Aggressiveness of *Ralstonia solanacearum* Strains and Evaluation of Tomato Genotypes for Resistance

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Abstract

Twenty-nine *Ralstonia solanacearum* strains collected from tomato, potato and pepper in Ethiopia were evaluated for their aggressiveness on four tomato cultivars differing in level of bacterial wilt resistance. The strains evaluated were categorized into three aggressiveness groups namely Agg1, Agg2 and Agg3 with proportion of 7, 41 and 52 % of tested strains respectively in an increasing virulence pattern. The most aggressive strain group (Agg1) consists of biovar 1 and race1 strains and the other groupings did not show correlations to biovar, host or geographical origin. Sources of resistance against the highly aggressive strain, *TomNa3* (biovar 1/race 1) was evaluated in growth chambers in comparison to Toudk2 (race 1 biovar 3) originated from Thailand. Wilt incidence, percent severity index and corresponding areas under disease progress curves were used to evaluate level of resistance. Among thirty-three tomato genotypes, six genotypes were identified as resistant and eleven as moderately resistant to the Ethiopia highly aggressive strain (*TomNa3*). Differential reactions were observed with some genotypes based on the strain of *R. solanacearum* used. All tomato cultivars commonly grown in Ethiopia were found either susceptible or highly susceptible to the most aggressive strain of *R. solanacearum* of Ethiopia and Thailand. The study recommends a breeding program incorporating resistant sources into existing tomato cultivars or evaluating the identified resistant sources for desirable agronomic traits.

Introduction

Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi et al. 1995) is a major disease of tomato in the tropics and subtropics (Hayward 1991). The pathogen is described as species complex forming four Phylotypes (Fegan and Prior 2005). In Ethiopia the disease is economically important both on tomato and potato (Stewart and Dagtechew 1967; Yaynu 1989). In terms of race/ biovar classification, biovar 2/race 3 and biovar 1/race 1 have been identified in Ethiopia (Lemessa and Zeller 2007).

Strains of *R. solanacearum* have been known for their genetic variability and aggressiveness (Darrasse et al.1998; Jaunet and Wang 1999). Strains of race 1 originated from Taiwan were identified for having six aggressiveness groups (Jaunet and Wang, 1999) and strains from the French West Indies were grouped into five aggressiveness groups (Darrasse et al. 1998). The term aggressiveness has been defined by Boucher et al. (1992) as the intensity of the symptoms induced by a particular strain on a compatible host which is estimated by measuring the kinetics of disease development, by rating the different types of symptoms, or by other quantitative traits.

There is scanty information on the general characteristics of *R. solanacerum* originating from Ethiopia. Furthermore, control of bacterial wilt has been proved to be difficult because the pathogen is

soil-borne and has a wide host range (Hayward 1991). Chemical control is ineffective. However, the use of resistant varieties remains a key strategy to control bacterial wilt (Wang et al. 1998, Boshou 2005). A major problem in resistance breeding however, is instability of resistance due to the diversity of the pathogen as resistance identified to specific strain break down to other strain(s) (Boshou 2005). Therefore, effective breeding for disease resistance in a particular region depends on a thorough understanding of host-pathogen interactions and identification of wilt resistance sources and aggressiveness nature of the pathogen. This study, therefore presents the aggressiveness of *R. solanacearum* strains from Ethiopia and evaluation of level of resistance among local and introduced tomato cultivars/genotypes against highly virulent strain from Ethiopia and reference strain from Thailand.

Materials and Methods

Bacterial strains and inoculation

A total of 29 *R. solanacearum* strains, all collected from the main tomato and potato growing regions in Ethiopia were used for this study (Table 1). Each bacterial strain was revived by streaking on triphenyl tetrazolium chloride (TTC) agar medium (French et al. 1995) containing 10 g Bacto peptone, 5 g D-glucose, 1 g casamino acid, 15 g Bacto agar and 1000 ml distilled water, where 10 ml of filter-sterilized solution of 0.5% (w/v) 2,3,5-triphenyl tetrazolium chloride (Sigma, Germany) were added.

