathotypes present at the research site in Puerto Rico. These sorghum accessions showed reddening of inoculated leaves as a result of phytoalexin production, but lacked lesion development with acervuli. The frequency of anthracnose resistant accessions varied based on region of origin. Germplasm from the Kenieba and Kita administrative districts showed the highest frequency of resistance, with more than 65% of the accessions showing a resistant response. The frequency of resistant germplasm ranged from 24 to 44% for the five other administrative districts of the Kayes region of Mali. Germplasm showing resistance was also more frequent in areas experiencing higher annual rainfall. More than 60% of the accessions collected from regions receiving over 800 mm of annual rainfall showed anthracnose resistance. In comparison, less than 30% of the accessions collected from areas receiving less than 800 mm of annual rainfall showed a resistant response. Results of the evaluation suggest that the Kayes region of Mali is an important source of anthracnose resistant germplasm and that ecogeographic information could aid in the selection of germplasm to increase the likelihood of identifying additional sources of resistance.

Key words: genetic resources, *Sorghum bicolor*, West Africa, ecological zones

RESUMEN

Germoplasma de sorgo proveniente de la región Kayes de Mali y mantenida por el Departamento de Agricultura de los Estados Unidos de Norteámerica, Servicio de Investigación Agrícola, Sistema Nacional de Germoplasma Vegetal (USDA, ARS, NPGS) fue inoculado con *Colletotrichum sublineolum* y evaluado para la resistencia foliar a la antracnosis durante 2003-04 en la estación de Investigación de Agricultura Tropical en Isabela, Puerto Rico (USDA, ARS). De las 277 accesiones evaluadas, 119 presentaron una respuesta de resistencia durante múltiples ciclos de crecimiento hacia patotipos de antracnosis mantenidos en la estación experimental (Puerto Rico). Estas variedades de sorgo presentaron enrojecimiento de las hojas inoculadas como resultado de la producción de fitoalexina, pero no presentaron desarrollo de lesiones. La frecuencia de accesiones resistentes a antracnosis varió de acuerdo a la región de origen. Germoplasma de los distritos de Kenieba y Kita mostraron la mayor frecuencia de resistencia (más de 65% de las accesiones). La frecuencia de germoplasma resistente varió de 24 a 44% en los restantes cinco distritos de la región Kayes de Mali. Los germoplasma que presentaron resistencia fueron más frecuentes en las regiones con una mayor precipitación pluvial. Más del 60% de las accesiones colectadas de regiones con más de 800 mm precipitación presentaron resistencia a la antracnosis en comparación con menos del 30% de las accesiones que provenían de regiones con menos de 800 mm precipitación. Los resultados sugieren que la región Kayes de Mali es una fuente importante de germoplasma resistente a la antracnosis y que la información ecogeográfica puede ser de utilidad para seleccionar germoplasma y mejorar la probabilidad de identificar fuentes adicionales de resistencia.

Palabras clave: Regursos genéticos, *Sorghum bicolor*, África Occidental, Zonas ecológicas.

INTRODUCTION

Colletotrichum sublineolum P. Henn., Kabát & Bubák is the fungal pathogen responsible for sorghum anthracnose (Crouch et al., 2006) and the disease occurs worldwide in most sorghum producing regions (Thakur and Mathur, 2000). However, disease epidemics are more frequent in tropical and subtropical regions where warm, humid climatic conditions contribute to the rapid development and spread of the disease. Disease epidemics can result in grain yield losses of more than 50% for susceptible cultivars (Harris et al., 1964; Ali et al., 1987; Thomas et al., 1996; Thakur and Mathur, 2000). In Puerto Rico, yields losses have been observed from severe foliar infection resulting in plant death prior to maturity, from panicle infection resulting in the formation of fewer seed, and more frequently from severe foliar infection prior to seed development resulting in poor grain fill and lodging.

