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Screening tomato accessions for resistance to bacterial wilt

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Abstract

Eighty seven Makerere University tomato (*Lycopersicon esculentum* L.) accessions were screened for resistance to *Ralstonia solanacearum* under natural infestation. Most of the accessions showed susceptible reactions (>20%wilt), but accessions MT9, MT19, MT40, MT49, MT54, MT55, MT74, MT78, and MT98 showed resistant reaction (<10%wilt). Accessions MT2, MT65, MT69, MT70, MT80, and MT100 previously reported to be resistant to bacterial wilt proved susceptible to the disease. Additionally, latent infection was present in most of the selected resistant accession. Five isolates of *R. solanacearum* isolated from plants collected from different parts of Uganda were characterised by physiological tests. Based on Haywards classification scheme, all the isolates were biovar 3.

Key words: Biovar, host resistance, *Lycopersicon esculentum, Ralstonia solanacearum*, Uganda

Introduction

Bacterial wilt (BW) of tomato incited by *Ralstonia solanacearum (*syn. *Pseudomonas, Burkhoderia solanacearum)* is a devastating disease in many humid tropical regions with losses of up to 100% reported in Uganda (Opio, 1988). Since the bacterium is soil borne and resides in host plant xylem vessels, protective measures such as chemical control, soil fumigation and crop rotation have all proven ineffective for control. Planting resistant varieties has proven to be the simplest and most efficient method of BWdisease control. However, resistance to bacterial wilt in many varieties is not stable over locations presumably due to the existence of different races or biovars of the pathogen and variation in cultivar reactions in the backgound of different environments (Hanson *et al.*,1996).

We thus screened the tomato germplasm at the Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) to identify accessions resistant to bacterial wilt. The presence of different biovars in Uganda was also investigated.

Materials and Methods

Field evaluation

During the second rains of 1997, bacterial wilt reactions of 87 accessions in the Makerere University Agricultural Research Institute Kabanyolo (MUARIK) gene bank were determined in a field known to be naturally infested with *R. solanacearum*. The experimental layout was a completely randomised block design (CRBD) with 3 replications. In each replicate plot, 10 plants were grown at a spacing of 60 cm within rows and 60 cm between rows. Prior to seedling transplant to the field, seedlings were

raised in plastic sleeves containing steam sterilised soil and transplanted to the field 30 days after sowing. Rows of a susceptible cultivar Marglobe, were inter-planted with test lines to increase inoculum pressure and spread in the field.

Foliar fungal diseases were controlled with dithane M-45 at 7-day intervals until the end of the experiment. Resistance/susceptibility to BW was evaluated by weekly recordings of the incidence of wilted plants during the study period. Severity ratings were done using the scale developed by Rujput *et al.* (1994), i.e., highly susceptible (HS) 51-100%, susceptible (S) 21-50%, mildly resistant (MR) 11-20%, resistant (R) 1-10% and highly resistant (HR) <1% wilt .

Isolate collection

Five farms with bacterial wilt were purposely selected in the districts of Mbale Pallisa, Mpigi, Mbarara and Kabale in order obtain samples habouring *R. solanacearum.* From these farms, diseased tomato samples (2-3 whole plants per field) were collected and brought to the laboratory for isolation and identification of the wilt pathogen. Isolation of *R. solanacearum* was then done as described below.

R. solanacearum isolation

Stem sections (15-20mm) were cut from infected samples and placed in test tubes containing 15ml sterile distilled water (SDW). Bacteria were allowed to flow from the vascular bundles for 20 minutes. A loopful of the extracted bacterial suspension was then streaked on triphenyl tetrazolium chloride (TZC) plates (Kelman, 1954) and incubated for 48 hours at 30°C. Characteristic *R. solanacearum* colonies were selectively subcultured to ensure their purity. Thereafter, single colonies of the fluidal type grown on TZC, (selected and maintained cultures) were stored as suspensions in sterile distilled water in screw capped bottles at room temperature. Biovar identification was based on the ability of strains to oxidise cellobiose, lactose, maltose, dulcitol, mannitol, and sorbitol (French, *et al.*,1995).

Data analysis

Final percentage wilting (%wilt) and final percentage survival (survival%) were computed from the last evaluation of each trial. Weekly percentage wilting data were then used to compute the area under disease progress curve (AUDPC) following the procedure of J.F.Wang (personal comm). It should be noted that percentage wilt and percentage survival data were arcsin transformed in order to normalise variances before performing analysis of variance (ANOVA) (Steel *et al.*, 1997). Fisher's protected least significant difference test (LSD) was used to compare means of significant study parameters. All statistical computations were done using MSTATC statistical package (Freed *et al.*, 1988).

Results and Discussion

In plants exhibiting compatible reactions, symptoms of BW were characterised by epinasty followed immediately by irreversible wilting beginning at the flowering stage. Disease incidence varied significantly ($P<0.01$) among the genotypes. Most of the entries were susceptible, but MT9, MT19, MT40, MT49, MT54, MT55, MT74, MT78, and MT98 showed a high level of resistance (Wilt incidence <10%). Five ascessions rated as mildly resistant (11-20%wilt), included MT8, MT48, MT61, MT72 and MT93. The remaining accessions were either susceptible or highly susceptible. The survival percentage of the cultivars ranged from 3%(MT14) to 96.67% (MT 55, MT74, MT78 MT9, MT19).