Table 1 Description of strains of *Ralstonia solanacearum* used for aggressiveness test

No	Strain code	Host	Collection place	Year	Biovar	Phylotype
1	TomNa3	Tomato	Nacha	2006	1	III
2	Tom6II	Tomato	Holeta	2003	1	III
3	Tom1 II	Tomato	Holeta	2003	2	II
4	Tom3	Tomato	Mutulu	2003	2	III
5	Pot91	Potato	Shashemene	2003	1	III
6	Pep7	Pepper	Gudar	2003	1	III
7	Tom53	Tomato	Shashemene	2003	1	III
8	TomBK4	Tomato	Bako	2006	2	III
9	Tom56	Tomato	Ziway	2003	2	III
10	Tom58	Tomato	Gudar	2003	2	iII
11	TomZy9	Tomato	Ziway	2006	2	III
12	Tomzy8	Tomato	Ziway	2005	2	II
13	TomAw2	Tomato	Shashemene	2006	2	II
14	Tom88	Tomato	Ziway	2003	2	II
15	TomGr6	Tomato	Gudar	2006	2	II
16	Pot92	Tomato	Shashemene	2003	2	II
17	Pot84	Potato	Ambo	2003	2	III
18	Pot10II	Potato	Holeta	2003	2	III
19	Pot10III	Potato	Bako	2003	2	III
20	Pot20III	Potato	Arjo	2003	2	III
21	Pot21III	Potato	Arjo	2003	2	III
22	Pot29JU	Potato	Jimma	2003	2	III
23	Pot34	Potato	Gedo	2003	2	III
24	Pot42	Potato	Jeldu	2003	1	III
25	Pot48	Potato	Ginchi	2003	1	III
26	Pot60	Potato	Awassa	2003	2	III
27	Pot62	Potato	Awassa	2003	1	III
28	Pot70	Potato	Jimma	2003	2	III
29	Tom768	Tomato	Ziway	-	2	III

The strains were confirmed by polymerase chain reaction (PCR) and pathogenicity test on the susceptible tomato line L-390. For pathogencity test, a single colony of the strain was further multiplied on Nutrient Glucose Agar (NGA) medium containing 0.3% beef extract 0.5% Bacto peptone, 0.25% D-glucose and 1.5% agar and incubated at 30 °C for 48 h at.

Determination of aggressiveness was performed according to Prior *et al.* (1990), Darrasse *et al.* (1998) and Jaunet and Wang (1999). Four tomato genotypes differing in their level of resistance against bacterial wilt were used; [Wva700 (susceptible), Moneymaker (moderately susceptible), King Kong 2 (moderately resistant) and Hawaii-7997 (highly resistant]. Seedlings of each cultivar were raised on plastic trays containing soil mixtures (sand, loamy soil, compost (1:3:1) and the seedlings were transplanted after three to four weeks into individual plastic pot (one seedling per pot) containing the same soil mixtures and supplemented with commercial fertilizers, urea and di-ammonium phosphate (DAP). Inoculum suspension was prepared by flooding each plate with distilled sterilized water and the suspension of optical density (OD) at 620 nm = 0.06 was adjusted corresponding to approximately 7.8 x 10^7 CFU/ml (colony forming units per milliliter) and about 30 ml suspension was inoculated to each seedling by soil drenching (Jaunet and Wang 1999). Seedlings inoculated with the same strain were placed in one flat, and spacing between flats was at least 15 cm to avoid contamination. The experiment was arranged in a completely randomized design (CRD) in a glasshouse where the temperature ranged from 24-27 ^{0}C and relative humidity ranged from 65-70%. Due to limitations in space, the experiment was carried out in four subsequent sets during March-June 2007.