Numerous studies have shown that the pathogen is highly variable (Ali and Warren, 1987; Cardwell et al., 1989; Pande et al., 1991; Rosewich et al., 1998; Marley et al., 2001; Valério et al., 2005). As a result, the longevity of single sources of host plant resistance is limited. Pyramiding of host plant resistance genes could reduce disease outbreaks, but additional sources of resistance are needed for cultivar development. The United States of America Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA, ARS, NPGS) maintains more than 43,000 sorghum accessions and field evaluation of germplasm from the collection has successfully identified sources of anthracnose resistance (Erpelding and Prom, 2004; Erpelding et al., 2005; Erpelding and Wang, 2007). However, additional evaluations of the NPGS sorghum collection are needed to determine whether passport and phenotypic evaluation data could be used to aid in the selection of germplasm to increase the potential of identifying sources of resistance to enhance the genetic diversity of resistance available for sorghum improvement.

The sorghum collection from the Kayes region of Mali was selected for field evaluation of anthracnose disease response. The objectives of the study were: 1) determine the frequency of anthracnose resistance in sorghum germplasm from the Kayes region and identify sources of resistance for genetic characterization; 2) determine if anthracnose resistance was associated with specific administrative districts within the Kayes region; 3) determine if resistance was associated with ecogeographic origin based on annual rainfall; and 4) determine if phenotypic evaluation data could aid in the identification of sources of resistance.

MATERIALS AND METHODS

The sorghum germplasm collection originating from Mali, West Africa maintained by the USDA, ARS, National Plant Germplasm System consists of 2,351 accessions (GRIN, 2003). Latitude and longitude information is available for 369 accessions from the Mali sorghum collection (GRIN, 2003). This information along with passport information was used to select 277 sorghum accessions representing the Kayes region of Mali. Latitude and longitude data was used to identify 232 accessions from the Kayes region. An additional 45 accessions were selected using passport information to determine the villages in the Kayes region were the landraces were collected. The Kayes region is located in the western part of Mali and sorghum accessions included in the evaluation were collected in 1987 and donated to the NPGS. Seed samples for the anthracnose evaluation were obtained from the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia.

The anthracnose evaluation of sorghum landraces from the Kayes region was conducted in 2003 and 2004 at the USDA, ARS, Tropical Agriculture Research Station in Isabela, Puerto Rico. The collection was planted on 15 October 2003, 13 January 2004, and 13 July 2004 in a partially balanced lattice design with three replications. The October and July planting dates coincided with the wet season and the January planting was evaluated during the dry season. In order to evaluate the variation within and between experiments, 27 sorghum genotypes were selected as controls. Two of the control genotypes were duplicated in the evaluation as inoculated and non-inoculated plots resulting in a total of 29 controls in the experiment. Four sorghum genotypes were selected as resistant controls and 23 were selected as susceptible controls based on previous field evaluations. The sorghum accessions were planted in single rows 1.8 m in length with 0.9 m row spacing. The experimental fields were surrounded by rows of anthracnose susceptible genotypes. Fertilizer was applied at a rate of 560 kg ha^{-1} (15-5-10 NPK) during planting. A second application of fertilizer was done approximately 30 days after planting for the January planting date. Field conditions prevented the second application of fertilizer for the October and July planting dates. Lorsban 15G (Chlorpyrifos) granular insecticide (Dow AgroSciences, Indianapolis, IN) was applied at a rate of 8 kg ha^{-1} during planting to prevent seed loss from fire ants. Supplemental irrigation was not necessary for the October planting date. Supplemental irrigation was necessary for the January and July planting dates and applied on a weekly basis. For the January planting date, no irrigation was applied after inoculation. Symptoms of drought stressed were observed in the July planting; therefore, supplemental irrigation was applied prior to inoculation followed by a single application after inoculation.