MT2, MT11, MT86, MT63, MT65, MT66, MT69, MT70, MT71 MT24, MT95 and MT100 were

Table 1. Mean survival (%) of selected tomato entries screened for bacterial wilt resistance at Kabanyolo

MT No.[a]	Cultivar/ Accession	Source[b]	Comments[c]	Wilt incidence (%)	Survival(%)
78	LA 2711	UC Davis	Salinity resistance	0.0	96.7
55	HYB Flash	Asgrow		0.0	96.7
74	Sun Coast	Florida		0.0	96.7
9	Nalfpacls 1	OARDC		3.3	96.7
19	CVF13-3	AVRDC		3.3	96.7
98	NC 820	NCARS		6.7	93.3
49	NVH4773	NK		10.0	90.0
40	GS12	NK		10.0	90.0
54	HYB Sunny	Asgrow		10.0	90.0
61	Pacesetter490	Asgrow		13.3	86.7
93	Stephens(S.Africa)	NCARS	V,F1&2	16.7	83.3
86	Cra66	NCARS	(French) BW	20.0	80.0
24	Kewelo	AVRDC	BW	23.3	76.7
12	Pink Red	OARDC		23.3	76.7
79	LA2710	UC Davis	Aluminium tol.	26.7	73.3
150	-	-		26.7	73.3
53	HYB Sunny	Asgrow		26.7	73.3
84	LA2662(Saladette)	UC Davis	Heat tol.	30.0	70.0
39	RomaVF	OARDC	V,F1&ASC	30.0	70.0
20	Rodade	AVRDC	BW	30.0	70.0
6	Ohio 7814	OARDC		30.0	70.0
22	PI 162679	AVRDC		30.0	70.0
66	CLN657BC1F2-285-21	AVRDC	BW,Tmv,N	33.3	66.7
81	LA2661(cv.Nagcarlang)	UC Davis	Heat&cold tol.	33.3	66.7
8.	Mountian pride	OARDC	TMV, N	20.0	80.0
48	NVH	NK	-	13.33	86.67
72	CLN657BCIF2-267-0-1AVRDC	-	-	16.67	83.33
52	HYB Humaya	Asgrow		33.3	66.7
34	Ohio CR-6	OARDC	Tmv,Fcrr,F1&2,V	33.3	66.7
62	Pacesetter882	Asgrow		33.3	66.7
30	Mbarara wild red2 (Malaya)	Uganda		36.7	63.3
15	86W15	OARDC		36.7	63.3
25	Carl Mart	AVRDC		40.0	60.0
95	Hawaii 7998	NCARS	BW	40.0	60.0
17	SenatorF2	AVRDC	-	40.0	60.0
11	Venus	OARDC	BW	43.3	56.7
63	CLN657BC1F2-274-0	AVRDC	BW,Tmv,N	46.7	53.3
47	NVH 4771	NK		46.7	53.3
71	CLN657BC1F2-267-0-3	AVRDC	BW,Tmv,N	46.7	53.3
41	GS20	NK		53.3	46.7
56	HYB Centurion	Asgrow		53.3	46.7
16	Flora-Dade	OARDC		53.3	46.7
60	Hybrid 898	Asgrow		60.0	40.0
94	8642D	NCARS	V,F.Cherry	60.0	40.0
100	Hawaii 7997	NCARS	BW	63.3	36.7
23	Healani	OARDC		66.7	33.3
59	Hybrid 896	Asgrow		66.7	33.3
1	Heinz	Uganda	From USA	66.7	33.3
38	Roma	OARDC		70.0	30.0
69	CLN475BC1F2-265-12	OARDC	BW,Tmv,N	70.0	30.0
65	CLN657BC1F2-285-20	OARDC	BW,Tmv,N	70.0	30.0
82	LA1272(88L1740)	UC Davis		73.3	26.7

Table 1. Contd.

MT No.[a]	Cultivar/ Accession	Source[b]	Comments[c]	Wilt incidence (%)	Survival(%)
70	CLN466BC1F2-45-34-9	AVRDC	BW,TMV,N	76.7	23.3
42	GS20	NK		76.7	23.3
87	87294	NCARS	V,F1&2	80.0	20.0
27	Kasese Medium	Uganda		83.3	16.7
4	Campbell28	OARDC		86.7	13.3
29	Mbarara Wild Yellow (Malaya)		90.0	10.0	
14	Marion	OARDC	F1,ASC,ST	96.7	3.3
80	CLN475BC1F1-265-4	AVRDC	BW,TMV,N	-	3.3
Marglobe				36.7	63.33
2	Saturn	OARDC	BW	63.3	36.7
Mean				43.6	56.36
LSD(0.05)				40.9	40.87
CV%				43.3	34.14

[a] MT = Makerere tomato accession code
[b] AVRDC- Asian Vegetable Research Development Center, NCARS = North Carolina Agricultural Research Station; Asgrow seed company; OARDC = Ohio Agricultural and Research Development Center.
[c] F= Fusarium wilt resistance, TMV = Tobacco mosaic virus resistance, V = Verticillium wilt resistance, N = Nematode resistance BW = bacterial wilt resistance

originally reported as highly resistant to bacterial wilt in North Carolina and Taiwan (Grimault *et al.*, 1994) but proved susceptible at MUARIK with the exception of MT 24, MT86 and which showed mild resistance. This suggested a locational breakdown in resistance, probably due to the existence of a different biovar.

All virulent isolates produced fluidal colonies with pink or light red centres after 48 hours on TZC medium. Of the five isolates so far tested, all belonged to biovar 3. Four of the 5 strains tested were from the lowland areas of Uganda, and the fifth from the highlands.

Based on the preliminary results, MT41, MT74, MT55, MT54, MT19, MT9, MT78, MT15, MT93, MT95, MT59, MT49, MT50, and MT86 appear to have resistance to *R. solanacearum* in Uganda. Additional field experiments are under way to determine the stability of resistance. More isolates of *R. solanacearum* are also being collected from other locations and hosts in Uganda in order to establish biovar distribution.

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