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Reaction to Rust by a Subset of Sorghum Accessions from Zimbabwe

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ABSTRACT

Sorghum rust is a common disease in most of the sorghum-growing areas of the world, which increase susceptibility to other major diseases. Although, plant resistance is the best control strategy, today, a limited number of resistance sources are available. Therefore, identification of new resistance sources is essential to extend the control of the disease. In this study 68 sorghum accessions from the Zimbabwe collection maintained by the USDA-ARS, Plant Genetic Resources Conservation Unit at Griffin, Georgia were evaluated in Isabela, Puerto Rico during two planting seasons in 2011 to identify new sources of rust resistance. Across the two growing seasons, 12 accessions showed resistance, 15 accessions exhibited a moderately susceptible response and 41 accessions showed a susceptible response. Variation in disease response was observed within and between experiments for 37 accessions. No rust infection was detected on PI482787 across the two growing seasons, while accession PI482795 exhibited the highest rust infection. This study identified new sources of rust resistance and shows that PI482787 possess gene(s) for rust resistance and that this accession could be used in sorghum improvement breeding programs.

Key words: *Puccinia purpurea*, Sorghum germplasm, foliar disease, rust, breeding programs

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important grain crop behind maize, wheat, rice and barley (FAO, 2010). In addition, it is of growing importance as a source of grain-based ethanol and a promising source of cellulosic ethanol. Nevertheless, its productivity and profitability are adversely affected by a number of foliar diseases such anthracnose and rust.

Sorghum rust is incited by *Puccinia purpurea* Cooke (syns: *U. sorghi* Pass.; *P. sanguinea* Diet.; *Dicaeoma purpureum* (Cooke) Kuntze) and *P. prunicolor* (Karunakar *et al*., 1996; Bandyopadhyay, 2000; Thakur *et al*., 2007) and is prevalent in most of the sorghum-growing areas of the world. In United States, the disease has minimal effect on yield; however, its importance is due to the fact that rust predisposes the plant to other major diseases, such as stalk rots (*Fusarium* sp.), charcoal rot and grain mold (Frederiksen and Odvody, 2000). Moreover, foliar diseases turn out to be an important factor in biomass production system (Saballos, 2008). Rust is most prevalent in cool and humid regions (Karunakar *et al*., 1996; Bandyopadhyay, 2000; Thakur *et al*., 2007), while in conducive environments, rapid development of the disease will occur resulting in poor panicle exsertion and grain yield (Thakur *et al*., 2007). Yield losses ranging from 30-65% due to rust infection have been reported in the Philippines, India and Australia (Bandyopadhyay, 2000; Anon, 2002). In Puerto Rico, estimated losses due to rust infection fluctuate from 29-50% in grain yield (Hepperly, 1990). Yield losses have also been reported in Malawi, Swaziland, Tanzania, Zambia, Brazil, Argentina and Zimbabwe (Anon, 2002). In areas where the disease is endemic, genetic resistance and tolerance are the most reasonable way of controlling the disease and maintaining an economic viable crop.

Rust symptoms may dependance on the plant color. The pathogen forms scattered purple, red, or tan flecks on both sides of the infected leaves and in highly susceptible lines, the flecks may coalesce to form blister-like, dark reddish brown pustules (Bandyopadhyay, 2000; Thakur *et al.*, 2007). In time, the color of the pustules will change from reddish brown to dark or blackish brown (Bandyopadhyay, 2000; Thakur *et al.*, 2007). In contrast, the rust flecks are restricted in resistant genotypes (Thakur *et al.*, 2007). Nevertheless, pairs of near isogenic lines which differed clearly in their pigment colors (red and tan) did not show significant differences in their reaction to rust and anthracnose when evaluated in the field (Siame *et al.*, 1993). In fact, the evaluation of 5,218 sorghum accessions for their rust reaction by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) identified fifteen accessions with high and moderate levels of resistance (Singh *et al.*, 1994). Among these 15 accessions, ten and five were purple and tan colored, respectively.