For resistance evaluation a highly aggressive bacterial strain *TomNa3* biovar 1/race 1 Phylotype III (Getachew 2009) isolated from tomato in Ethiopia and strain Toudk2 biovar 3 race 1 (standard reference strain at the Institute of Plant Diseases and Plant Protection, Leibniz University of Hannover, Germany) originated from Thailand were used. Bacterial culture and inoculum suspension was prepared as described above. Tomato cultivars/genotypes evaluated for resistance include those released /recommended for production in Ethiopia and those introduced from the World Vegetable Center-Asian Vegetable Research and Development center (AVRDC) (Table 2). The experiment was conducted under controlled growth chamber at the Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Germany. Seeds of each genotype were sown on seedling trays (30 x 47 cm), containing 'Fruhstorfer' soil (Type P, with 150 mg/l N, 150 mg/l P_2O_5 and 250 mg/l K_2O) (Industrie-Erdenwerk Archut GmbH & Co KG, Auterbach-Wallenrod, Germany) at a depth of 0.5 cm. The seedlings were raised in the glasshouse with temperature of 20°C day/night, 14 hours of light per day, 30,000 lux and 80% relative humidity. Four weeks after sowing, the seedlings were transplanted in plastic pots (12-cm diameter) containing about 330g of the same soil type used for raising seedlings and transferred to a climate chamber with 30^0 /27^0 C day/night temperature, 85% relative humidity, 14 h light, and 30 K lux. The treatments were arranged in a completely randomized design (CRD) with three replications and twelve plants per replication. Five plants from each cultivar/genotype were inoculated with tap water as the negative control. Each seedling was inoculated by soil drench with 33 ml of bacterial suspension per pot. After inoculation, pots were carefully watered up to the field capacity without causing a surplus and for the negative control. Due to limited space in the growth chamber the experiment was conducted in three subsequent batches at one month intervals during October 2006 to January 2007.

Assessment of Disease Development

In all cases disease development was monitored daily until four days post- inoculation and then at 4, 7, 10, 14, 21 and 28 days post inoculation by recording the degree of wilting in each strain and cultivar/genotype combination. A six point rating scale (0-5) modified from Winstead and Kelman (1952) was used, where 0 = no wilt symptoms, 1 = one leaf wilted, 2 = two or more leaves wilted, 3 = all leaves except the tip wilted, 4 = whole plant wilted and 5 = death (collapse) of the whole plant was used to record data at each scoring date. The final frequency of dead plants for each strain was used to calculate the average wilt incidence on the four genotypes and the same value was used in the

biplot analysis (Yan and Falk 2002) to determine the aggressiveness groups.

For resistance screening symptom development was observed and recorded as described for the aggressiveness test. The percentage of wilted plants (PWP) at each scoring date was calculated according to the following formula:

$[PWP = (NW / NT) * 100]$, where, NT is the number of total tested plants and NW is the number of wilted plants.

Similarly, the severity record on the basis of 0-5 scoring scale was converted to the percent severity index (PSi) as described by Cooke (2006).

PSi = $\sum (Scores * 100)$ / (number of plants rated*maximum scale of the scores) for each scoring date.

The reaction of each cultivar/genotype was categorized into four resistance levels based on average wilt incidences and percent severity index at 28 dpi as described by Adhikari and Basnyat (1998) where R = Resistant (where <20% wilt incidence/percent severity index), MR = Moderately resistant (20-40% wilt incidences/percent severity index), MS = moderately susceptible (41-60 %) wilt incidence/percent severity index and S = susceptible (>60 % wilt incidence/percent severity index). Further more, percentage of wilted plants and percent severity index at the evaluation dates of 4 to 28 days post inoculation of each score was used to calculate area under wilt incidence progress curve (AUDIC) and area under percent severity index curve (AUDSIC) using the trapezoid integration of the disease progress curve over time with the following formula adopted from Jeger and Vijanen-Rollinson (2001).

$$AUDIC = \sum_{i=1}^{n} [(PWPi_{i+1} + PWPi_i) / 2] \times [t_{i+1} - t_i]$$

in which PWP_i = Percentage of wilted plants at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.
Similarly, the area under percent severity index progress curve (AUDSIC) was calculated with the following formula;

$$AUDSIC = \sum_{i=1}^{n} [(PSi_{i+1} + PSi_i) / 2] \times [t_{i+1} - t_i]$$

Where PS_i = mean percent disease severity index at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.

Data Analysis

Data of the aggressiveness test were analyzed using the biplot analysis (Yan and Falk, 2002) with the table consisting of five columns and 30 rows. The columns correspond to the dependent number of wilt incidence of each tomato cultivar/genotype after inoculation at 28 days post inoculation (dpi) and the rows correspond to the strains of *R. solanacearum*. Although biplot analyses have been frequently used in visual analysis of genotype-by-environment data, the use has been illustrated on host-by-pathogen interactions, to express in a single scatter plot for visual evaluation of susceptibility/ resistance of genotypes and virulence/avirulence of strains, respectively (Yan and Falk, 2002).