The experimental fields were inoculated with anthracnose colonized sorghum seed to enhance anthracnose disease response. No information is available on the anthracnose pathotypes present at the Isabela research station in Puerto Rico; therefore, anthracnose infected leaf tissue was randomly collected from susceptible sorghum genotypes to represent the pathogen population. Preparation of anthracnose cultures, field inoculation, and disease evaluation were as described by Erpelding and Prom (2006); anthracnose lesions were excised from leaf tissue, surface sterilized, and placed on ½ potato dextrose agar (PDA) media. Small portions of the developing fungal masses were transferred to fresh ½ PDA plates and cultured for approximately 7 days. Sorghum seed was soaked overnight, rinsed, placed in glass jars, and autoclaved prior to inoculation. The outside edge of the 7 day old anthracnose culture was excised and added to the seed. Several different fungal cultures were added to each jar of seed, mixed, and incubated at room temperature. Approximately ten anthracnose colonized seed were placed in the leaf whorl of each plant with approximately 90% of the plants in the row inoculated. The anthracnose inoculation was conducted 31 days after planting for the first evaluation, 39 days after planting for the second evaluation, and 33 days after planting for the third evaluation. The assessment of anthracnose disease response was based on a 1 to 5 scale as described by Erpelding and Prom (2004) with disease response evaluated on inoculated leaves and disease progression on non-inoculated leaves. The sorghum collection from the Kayes region of Mali is composed of landraces and heterogeneity for disease response is possible. Therefore, the observed predominate disease phenotype was used to rate the accessions. Disease evaluations were conducted at 27, 45, and 65 days after inoculation for the first evaluation. For the second evaluation planted in January, disease evaluations were conducted at 35 and 56 days after inoculation. The third evaluation was rated at 15, 33, and 65 days after inoculation. For the second and third evaluation, the percentage of infected leaf area was also estimated during the final rating of the susceptible accessions to evaluate infection severity, which was based on a visual estimate of leaf infection for each row. Statistical analysis of the data was conducted using the disease response from the final rating. At this stage, plants are approaching maturity and further disease progression is associated with colonization of senescencing leaf tissue. The Statistix software package (Analytical Software, Tallahassee, FL) was used to conduct the analysis of variance for the data.

RESULTS

The anthracnose field evaluation of the 277 accessions from the Kayes region of Mali conducted in Isabela, Puerto Rico during the 2003 and 2004 growing seasons resulted in the identification of 119 accessions showing resistance to anthracnose infection. These accessions showed reddening of inoculated leaves from the production of phytoalexins at the site of infection and lacked lesion development with acervuli. Additionally, no variation in disease response was observed within and between growing seasons for these accessions.

Approximately 51% of the accessions showed a resistant response for the first evaluation planted in October (Table 1). For the 135 accessions rated as susceptible, 64 showed variation for infection response between replications and 22 accessions showed a consistent highly susceptible response. Six accessions showed a susceptible response only during the first evaluation. The lowest frequency of resistant accessions was observed during the second evaluation planted in January, with approximately 48% of the accessions showing a resistant response (Table 1). Variation in disease response between replications was observed for 31 accessions and 37 accessions showed a consistent highly susceptible response. Infection severity averaged 41.7% for the 145 accessions rated as susceptible during the second evaluation. A susceptible response for 19 accessions was only observed in the second evaluation. The highest frequency of resistant accessions was observed for the third evaluation planted in July (Table 1). Approximately 68% of the accessions showed a resistant response. For the accessions rated as susceptible, 47 showed variation in infection response between replications and only one accession was rated as highly susceptible. Infection severity was lowest for the third evaluation and averaged 19.8% for the 89 susceptible accessions. Four accessions showed a susceptible response only in the third evaluation. All accessions showed the hypersensitive response, leaf reddening, within 15 days after inoculation, with acervuli development on inoculated leaves for the susceptible accessions.

The anthracnose infection response was as expected for the 27 control genotypes included in the evaluation (data not shown). The four resistant control genotypes, BTx378, RTx2536, SC326-6, and SC748-6, included in the field evaluation showed a resistant, hypersensitive response for the three growing seasons. The 23 susceptible control genotypes, B35, BTx398, BTx622, BTx3197, PI 534040, PI 534043, PI 534163, PI 536016, PI 552861, PI 561071, PI 561072, PI 561073, PI 564163, PI 576390, PI 609151, PI 609251, PI 609582, PI 609634, PI 609636, PI 609746, PI

612810, RTx7078, and SC1056, generally showed a highly susceptible response for the three evaluations. Four of the susceptible controls genotypes, BTx398, PI 534043, PI 536016, and RTx7078, did show variation in disease response within a growing season and this variation was generally observed during the third evaluation planted in July. For the inoculated and non-inoculated controls, PI 576390 and PI 609251, included in the evaluation, a highly susceptible infection response was observed within and between growing seasons.

