Pathogenic Variability in *Uromyces appendiculatus* from Tanzania and Rust Resistance in Tanzanian Bean Cultivars

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ABSTRACT

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Nine Tanzanian races of the rust pathogen, *Uromyces appendiculatus*, were identified on 19 differential bean lines and cultivars. All nine races differed from previously described races. Differential cultivars Ecuador 299, Mexico 235, Mexico 309, and Compuesto Negro Chimaltenango were resistant and cultivars Kentucky Wonder 780, Golden Gate Wax, U.S. 3, and Pinto 650 were susceptible to all of these races. Some Tanzanian bean cultivars previously selected for field resistance in Tanzania were resistant to most Tanzanian and several U.S. races of the pathogen.

The common bean (*Phaseolus vulgaris* L.) is an important grain legume in Tanzania. It is the main source of protein, and diseases, pests, and low yielding cultivars result in low bean yields. Rust, caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*, is second only to anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., in importance of bean diseases in Tanzania (10).

Efforts to control rust in Tanzania through genetic resistance were initiated in the early 1960s with selections of resistant cultivars (13). Since then, evaluation of local and foreign germ plasm for resistance to rust at different localities in Tanzania has indicated pathogenic variability in local populations of the fungus. Cultivars found resistant at one locality were susceptible in another area (9,12,15). Pathogenic variability of *U. appendiculatus* in other places is well documented, with over 55 races reported from the United States (7,8,17) and about 150 races reported from other countries, including Brazil (2,3), Australia (1), Mexico (5), and Colombia and other Latin American countries (4,20).

The objectives of this study were to confirm the variability of the rust pathogen in Tanzania and to determine reactions of some Tanzanian cultivars, previously selected for their resistance in the field, to Tanzanian and U.S. races of the pathogen.

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MATERIALS AND METHODS

Urediniospore collection and single uredinium isolations. Urediniospores were collected in May 1985 from Mbeya, Uyole Agricultural Research Center experimental farms and from the experimental farms of Sokoine University of Agriculture, Morogoro, Tanzania. They were collected from leaves of bean plants seeded in March for July harvest. Leaves with numerous uredinia were collected from different cultivars, and the urediniospores were shaken onto paper, then poured into glass vials. The urediniospores were dehydrated before storage by placing the open vials for 6 hr in a desiccator containing a beaker of concentrated sulfuric acid. The urediniospores were stored in a freezer at −15 C and kept frozen, except during transit to the United States, where they were used to inoculate bean seedlings for single uredinium isolation.

Suspensions of 18,000–22,000 urediniospores per milliliter were made from each collection in tap water containing 0.1 ml of Tween 20 per liter. Six days after seeding, 35–65% expanded unifoliolate leaves of differential cultivars were spray-inoculated on both surfaces as described elsewhere (16,17). Inoculated plants were incubated in a dew chamber at 19 ± 1 C and 100% relative humidity for 18 hr, then moved to a glasshouse maintained at 23 ± 2 C. Cultivars that developed large uredinia were noted, and additional seeds of the same cultivars were germinated for use in single uredinium isolation. Plants not previously exposed to *U. appendiculatus* were inoculated with urediniospores from single uredinia by transfers with a moist camel's-hair brush. Inoculated plants were incubated

in a dew chamber under the above temperature, humidity, and time conditions, then kept in isolated glasshouse locations at 24–28 C to avoid mixing of isolates. Urediniospores from single uredinium isolations were collected after 9–14 days by tapping infected leaves to dust urediniospores onto clean paper. The urediniospores were then dehydrated for 6 hr with anhydrous calcium chloride and frozen.

Testing of single uredinium isolates on differential cultivars. The standard set of 19 differential cultivars of *P. vulgaris* for identifying races of *U. appendiculatus* (17,19) were grown in 10-cm-diameter plastic pots, four seeds per pot as described elsewhere (16). Unifoliolate leaves were inoculated at the proper stage with suspensions containing 22,000 urediniospores per milliliter (16,17).

Reaction grades were rated 14 days after inoculation, using the standard scale of 1–6 developed at the 1983 International Bean Rust Workshop (17,19). Each isolate was tested at least twice on the differential cultivars. Isolates that gave similar reactions on the differential cultivars were assumed to have similar virulence patterns, so only one representative of the group was tested further.