The analysis of resistance level of 33 tomato cultivars/genotypes was based on analysis of variance using the SAS general linear model procedure (SAS for Windows, 1999-2003, SAS Institute, carry, USA) and means separation was based on least significance difference at 5% using Waller-Duncan K-ratio t test for the average final wilt incidence and mean percent of severity index at 28 dpi. Similarly, analysis and mean separation on area under disease incidence progress curve and area under percent severity index progress curve were done in the same way as above.

Results

Aggressiveness Group

The biplot analysis of aggressiveness data of the 29 Ethiopian *R. solanacearum* strains and four tomato genotypes using the final wilt incidence values at 28 dpi revealed three aggressiveness groups designated as Agg1, Agg2 and Agg3 (Figure 1). Less aggressive strains and susceptible genotypes located on the second quadrant of the plane while highly aggressive strains and more resistant genotypes were located in the upper part of the first quadrant of the plane. The intermediate aggressive

strains and moderately resistant tomato genotypes located in the fourth quadrant close to the axis. Agg1 represent the most aggressive group infecting all tested cultivars causing a mean wilt incidence ranging from 8.3 to 79%, Whereas Agg2 strains caused wilting symptom only on the moderately resistant (King Kong 2) and susceptible genotypes (Wva700, Moneymaker) with mean wilt incidence of 34.51% and 56% respectively. Agg3 strains represented the lowest aggressive strains which did not cause wilt symptom on both the resistant and moderately resistant tomato genotypes but on the susceptible tomato genotypes (Wva 700 and Money Maker) with mean wilt incidences of 29% and 33%, respectively. The most aggressive group Agg1 contains only strains belonging to biovar 1 race 1 isolated from tomato. The grouping pattern in other groups Agg2 and Agg3 did not show a relation to biovars, host or geographical origin.