The inheritance of rust resistance is complex and has not been well clarified. While the first inheritance study determined that rust resistance is controlled by the dominant gene Pu (Coleman and Dean, 1961), subsequent research identified different allelic interactions which confer different kinds of resistance (Miller and Cruzado, 1969). Moreover, a complex inheritance was observed by Patil-Kulkarni *et al.* (1972) when highly susceptible and resistant lines were crossed, concluding that many genes could be involved in the rust resistance. Recently, four genomic regions associated with rust resistance were identified through the segregation analysis of recombinant inbred lines derived from the cross of resistant and susceptible lines (Tao *et al.*, 1998). The genomic region (i.e., QTL) with the largest effect was located in chromosome eight and explained 40% of the phenotypic variation. Comparative mapping studies employing sugarcane, maize and sorghum situated the homologous of Rp1-D, a maize rust resistance gene, within the major rust resistance QTL of sorghum (McIntyre *et al.*, 2004).

Host plant resistance is considered to be the best control strategy for this disease. The developing of durable resistance involve gene-stacking of different sources of resistance in order to reduce the ability of the pathogen to overcome the resistance genes. Experiments conducted on pearl millet (*Pennisetum glaucum* L. R. Br.) suggest that the dynamic multiline breeding strategy is effective in developing rust-resistance hybrids (Wilson and Gate, 2002). Thus, the objective of this research was to evaluate subsets of sorghum accessions collected from Zimbabwe for resistance against *P. purpurea* in order to increase the resistance gene pools.

MATERIALS AND METHODS

Germplasm: Sixty-eight sorghum accessions were randomly selected from the Zimbabwe germplasm collection maintained by the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia. In addition, 15 control genotypes (Prom and Erpelding, personal communication) were included in the evaluation. The susceptible genotypes were PI247136 (Serbia), PI152714 (Sudan), PI609251 (Mali), PI609634 (Mali), PI211633 (Afghanistan), PI533794 (Ethiopia), PI173112 (Turkey) and the USA improved line N107 (PI535792), while resistance genotypes were PI533991 (Sudan), PI533831 (Sudan), PI534131 (Ethiopia), PI534157 (Ethiopia) and the three USA improved lines BT×623 (PI564163), SC1313 (PI595735) and Sureno (PI561472).

Experimental design: Accessions were evaluated at the Tropical Agriculture Research Station experimental farm at Isabela, Puerto Rico during two seasons: the dry (January-April) and wet (August-Dec) of 2011. The experimental design for both seasons was a randomized complete block design (RCBD) consisting of three blocks. Seeds from each accession were planted in a single row of 1.8 m in length with 0.9 m spacing. Fertilizer was applied at a rate of 560 kg ha^{-1} (15-5-10 NPK) during planting and sprayed with DiPel (Valent Bioscience Corporation, IL). Weeds were controlled both manually and with mechanical tillage.

Rust and phenotype evaluation: All accessions were evaluated at the soft dough to hard dough stages of development. Disease assessment was based on the rust severity of leaves from the middle of the stalk to the top of the plant. Rust severity was based on a scale of 1-5; where 1 = no rust, leaves free of the disease; 2 = 1-10% leaf area infected; 3 = 11-40% leaf area infected; 4 = 41-65% leaf area infected; 5 = 66-100% leaf area infected (Wang *et al*., 2006). This scale was further categorized into three reaction classes i.e., accessions rated as 1 or 2 are considered resistant, accessions rated 3 are considered as moderately susceptible, while accessions rated as 4 or 5 are susceptible.

Flowering time, plant height, panicle length and plant color were recorded for each accession in order to morphologically characterize the resistant accessions. Flowering time was defined as the number of days 50% of the plants within a row reached anthesis, while plant height and panicle length refer to the distance from the base of the plant (i.e., soil) to the top of the panicle and the distance from the first rachis to top of the panicle, respectively. Plant color classification was according to the Sorghum Crop Germplasm Committee (SCGC) which consists of 9 color categories: (1) purple, (2) red, (3) tan, (4) purple-red, (5) red-purple (6) tan-red, (7) tan-purple, (8) mixed and (9) black.