Each isolate for which the differential cultivars gave separable reactions also was tested on the Mexican and Australian differential cultivars (1,5). The reactions of these cultivars to the isolates were compared with those reported for Mexican and Australian races of the pathogen. The reactions to the Tanzanian isolates also were compared with those to U.S. races (8,17). Isolates that gave similar reactions to any of the available U.S. races (17,18; J. R. Stavely and J. R. Steadman, *unpublished*) were tested on the differential cultivars alongside the appropriate U.S. race.

Determination of rust reactions of Tanzanian cultivars. Previous evaluations in Tanzania of 206 local *P. vulgaris* cultivars identified those resistant to rust in the field (15). The seven most resistant cultivars were tested at Frederick and Beltsville, MD, with individual races to determine the extent of their resistance to nine Tanzanian races and 15 U.S. races. Cultivars that showed the broadest resistance were then tested at Beltsville with 16 additional U.S. races. At least two replicates of four plants were inoculated as described above.

Cultivar reactions were categorized as resistant to susceptible on the basis of reaction grade and uredinium size: immune (I), no visible symptoms; highly resistant (HR), necrotic flecks without sporulation; resistant (R), uredinia predominantly less than 300 μm in diameter (when necrotic flecks and small uredinia occurred together, the reaction was R); moderately resistant (MR), uredinia predominantly 300–500 μm in diameter, with none larger than 500 μm; moderately susceptible (MS), uredinia 500–800 μm in diameter but none larger than 800 μm; and susceptible (S), uredinia 500–800 μm in diameter and some or most larger than 800 μm.

RESULTS

Infection of differential cultivars by Tanzanian urediniospore collections. Light infections, producing a few uredinia on certain differential cultivars, were obtained from only one urediniospore collection from Mbeya and one from Morogoro. From these uredinia, 17 single uredinium isolations were successful.

Testing of single uredinium isolates on differential cultivars. From the 17 single uredinium isolates, nine distinct races were identified on the 19 standard differential cultivars (19) (Table 1). These races have been progressively numbered, from narrowest to broadest in virulence, as Tanzanian (T) races 1 through 9. Four of the isolates were race T-2, four were T-3, and three were T-6, with one isolate of each of the other six races. All nine races produced uredinia larger than 800 μm on U.S. 3 and Kentucky Wonder (KW) 780 and larger than 500 μm on Golden Gate Wax. Ecuador 299, Mexico 235, Mexico 309, Actopan × Sanilac selection 37 (A×S 37), and Compuesto Negro Chimaltenango (CNC) were HR or R to all nine races, developing either necrotic flecks without sporulation or uredinia predominantly less than 300 μm in diameter. Differential cultivars NEP-2, Aurora, and 51051 were HR to seven races and S to races T-8 and T-9 (Table 1).

Most of the Tanzanian races were easily separable by the reactions of several of the differential cultivars (Table 1). Comparisons of races T-3 with T-4 and of T-5 with T-6 indicate much common virulence. However, they were clearly differentiated by small uredinia of T-4 vs. lack of sporulation by T-3 on CNC and A×S 37 and differences in uredinium sizes between T-5 and T-6 on Golden Gate Wax and Brown Beauty.

Race T-8 was similar to U.S. race 13 (8) in virulence on the first six differential cultivars, the only ones used when race 13 was originally described. However, when tested on 13 additional cultivars that had been tested with race 13 (8), race T-8 differed from race 13 by producing a necrotic reaction on Swedish Brown, an

Table 1. Reactions of 19 differential bean cultivars to nine Tanzanian races of *Uromyces appendiculatus*