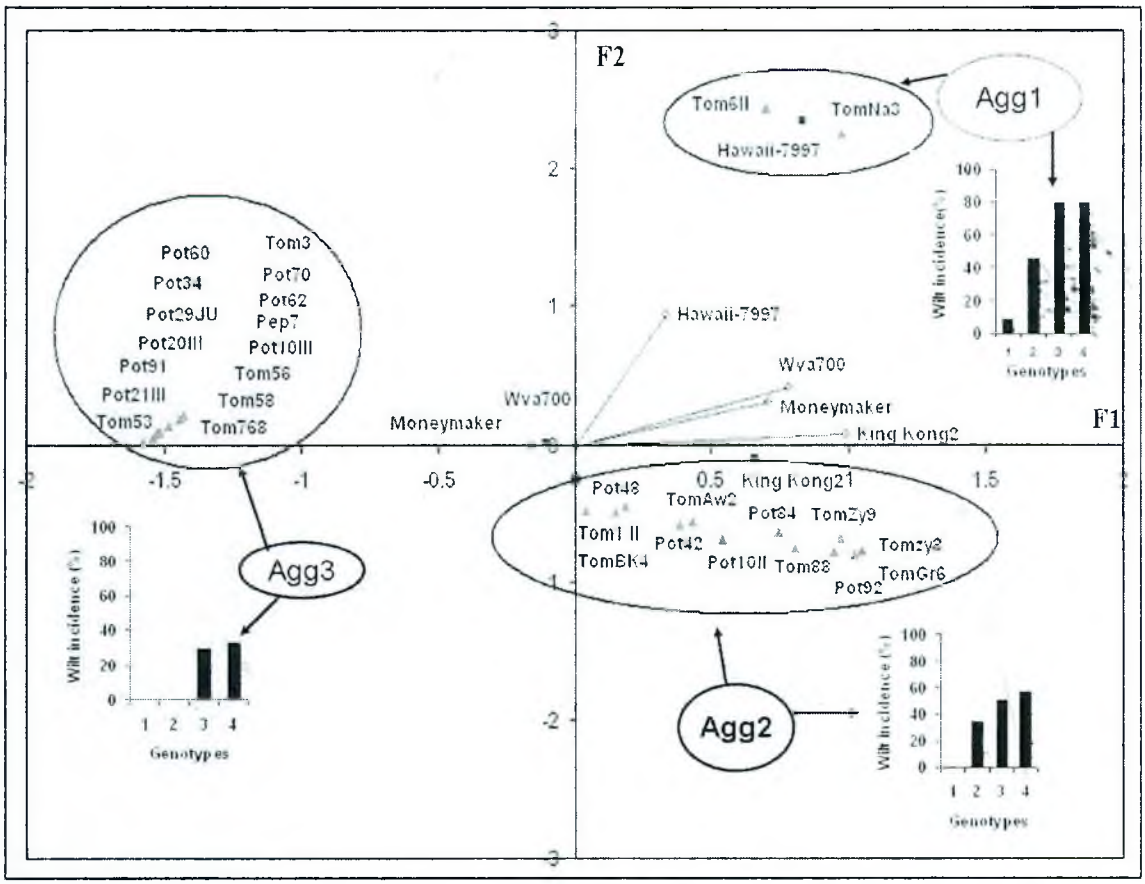

Figure 1. Aggressiveness groups of *R. solanacearum* strains from Ethiopia. Project of points corresponding to the different strains in the factorial plane defined with factors 1 and factors 2 (F1*F2). Each group of strains is characterized by histogram of mean wilt incidence (%) on the four tomato genotypes (1 = Hawaii-7997, 2 = King Kong 2, 3 = Wva700, 4 = Moneymaker)

Variation in host resistance level

Significant differences were observed in percent severity index, final wilt incidence and corresponding areas of under the disease progressive curves ($P \leq 0.001$) among the 33 tomato genotypes evaluated for their resistance against the strains *TomNa3* and *Toudk2* (Tables 2, 3). The final wilt incidence ranged from 0 to 92% wilt symptoms for *TomNa3* strain and from 0 to 100% for *Toudk2*. Similarly, percent severity index

at 28 dpi ranged from 0 to 82.8 % and 0 to 100% for strain of *TomNa3* and *Toudk2* respectively. On the basis of final wilt incidence tomato genotypes Hawaii-7996, Hawaii-7997, CL-1131-0-0-43-10-1, CRA84-263 (BL-333), CLN-2418A and CLN-1621L were grouped as resistant to strain *TomNa3* with final wilt incidence ranging from 0 to 19.5%. On the basis of percent severity index at 28 dpi also same genotypes also fell into resistant category with percent severity index ranging from 0 to 18.9%. Tomato genotypes Royallball, CLN-59-206-D4-2-2-0, CLN-2037H, R-3034-3-10-NUG (BL-1004), CLN-2366A, BL-439, CLN-2037A, H-2543, CLN-2037F, BF-OKISTU 101, and H-1350 were grouped in the moderately resistant category on the basis of wilt incidence ranging from 22 to 39%. On the basis of percent severity index the same tomato genotypes were also classified in the moderately resistant category with additional tomato genotypes such as King Kong 2 and CLN-2037Z. However, on the basis of wilt incidence King Kong2, CLN-2037Z and CLN-2037D categorized to the moderately susceptible groups with wilt incidences ranging from 44.4 to 49.9%. On the basis of percent severity index, genotype CLN-2037C was classified in the moderately susceptible category. The remaining thirteen tomato genotypes which include all tomato cultivars released in Ethiopia for production and the reference susceptible checks were observed to fall in the susceptible classification with final wilt incidence ranging from 61-92%.

Comparing the reaction of genotypes against the two test strains with category of resistant to *TomNa3* only Hawaii-7996 and Hawaii-7997 were identified as resistant to the strain of *Toudk2*. The other tomato genotypes such as CRA-84-263 (BL-333) observed as resistant to the strain of *TomNa3* fell into the moderately resistant group in reaction to *Toudk2* and 8 tomato genotypes showed moderately susceptible reaction to *Toudk2*. The remaining 22 genotypes were classified in the susceptible category against *Toudk2* (Table 2).