Table 1. Number of anthracnose resistant and susceptible accessions from the 277 sorghum accessions evaluated from the Kayes region of Mali during the 2003 and 2004 growing seasons in Isabela, Puerto Rico.

Evaluation[1]	Resistant Accessions	Susceptible Accessions
1	142	135
2	132	145
3	188	89

[1]The first anthracnose evaluation was planted 15 October 2003, the second evaluation was planted 13 January 2004, and the third was planted 13 July 2004. Accessions were rated as resistant if no lesion development with acervuli was observed and the resistant response, reddening of inoculated leaves, was observed for the three replications.

The Kayes region of Mali includes seven administrative districts and landraces are available in the NPGS sorghum collection from each district (Table 2). Passport information was lacking for five accessions, thus the district was unknown for these accessions. Variation in the frequency of resistant landraces was observed between the districts. The highest frequency of resistant landraces was observed for the Kenieba and Kita districts with more than 65% of the accessions from these districts rated as resistant. These two districts are the southern most districts in the Kayes region. Less variation in disease response within and between growing seasons was also observed for germplasm from these two districts. The lowest frequency of anthracnose resistant landraces was observed for the Kayes district with 24% of the accessions from this district showing a resistant response. A low frequency of anthracnose resistant landraces was also observed for the Nioro and Yelimane districts, which are the northern most districts of the Kayes region. Approximately 43% of the accessions from the Bafoulabe and Diema districts showed a resistant response. The highest average infection severity was observed for susceptible landraces from the Diema district, with an average infected leaf area of approximately 50%. The susceptible landraces from the Kenieba district had the lowest average infection severity, with an average infected leaf area of 27%. Infection severity for the susceptible accessions was generally higher for germplasm collected from the northern districts of the Kayes region of Mali.

Annual rainfall is highly variable for the Kayes region of Mali with less than 400 mm of annual rainfall received in the northern region compared to more than 1,100 mm of annual rainfall received in the southern region. A comparison between anthracnose infection response and annual rainfall was conducted to determine if disease response was associated with rainfall (Table 3).

Table 2. Number of anthracnose resistant and susceptible accessions from the seven administrative districts of the Kayes region of Mali.

Administrative District[1]	Resistant Accessions	Susceptible Accessions	Total[2]	Villages[3]
Yelimane[a]	6	17	23	5
Nioro[a]	14	33	47	13
Diema[ab]	7	9	16	4
Kayes[ac]	14	44	58	17
Bafoulabe[bcd]	16	21	37	9
Kita[bd]	34	18	52	16
Kenieba[d]	26	13	39	12

[1]Districts followed by the same letters were not significantly different ($p=0.05$) for disease response, based on infection severity for all accessions from each district.
[2]Five accessions from the 277 evaluated were not mapped to an administrative district.
[3]Number of villages in each administrative district where sorghum landraces were collected.

The frequency of anthracnose resistant landraces increased with an increase in annual rainfall. Nearly 90% of the landraces from regions receiving more than 1,100 mm of annual rainfall showed a resistant response. In contrast, 17% of the landraces from regions receiving less than 600 mm of annual rainfall were rated as resistant. Average infection severity followed a similar pattern with the lowest infection severity observed for susceptible accessions collected from the wettest regions and the highest infection severity observed for susceptible accessions collected from the driest regions. More than 60% of the accessions showed a resistant response in regions receiving more than 800 mm of annual rainfall, with less than 30% of the accessions showing a resistant response in regions receiving less than 800 mm of annual rainfall. Averaged infection severity was more than 40% for the susceptible accessions from regions receiving less than 800 mm of annual rainfall. In regions receiving more than 800 mm annual rainfall, average infection severity for the susceptible accessions was less than 30%.

Phenotypic variation is also present in the sorghum germplasm collection from the Kayes region of Mali. Accessions from the Kayes region can be classified into five sorghum races (Table 4).