Statistical analysis: Rust data for the two seasons (scale of 1 to 5) were combined and subjected to the analysis of variance using PROC GLM of SAS (SAS version 9.2, SAS Institute, Cary, NC) to determine the main effect of sorghum accessions. The linear random effects model for such ANOVA was the following: $Y = \mu+S+B(S)+A+A\times S+e$; where Y is the rust score, μ is the common effect, S is the season effect, B(S) is block within season effect, A is the effect of accessions, A×S is the interaction of accession×season and e is the plot to plot variation within accession. Because of an interaction, analysis of the accession was also performed by season. Rust resistance performances of accessions were compared by least-squares means using the Tukey-Kramer p-value adjustment for multiple comparisons. Likewise, means and standard deviations for flowering time, plant height and panicle length were determined by the command PROC MEANS.

RESULTS AND DISCUSSION

Rust response was significantly different among accession ($p<0.001$), while, resistant and susceptible controls showed the expected rust response, indicating diseases incidence was appropriate during both experimental seasons. Across the two growing seasons, 12 accessions (PI482709, PI482718, PI482802, PI482813, PI482820, PI482727, PI482784, PI482788, PI482809, PI482801, PI482804, PI482787) conferred a resistant response (<2.5), 15 accessions exhibited a moderately susceptible response (2.5-3.5) and 41 accessions showed a susceptible response (>3.5) (Table 1). No rust infection was detected on PI482787, while accession PI482795 exhibited the highest rust infection. Results coincide with the obtained by Wang *et al.* (2006) when evaluated 96 random accessions, including four Zimbabwe accessions with variable resistance response. These four lines were susceptible (PI287578), intermediate resistance (PI287598 and PI287595) and high resistance (PI287597). Likewise, Singh *et al.* (1994) evaluated 5,218 accessions from the ICRISAT sorghum collection to identify 16 resistances accessions, including four from Zimbabwe. The identification of rust resistance accessions by the evaluation of limited number of samples (Wang *et al.*, 2006) suggests the Zimbabwe region might be adequate for the evolution of new resistance sources. Remarkable, the 12 resistance accessions identified herein were collected in the Midlands Province located at the center region of Zimbabwe.

Significant interactions were observed between accession and season ($p<0.001$) and accession and blocks ($p<0.001$). In fact, variation in disease response among seasons was observed for 37 accessions, while the responses of resistant and susceptible controls did not differ across experiments. Likewise, of the twelve resistant accessions identified herein, eight had consistent results across seasons (PI482709, PI482718, PI482813, PI482784, PI482788, PI482809, PI482801 and PI482787) and four (PI482802, PI482820, PI482727 and PI482804) were classified as showing resistance or moderate resistance (Table 2). This variation may be due to the fact that some of the sorghum landraces or accessions in the collection are heterogeneous. For instance, accessions obtained from several African countries and India exhibited variable response to rust when evaluated in Isabela, Puerto Rico (Wang *et al.*, 2006). Likewise, Singh *et al.* (1994) had variable rust resistance response when evaluated 16 Africa accessions across three years. In fact, majority of resistance accessions did not show rust infection during first year, however, the infection up to 40% in subsequently years. Moreover, Karunakar *et al.* (1996) has noted that the level of rust severity can be influenced by growth stage, inoculum density, temperature and relative humidity.