The use of wilt incidence and percent severity index at 28 dpi in delineating the resistance levels of 33 tomato genotypes tested against the two strains gave relatively similar proportions for the same strain. For the strain *TomNa3*, using percent severity index value enabled to identify 7, 13, 1 and 12 genotypes as resistant, moderately resistant, moderately susceptible and susceptible, respectively. On the basis of final wilt incidence also 6, 11, 3, and 13 tomato genotypes were also identified in the category of resistant, moderately resistant, moderately susceptible and susceptible respectively. For the strain *Toudk2*, 3, 2, 6 and 22 tomato genotypes in the category of resistant, moderately resistant, moderately susceptible, and susceptible respectively were identified on the basis of percent severity index. On the basis of final wilt incidence, 2, 1, 8 and 22 tomato genotypes were classified in the category of resistant, moderately resistant, moderately susceptible and susceptible, respectively.

Comparison of the analysis of mean grouping and separation of the average wilt incidence, percent severity index and corresponding areas under disease progress curves also indicated almost similar levels of mean groupings for the 33 tomato genotypes evaluated against strain *TomNa3* but slightly different figure for *Toudk2*. For the strain of *TomNa3*, there were 19 and 18 significant difference levels on the basis of percent severity index and area under percent severity index progress curve, respectively. On the basis of final wilt incidence and area under disease incidence progress curve there were also 19 significant levels for both parameters. However, for the strain of *Toudk2* there were 28 and 12 significance difference based on percent severity index and area under percent severity index progress curve. Furthermore, there was also 14 and 24 level of significance differences observed for average wilt incidence and area under disease incidence progress curve (Tables 3).

Table 2. Average wilt incidence (Awi) at 28 dpi percent severity index (PSi) of tomato genotypes inoculated with the virulent strain from Ethiopia and Thailand

No	Genotype	*TomNa3 (Ethiopia)*			*Toudk2 (Thailand)*		
		AWi	PSi	R^{E*}	AWi	PSi	R^{T*}
1	Marglobe[a]	86.1ba	76. 8*ba*	S	94.5*ba*	91.1*bdc*	S
2	Moneymaker[a]	77.8dc	73.3*bc*	S	80.5*de*	79.5*ef*	S
3	Melkasalsa[a]	75.0de	68.3*dc*	S	100.0*a*	100.0*a*	S
4	L-390[a]	88.9*a*	82.8*a*	S	100.0*a*	93.9*bac*	S
5	RomaVF[a]	89.9*a*	82.8*a*	S	100.0*a*	98.3*ba*	S
6	Melkashola[a]	91.6*a*	82.2*a*	S	100.0*a*	100.0*a*	S
7	L-3708[b]	80.6*bc*	76.7*ba*	S	88.9*bc*	76.7*ba*	S
8	WVa700	77.8*dc*	68.9*dc*	S	77.8*e*	76.7*h*	S
9	UC-204-A [b]	72.2*de*	68.9*dc*	S	94.5*ba*	85.6*ed*	S
10	CLN-2037B	72.2*de*	66.7*d*	S	72.2*de*	66.7*d*	S
11	Floradade[a]	69.5*fe*	66.7*d*	S	77.8*e*	72.2*gh*	S
12	CLN-51915--553-D4-3-0	61.3*g*	63.3*de*	S	80.6*de*	77.8*gf*	S
13	CLN-2037C [b]	63.9*gf*	57.2*e*	S	83.3*de*	78.9*ef*	S
14	CLN-2037Z [b]	47.2*h*	40.0*f*	MS/MR*	50.0*h*	40.0*f*	MS/MR
15	CLN-2037D [b]	44.5*ih*	38.3*gf*	MS/MR	80.6*de*	76.1*h*	S
16	H-2543(BL-439) [b]	36.1*j*	32.8*gh*	MR	88.9*bc*	84.9*ef*	S
17	CLN-2037F [b]	36.1*j*	32.8*gh*	MR	47.2*h*	49.4*lk*	MS
18	King Kong2[c]	49.9h	31.7*ih*	MS/MR*	50.1*hg*	34.4*no*	MS/MR*
19	BF-Okistu 101 (BL-994) [b]	36.1*j*	31.1*ih*	MR	80.6*de*	65.0*i*	S
20	H-1350[a]	38.9*ij*	29.9*ihj*	MR	69.5*f*	61.7*ji*	S
21	CLN-2366A[b]	27.8*j*	29.5*ihj*	MR	50.0*h*	8.9*p*	MS/MR*
22	BL-439[b]	27.8*j*	27.2*ij*	MR	47.2*h*	41.1*nm*	MS
23	R-3034-3-10-N-UG (BL-1004) [b]	27.8*j*	27.2*ij*	MR	80.6*de*	74.4*h*	S
24	CLN-2037A[b]	27.8*j*	26.1*ij*	MR	88.9*bc*	87.8*dc*	S
25	CLN-5915-206-D4-2-2-0[b]	27.8*j*	24.4*kj*	MR	58.3*g*	55.6*jk*	MS
26	CLN-2037H[b]	27.8*j*	23.3*kl*	MR	66.7*f*	64.4*i*	S
27	Royal Ball[a]	22.2*lk*	18.9*l*	MR/R*	58.3*g*	56.1*jk*	MS
28	CLN-1621L[b]	19.5*lm*	18.9*l*	R	86.1*dc*	85.0*edf*	S
29	CLN-2418A[b]	13.9*nm*	10.6*m*	R	47.2*h*	46.1*m*	MS
30	CRA-84-263(BL-333) [b]	11.1*n*	9.4*m*	R	38.9*i*	31.7*o*	MR
31	CLN-1131-0-0-43-10-1[b]	11.1*n*	8.3*m*	R	69.5*f*	64.5*i*	S
32	Hawaii-7996[b]	0.0*o*	0.0*n*	R	11.1*j*	5.0*qp*	R
33	Hawaii-7997[b]	0.0*o*	0.0*n*	R	0.0*k*	0.0*q*	R
	Mean	46.6	42.3		70.9	65.7	
	Coefficient of variation	10.46	10.06		8.0	7.65	
	WD LSD[b]	6.14	6.14		8.24	7.3	