Table 3. Number of anthracnose resistant and susceptible accessions from the Kayes region of Mali associated with annual rainfall.

Annual Rainfall[1] (mm)	Resistant Accessions	Susceptible Accessions	Total[2]
350-599[a]	9	43	52
600-799[a]	40	72	112
800-1,100[b]	48	36	84
>1,100[b]	14	2	16

[1]Annual rainfall corresponds to data presented by Hess et al. (2002). Rainfall regions followed by the same letter were not significantly different (p=0.05) for anthracnose disease response, based on infection severity for all accessions in the rainfall regions.
[2]Passport information was lacking for 13 accessions from the 277 evaluated and these accessions were not mapped to a rainfall region.

Sorghum landraces classified as race guinea were the most frequent in the germplasm collection from the Kayes region, with 71% of the accessions classified as race guinea. Approximately 25% of the landraces from the Kayes region were classified as race durra. Landraces classified as race caudatum, durra-caudatum, and guinea-caudatum were also present in the germplasm collection from the Kayes region, but due to the low number of accessions for these races in the collection, these accessions were not included in the statistical evaluation. The highest frequency of anthracnose resistant landraces was observed for sorghum accessions classified as race guinea, with approximately 45% of the guinea landraces showing a resistant response. In comparison, 33% of the landraces classified as race durra showed a resistant response. Average infection severity was also higher for the landraces classified as race durra. Infection severity averaged approximately 55% for the susceptible race durra accessions compared to an average infection severity of 34% for the susceptible race guinea accessions. However, the guinea landraces showed more variation in disease response within and between growing seasons.

Table 4. Phenotypic race classification for the sorghum accessions from the Kayes region of Mali and the corresponding number of anthracnose resistant and susceptible accessions for each race.

Race[1]	Resistant Accessions	Susceptible Accessions	Total
Durra	23	46	69
Guinea	90	107	197
Caudatum	3	3	6
Durra-Caudatum	3	1	4
Guinea-Caudatum	0	1	1

[1]Race durra accessions were significantly more susceptible than race guinea accessions (p=0.05), based on infection severity for all accessions classified as race durra and guinea.

Landraces classified as race durra and guinea were present in the seven administrative districts of the Kayes region, but the majority of the accessions from the Bafoulabe, Kayes, Kenieba, and Kita districts were race guinea (Table 5). The race guinea accessions from the Kenieba and Kita districts were more frequently rated as resistant. Disease severity was greater for the susceptible race guinea accessions from the Kita, Nioro, and Yelimane districts, with the lowest infection severity observed for the guinea accessions from the Kenieba district. Race durra accessions were more frequent in the collection from the Nioro district, with the majority of the durra landraces rated as susceptible. However, the few race durra accessions from the Bafoulabe, Kenieba, and Kita districts frequently showed a resistant response. Race durra accessions were also more frequent in regions receiving less than 600 mm annual rainfall (Table 6). The frequency of durra landraces showed a decrease with an increase in annual rainfall. No durra landraces were present in the collection from regions receiving more than 1,100 mm of annual rainfall and only 3% of the accessions in regions receiving more than 800 mm of annual rainfall were classified as race durra. In contrast, the frequency of race guinea accessions increased with an increase in annual rainfall. Also, the frequency of race guinea accessions rated as resistant increased with an increase in annual rainfall and nearly all the race guinea accessions collected from regions receiving over 1,100 mm of annual rainfall showed a resistant response. Disease severity was similar for the susceptible race guinea accessions from the four annual rainfall regions.