Differential cultivars[a]	Reactions[b] to pathogenic races								
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9
U.S. 3	5,6/6,5	5,6/6,5	5,6	6,5	5,4,6/6,5	5,6/6,5	6,5	5,6/6,5	5,6/6,5
CSW 643	2,2+,3	2,3	3,2	3/3,2	3,2/3,2,4	3,2/3,2,4	4,3,5	5,6/6,5	5,6,4/6,5
Pinto 650	4,3	5,6,4/6,5	6,5	6,5	5,6,4/6,5	6,5/5,6,4	6,5	6,5	6,5/5,6,4
KW 765	3	3,4/4,3	3,2/3,4	2,2+,3	4,5,3/5,4	4,5/4,5,3	4,5	5,6/6,5	5,6,4
KW 780	5,6/6,5	5,6,4/6,5	5,6,4/6,5	5,6,4/6,5	5,4/5,6	5,6/5,6,4	6,5	5,6,4	5,6,4
KW 814	2,3	2,3/3,2	2,3/3,2	2/2,3	4,5/5,4	5,4	4,3,5/4,5	5,4/5,4,6	4,3,5/4,5
Golden Gate Wax	5,6	4,5/5,4,6	5,4,6/6,5	5,6/6,5	5,4,6/5,6	4,5/4,5,3	4,5/5,4,6	4,5/5,6,4	4,5/5,4,6
Early Gallatin	4,5	4,5/5,4	4,5	4,5/5,4	3,4/4,3	3,4/4,5	4,5/5,4	5,6/6,5	4,5/5,4
Redlands Pioneer	2/2,3	3,2/3,4	3,4/4,3,5	4,5/5,4,6	3,2/3,4	3,2/3,2,4	3,4	4,5/5,4,6	5,4/5,4,3
Ecuador 299	2	2,2+	2/2,2+	2,2+	2/2,2+	2/3,2+	2,2+	3,2/3	3,2/3
Mexico 235	2	2,2+	2	2	2	2	2,2+	3,2	3,2/3
Mexico 309	2/2,3	2,2+/3,2	2,3	3,2	3,2/3	3,2/3	3,2	3,2	3,2/3
Brown Beauty	4,5	4,5/5,4	4,3/4,5	5,4,6/5,6,4	3,4/4,3	4,3/4,5	4,5	5,6/5,6	4,5/5,4
Olathe	3,4	3,4	3,2/3,4	3,2/3,4	5,6/6,5	5,6/6,5	5,6,4/6,5	5,6/6,5	5,6,4/5,4,6
A×S 37	2,3	2,3/3,2	2,2+	2,2+/3,2,2+	2/2,3	2/2,3	2,2+	2,2+/2,2+,3	3,2
NEP-2	2,2+	2,2+	2,2+	2,2+	2,2+	2,2+	2,2+	4,5/5,4,6	5,4/5,6
Aurora	2,2+	2,2+	2,2+	2,2+	2,2+	2/2,2+	2,2+	5,4,6/5,6,4	4,5/5,6,4
51051	2,2+	2,2+	2,2+	2,2+	2,2+	2,2+	2,2+	4,5/5,4	4,5/5,6,4
CNC	2,2+/3	3,2/3	2,2+	3,2/3,4	2,3/3,2	2/3,2	3,2/3	2,2+/3,2	2/3

[a] U.S. = United States, CSW = California Small White, KW = Kentucky Wonder, A×S = Actopan × Sanilac, CNC = Compuesto Negro Chimaltenango.
[b] Reaction grades: 1 = immune, 2 = necrotic flecks without sporulation, 2+ = necrotic flecks 300–1,000 μm, 3 = uredinia less than 300 μm in diameter, 4 = uredinia 300–500 μm in diameter, 5 = uredinia 500–800 μm in diameter, and 6 = uredinia larger than 800 μm in diameter. Where a slash is given, the numerator is for the adaxial surface and the denominator is for the abaxial leaf surface. Where several figures are given, they are listed in order of predominance from most to least.

MR reaction on Low Champion, and MS reactions on Commodore, Pencil Pod Wax, and Tendergreen. In instances where simultaneous testing seemed justified, no Tanzanian race produced the same reactions on the differential cultivars as any recently described U.S. race (17). None of the Tanzanian races was similar to any Mexican or Australian race (1,5).

Rust reactions of Tanzanian cultivars. Reactions of Tanzanian cultivars to U.S. and Tanzanian races are given in Table 2. TMO 31 had the greatest and the broadest resistance to Tanzanian and U.S. races. In addition to the races shown in Table 2, TMO 31 was I to U.S. races 38, 43, 53, 55, 56, and 68; HR to race 48; and R to races 54, 57, 59, 60, 61, and 64. Cultivars TMO 25, TMO 33, and TMO 130 segregated in their reactions to many races. Cultivars TMO 58 and TMO 66 had a few R or MR reactions with Tanzanian and U.S. races but were not more than MS to any race. Even though cultivars TMO 25 and TMO 33 were S to many U.S. races, they were mostly HR to MS to the Tanzanian races (Table 2).