R^{E*}= Reaction against TomNa3 strain and *R^{T}= Reaction against Toudk2 strain R= Resistant (<20% plants wilted), MR= Moderately resistant (20-40% plants wilted), MS= moderately susceptible (41-60 %) plants wilted and S= Susceptible >60 % plants wilted, a= means from three replication, [b]LSD value at p=0.05 level based on Waller-Duncan K-ratio t- test. Means followed by the same letter(s) in same column are not significantly different from each other according to Waller-Duncan K-ratio t test ($p \leq 0.05$). *=variable reaction based on the value considered.

[a]=Melkassa Agricultural Research Centre, Ethiopia), [b]=Asian Vegetable Research and Development Centre, Taiwan, [c]=Known-You Seed Co., Ltd, Taiwan

Table 3. Area under disease severity index curve (AUDSIC) and area under disease incidence curve (AUDIC) for 33 tomato genotypes tested against the virulent strain from Ethiopia and Thailand

No	Genotype	*TomNa3* (Ethiopia)		*Toudk2* (Thailand)	
		AUDIC	AUDSIC	AUDIC	AUDSIC
1		1836.3*a*	1536.0a	2123.3*a*	2017.7*a*
2	Marglobe	1662.5*c*	1474.2bc	1882.4*cd*	1629.2*c*
3	L-390	1649.9*c*	1504.0bc	1897.2*cd*	1793.9*b*
4	RomaVF	1781.5*ba*	1436.7bc	2225.0*a*	3093.0*a*
5	L-3708	1745.8*bc*	1523.7ba	1940.6*cb*	1845.8*b*
6	Moneymaker	1680.5bc	1419.3c	1763.2*fe*	1629.7*c*
7	WVa700	1536.2*d*	1295.11d	1570.2*ih*	1383.5*e*
8	Melkasalsa	1523.9*d*	1311.8d	2198.6*a*	2061.0*a*
9	UC-204-A	1501.4*ed*	1469.2bc	3120.3*a*	2133.0*a*
10	CLN-2037B	1533.2*d*	1215.8d	1874.9*cd*	1540.4*dc*
11	Floradade	1409.8*e*	1232.1d	1699.7*fg*	1602.3*c*
12	CLN-51915–553-D4-3-0	1128.8*g*	1012.2e	1503.8*ij*	850.2*h*
13	CLN-2037C	1269.4*f*	1070.3e	1662.8*fg*	1443.9*de*
14	CLN-2037Z	859.4*i*	675.8gf	1026.6*nm*	943.6*h*
15	CLN-2037D	969.8*h*	734.2f	1786.8*ed*	1609.8*c*
16	H-2543(BL-439)	673.6*j*	567.5jh	2004.2*b*	1839.0*b*
17	CLN-2037F	802.3i	665.3gfh	921.4*no*	873.6*h*
18	King Kong2	804.1i	499.8jk	851.1*pq*	638.6*i*
19	BF-Okistu 101 (BL-994)	629.2kj	493.6jk	1670.8*fg*	1173.1*g*
20	H-1350	779.1*i*	601.4gh	1364.05*kl*	1147.8*g*
21	CLN-2366A	580.6*kj*	530.6ji	804.4*pq*	93.3*j*
22	BL-439	447.2*l*	381.1l	904.1*po*	671.9*i*
23	R-3034-3-10-N-UG (BL-1004)	554.2*k*	485.0jk	1608.9*lg*	1378.8*fe*
24	CLN-2037A	625.7*kj*	524.7ji	1738.5*fe*	1574.2*dc*
25	CLN-5915-206-D4-2-2-0	423.6*l*	346.1l	1089.3*m*	967.9*h*
26	CLN-2037H	444.4*l*	404.4lk	1297.1*l*	1159.4*g*
27	Royal Ball	398.6*ml*	313.3*l*	1116.8m	971.9*h*
28	CLN-1621L	422.9*l*	376.4l	1762.3*fe*	1558.6*dc*
29	CLN-2418A	299.8*mn*	133.54m	803.2*pq*	692.3*i*
30	CRA-84-263(BL-333)	241.6*n*	147.29m	791.7*g*	640.7*i*
31	CLN-1131-0-0-43-10-1	208.4*n*	134.74m	1434.8*kj*	1232.0*fg*
32	Hawaii-7996	0.0*o*	0.0*o*	116.6r	17.5*j*
33	Hawaii-7997	0.0*o*	0.0*o*	0.0s	0.0*j*