Resistant accessions identified herein differ significantly in flowering time, panicle length, plant height and color (Table 2). Flowering time ranged from 54-69 and 70-80 days for the first and second experimental season, respectively. This flowering time difference from the early to later season could be a consequence of the change in day-length conditions. Although, day-length difference are not as obvious in tropical regions, the first experimental season (January-April) involved transition from shorter to longer days, while second season (July-Dec) was the conversion from longer to shorter day length. In fact, plants were also taller during the second experimental season, ranging from 157-327 cm versus 135-227 cm, in the first planting season. Photoperiod sensitive sorghums are taller during long day conditions where the vegetative stage period becomes longer (Pao and Morgan, 1986). Most resistant accessions had shorter panicles than standard commercial lines (BT×623 and RT×430) during both growing seasons. Moreover, the shortest panicle was observed in the most resistant line, PI482787. Associations among plant color and rust resistance were not observed in this germplasm. Nevertheless, the tan and purple color have been previously associated with resistance to rust (Singh *et al.*, 1994) and resistance identified herein involve these two color, therefore, both might associated with the resistance response. Further

Table 1: Disease reaction of 68 sorghum accessions from Zimbabwe to rust evaluated at Isabela, Puerto Rico during two experimental seasons in 2011

Accession	Rust reaction	Accession	Rust reaction
PI482795	5.0^{a}	PI482812	3.3^{abcd}
PI482706	4.7^{ab}	PI482764	3.0^{abcd}
PI482708	4.7^{ab}	PI482767	3.0^{abcd}
PI482719	4.7^{ab}	PI482775	3.0^{abcd}
PI482771	4.7^{ab}	PI482777	3.0^{abcd}
PI482803	4.7^{ab}	PI482780	3.0^{abcd}
PI482819	4.7^{ab}	PI482791	3.0^{abcd}
PI482821	4.7^{ab}	PI482794	3.0^{abcd}
PI482768	4.3^{abc}	PI482714	2.7^{abcd}
PI482774	4.3^{abc}	PI482766	2.7^{abcd}
PI482778	4.3^{abc}	PI482772	2.7^{abcd}
PI482790	4.3^{abc}	PI482793	2.7^{abcd}
PI482796	4.3^{abc}	PI482815	2.7^{abcd}
PI482799	4.3^{abc}	PI482709	2.3^{abcd}
PI482810	4.3^{abc}	PI482718	2.3^{abcd}
PI482776	4.2^{abcd}	PI482802	2.3^{abcd}
PI482728	4.0^{abcd}	PI482813	2.3^{abcd}
PI482769	4.0^{abcd}	PI482820	2.3^{abcd}
PI482773	4.0^{abcd}	PI482727	2.0^{bcd}
PI482781	4.0^{abcd}	PI482784	2.0^{bcd}
PI482783	4.0^{abcd}	PI482788	2.0^{bcd}
PI482786	4.0^{abcd}	PI482809	2.0^{bcd}
PI482789	4.0^{abcd}	PI482801	1.7^{cd}
PI482792	4.0^{abcd}	PI482804	1.7^{cd}
PI482797	4.0^{abcd}	PI482787	1.0^{d}
PI482798	4.0^{abcd}		
PI482807	4.0^{abcd}		
PI482811	4.0^{abcd}		
PI482814	4.0^{abcd}		
PI482816	4.0^{abcd}		
PI482817	4.0^{abcd}		
PI482707	3.7^{abcd}		
PI482715	3.7^{abcd}		
PI482765	3.7^{abcd}		
PI482779	3.7^{abcd}		
PI482782	3.7^{abcd}		
PI482785	3.7^{abcd}		
PI482800	3.7^{abcd}		
PI482805	3.7^{abcd}		
PI482806	3.7^{abcd}		
PI482818	3.7^{abcd}		
PI482770	3.3^{abcd}		
PI482808	3.3^{abcd}		

Means within column followed by the same letter(s) are not significantly different based on the Tukey-Kramer adjustment for multiple comparisons at the 5% probability level

Table 2: Means of disease reaction, flowering time, plant height, panicle length and plant color of sorghum (*Sorghum bicolor* L. (Moench)) accessions from Zimbabwe (ZW) resistant to rust and the performance of two commercial lines evaluated at USDA Research Station, Isabela, Puerto Rico during two experimental seasons in 2011