Table 2. Reactions of certain Tanzanian bean cultivars to Tanzanian and certain United States races of *Uromyces appendiculatus*

	TMO cultivars[a] and reactions[b]						
Race	**25**	**31**	**33**	**58**	**66**	**75**	**130**
T-1	HR	I	R	MR	MS	R	MS
T-2	MS,S	I	R	MR	MS	R	MR,MS
T-3	R	HR	HR	MS	MS	MS	MS
T-4	HR	HR	MS	MS	MS	MS	MS
T-5	MR,S	HR	HR	MS	MR	MS	MR,MS
T-6	HR	I	R	MS	MS	MS	MS
T-7	R	I	HR	MS	MS	R	HR
T-8	MS,S	MS	MR,MS	MS	MS	S	MR,MS
T-9	MR	MS	MR,S	MS	MS	S	MS
39	HR	I	I,R	R	R	MR	R,S
40	S	R	HR	MS	MS	MS	MS,S
44	S	I	MS,S	MS	MS	MS	HR,S
46	MS	MS	S	MS	MS	MS	R
48	S	MS	S	MS	MS	MS	MR,S
49	S	MS	S	MS	MS	MS	MS
51	S	MS	R,S	MS	MS	MS	MS
53	R,S	R	R,S	MS	MS	MS	MS
58	I	I	HR	MR	MR	MS	MS
62	S	MS	S	MS	MS	MS	HR,MS
63	S	MS	R,S	MS	MS	MS	HR,MS
64	S	MS	R,S	MS	MS	MS	MS
65	S	MS	S	MS	MS	MS	HR,MS
66	S	MS	S	MS	MS	MS	MS
67	S	MS	S	MS	MS	MS	HR,MS

[a] The native and introduced bean cultivars or lines in the Tanzanian seed collection at Morogoro have all been given Tanzanian Morogoro (TMO) numbers. The original names for those having another local name were given by Misangu (14).

[b] Reactions: I = immune, no visible symptoms; HR = highly resistant, necrotic flecks without sporulation; R = resistant, uredinia predominantly less than 300 μm in diameter; MR = moderately resistant, uredinia predominantly 300–500 μm in diameter, with none larger than 500 μm; MS = moderately susceptible, uredinia 500–800 μm in diameter, with none larger than 800 μm; S = susceptible, uredinia 500–800 μm in diameter, with some or most larger than 800 μm. Where two reactions are given, the cultivar was segregating with most plants giving the first reaction and with some giving the second.

DISCUSSION

This research has demonstrated that a high degree of variability in the bean rust pathogen exists in Tanzania. If the viability of the field-collected spores had been better maintained, a larger number of races might have been obtained from the area sampled. The light infection that was obtained from urediniospores collected from the field indicates loss of viability during dehydration or transit from Tanzania to the United States. Further work will be necessary to identify the predominant races and full extent of pathogen variability in Tanzania. The degree of variability identified here indicates *U. appendiculatus* may have a similar high degree of variability in Tanzania to that already identified in Australia (1), Brazil (2,3), and the United States (6,7,8,17). This suggests the need for selecting breeding approaches that will maximize chances for stabilizing resistance. For most such approaches, obtaining the widest possible range of pathogen virulence is likely to enhance chances for developing resistance that will be stable in Tanzania.

Among the cultivars that have been reported to have resistance to many U.S. rust races (17), KW 780, Golden Gate Wax, Early Gallatin, Brown Beauty, Olathe, KW 765, and KW 814 were either MS or S to at least five of the nine Tanzanian races (Table 1). NEP-2, Aurora, 51051, and CSW 643 were S to only two races, T-8 and T-9. Cultivars Ecuador 299, Mexico 235, Mexico 309, A×S 37, and CNC were R to all nine T races. Cultivars TMO 31, TMO 33, TMO 25, TMO 58, TMO 66, TMO 130, and TMO 75 were broadly R and/or MS to at least a major portion of the rust population in Tanzania. Results from testing Tanzanian cultivars against the U.S. races confirmed the relative breadth of the resistance of some of these lines. Cultivars TMO 31, TMO 58, and TMO 66 were not S to any race.

Several approaches seem to have potential for supplying stable rust-resistant bean lines or cultivars for Tanzania. The reduction in rust severity and crop losses given by the MS reaction might suggest its usage. However, occurrence of races giving S reactions on some such cultivars, as reported here and earlier (17), seems to reduce this possibility. Pyramiding of various genes or linkage groups of genes giving HR, R, MR, or MS reactions to multiple races could produce cultivars with sufficient resistance genes for the available races to be adequate buffer against other races. Incorporation of these resistance genes into multiline cultivars seems to be another useful alternative for Tanzania (11).

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