DISCUSSION

Approximately 43% of the sorghum accessions from the Kayes region showed a resistant response to anthracnose pathotypes in Isabela, Puerto Rico, which would suggest this region of Mali is an important source of anthracnose resistant germplasm. Variation in disease response was observed within and between growing seasons for 129 accessions classified as susceptible and differences in climatic conditions are a potential factor contributing to the observed variation. Hess et al. (2002) indicated that anthracnose infection severity was significantly influenced by a genotype by environment interaction. Climatic conditions for the second evaluation planted in January 2004 appeared to be more favorable for anthracnose disease development. Several studies have indicated that regions of higher annual rainfall provide more favorable conditions for anthracnose disease development (Pande et al., 1994; Néya and Le Normand, 1998; Hess et al., 2002; Ngugi et al., 2002). However, more than twice as much rainfall was received during the first evaluation, planted in October 2003, as compared to second evaluation. Even though less rainfall was received during the second evaluation, the duration of rainfall was greater with frequent rain showers occurring throughout the day as compared to intense rain storms that generally occurred in the afternoon during the wet growing season. Cooler temperatures during the second evaluation may have also prolonged the dew period contributing to greater anthracnose infection. For the third evaluation, less frequent rain storms and higher temperatures may have contributed to the lower susceptibility observed during the growing season, since reddening of inoculated leaves was observed for the accessions. Variation in virulence within the pathogen population may also contribute to the variation in disease response observed within and between anthracnose evaluations. However, no variation in disease response between evaluations was observed for the 27 control genotypes and more than 53% of the accessions included in the evaluation showed the same disease response over growing seasons. Presumably, the majority of the variation within and between evaluations would have resulted from environmental interactions between the pathogen and host.

Table 5. Number of anthracnose resistant and susceptible accessions classified as race durra and guinea for the seven administrative districts of the Kayes region of Mali.

	Durra			Guinea		
District	Resistant	Susceptible	Total	Resistant	Susceptible	Total
Nioro	8	17	25	5	13	18
Diema	2	6	8	5	3	8
Yelimane	2	9	11	1	8	9
Kayes	4	10	14	10	34	44
Bafoulabe	3	0	3	11	20	31
Kita	2	2	4	32	15	47
Kenieba	1	0	1	25	13	38

Table 6. Number of anthracnose resistant and susceptible accessions for each annual rainfall region classified as race durra and guinea for the germplasm collection from the Kayes region of Mali.

	Durra			Guinea		
Rainfall (mm)	Resistant	Susceptible	Total	Resistant	Susceptible	Total
350-599	4	21	25	4	19	23
600-799	16	22	38	19	50	69
800-1,100	2	1	3	46	33	79
>1,100	0	0	0	14	2	16

Anthracnose disease response for the sorghum landraces from the Kayes region showed an association with region of origin. More anthracnose resistant landraces were observed from the Kenieba and Kita administrative districts, which are located is the southern areas of the Kayes region. Anthracnose resistant landraces were less frequent from the northern administrative districts of Bafoulabe, Diema, Kayes, Nioro, and Yelimane, with the lowest frequency of resistance observed for the landraces from the Kayes and Yelimane districts. This association between anthracnose disease response and region of origin for sorghum accessions was also shown to be associated with annual rainfall. The lowest frequency of anthracnose resistant accessions was observed for northern regions experiencing less than 600 mm of annual rainfall. In contrast, southern regions receiving more than 800 mm of annual rainfall showed a greater frequency of anthracnose resistant accessions. This association could aid in the selection of accessions from the NPGS sorghum collection for disease evaluation to identify additional sources of anthracnose resistance and aid germplasm acquisition by identifying regions where disease resistance could be more frequent.

The sorghum landraces from the Kayes region also showed phenotypic diversity, with anthracnose disease response showing an association with phenotypic race classification. Race guinea accessions were more frequently rated as resistant as compared to race durra accessions. However, this association between anthracnose disease response and sorghum race classification may be fortuitous in that the distribution of the race guinea and durra accessions is also influenced by annual rainfall in the Kayes region. Race durra accessions are typically found in drier regions; whereas, race guinea accessions are associated with wetter sorghum production regions. Additionally, the frequency of resistant guinea landraces was generally similar to the frequency of resistant durra landraces for the accessions collected from the drier, northern regions. Although rainfall may influence the distribution of race durra and guinea accessions, the observed association between anthracnose resistance and sorghum race classification could aid in the selection of germplasm for disease evaluation when passport information is lacking to determine ecogeographic origin. The association between anthracnose disease response and ecogeographic origin would indicate resistant germplasm is more frequent in wetter regions; however, genetic characterization of resistant sorghum accessions will be essential to determine if genetic diversity for resistance is also associated with ecogeographic origin.

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