		Rnst reaction score			Flowering time (day)		Plant height (cm)		Panicle lenght (cm)		
Accession	Origin	Season 1	Season 2	LS mean	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Plant color
PI482787	Sadza, ZW	1.0±0.0	1.0±0.0	1.0	62.78±3.02	78.11±6.00	172.67±10.54	238.61±37.18	09.33±0.88	12.50±2.20	Purple-red
PI482804	Masvingo, ZW	3.0±0.0	1.3±0.6	1.7	59.66±2.08	70.33±0.58	160.00±10.44	231.67±15.00	22.00±2.64	21.67±1.67	Purple-red
PI482801	Masvingo, ZW	1.5±0.7	1.7±0.6	1.7	62.50±2.12	78.33±0.67	166.83±0.71	216.67±25.66	13.50±3.54	16.39±1.27	Tan
PI482784	St. Joseph Mission, ZW	2.0±0.0	2.0±0.0	2.0	68.67±4.24	78.11±1.02	158.17±7.78	225.83±3.82	16.67±4.71	15.56±4.19	Tan
PI482727	Selukwe, ZW	3.5±0.7	1.3±0.6	2.0	65.00±2.83	78.11±3.21	184.83±5.42	196.94±15.86	17.67±0.47	21.67±1.67	Red-purple
PI482809	Mataka, ZW	2.0±0.0	2.0±0.0	2.0	65.00±6.69	76.83±2.59	163.33±8.41	207.50±10.61	11.56±0.77	13.75±1.77	Tan
PI482788	Sadza, ZW	1.6±0.6	1.3±0.6	2.0	53.89±3.56	78.67±0.47	164.67±10.37	259.17±3.54	10.44±0.84	12.83±1.65	Purple-red
PI482820	Shabani, ZW	3.5±2.1	1.7±0.6	2.3	56.67±3.30	79.78±2.55	227.33±6.60	327.50±23.85	25.50±4.48	20.42±4.73	Purple-red
PI482813	Mataka, ZW	2.3±0.6	1.7±0.6	2.3	68.50±10.61	76.22±3.17	150.67±31.58	211.94±11.98	14.17±3.54	16.11±1.27	Tan
PI482802	Masvingo, ZW	3.5±0.7	1.7±0.6	2.3	62.50±1.65	79.67±2.08	219.33±9.43	324.72±33.23	24.33±4.24	23.06±1.73	Red-purple
PI482718	Bulawayo, ZW	2.0±1.4	2.0±0.0	2.3	63.50±5.89	73.56±3.10	135.50±5.89	157.22±10.58	11.00±0.94	15.56±4.19	Tan
PI482709	Bulawayo, ZW	2.0±0.0	2.3±0.6	2.3	64.17±0.71	79.33±2.52	155.67±11.31	222.22±13.98	12.67±0.94	16.39±5.36	Tan
BT×623	Texas, USA	3.0±0.0	3.0±0.0	3.0	60.55±2.83	79.67±4.33	139.77±3.89	125.14±6.36	22.11±1.72	17.01±2.56	Red
RT×430	Texas, USA	3.0±0.0	3.0±0.0	3.0	69.11±5.72	78.88±5.48	98.33±4.41	112.08±4.33	22.22±3.47	16.63±3.47	Purple-red

LS mean: Least aquare means

genetic diversity studies involving molecular markers (e.g., SSR, single nucleotide polymorphism) might provide insight as to the origin or relationship among these rust resistance accessions and better strategies to evaluate this germplasm.

CONCLUSION

The rust response evaluation of 68 accessions from the Zimbabwe collection maintained by the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia resulted in the identification of 12 resistance accessions. These twelve accessions were originally from the Midlands Province of Zimbabwe suggesting the regions might be an important source of rust resistance. The highest resistance response was observed in PI482787, however, all resistance accessions could be useful in sorghum improvement breeding programs; in particular the rust resistance genes present in PI482787 might be introgressed into elite sorghum lines.

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