	Mean	921.94	773.21	1441.05	1248.7
	Coefficient of variation	7.75	8.89	5.36	8.34
	WD LSD[z]	102.96	99.2	111.6	150.7

[z] least significant difference value at ($p < 0.05$) level based on Waller-Duncan K-ratio t- test. Means followed by the same letter(s) in the same column are not significantly different from each other according to Waller-Duncan K-ratio t- test ($p \leq 0.05$).

Discussion

Inoculation of four tomato cultivars with 29 *R. solanacearum* strains originated from Ethiopia revealed three aggressiveness groups. The strains in the aggressiveness group Agg1 belong to biovar 1. Majority of strains were clustered in to the two aggressiveness groups Agg2 and Agg3, which contained 41% and 52%, respectively of the total

strains tested, respectively. Agg1 represents the most aggressive group which caused wilting on all genotypes while Agg2 and Agg3 contained the moderately aggressive and less aggressive strains. The grouping pattern in Agg2 and Agg3 was not related to biovar or host origin. Studies by Darrasse *et al.* (1998) and Jaunet, and Wang (1999) also revealed lack of any relation among the studied strains in a similar aggressiveness group. However, different aggressiveness groups have been reported for the *R. solanacearum* strains originated from different locations (Darrasse *et al.,* (1998; Jaunet, and Wang 1999) and this observation has been hypothesized to be related to location specificity of resistance in tomato genotypes. The need to study the genetic basis for variation in the aggressiveness has been recommended. Similarly, the existence of different aggressiveness groups in *R. solancearum* strains from Ethiopia signifies the need to consider the different groups in planning for disease management options and evaluation resistance sources to bacterial wilt pathogen.

Tomato genotypes found resistant to strain from Ethiopian have been also reported as resistant to *R. solanacaerum* strains in other resistance evaluation trials (Wang *et al.*, 1998). Furthermore, Hanson *et al* (1998) also reported that genotype Hawaii 7997, CRA84, and L285 were among the best resistance sources. Tomato genotypes differed in their resistance to the strains while the strains differed in their aggressiveness. This study also has demonstrated that level of resistance depends on the strain type and similar case was reported by Wang et al. (1998) and Carmeille et al. (2006).

Since tomato breeding mainly focuses on the development of varieties resistance to major diseases and have desirable agronomic features, the resistant and moderately resistant groups identified in this study could be used as sources for resistance in incorporating into cultivars of Ethiopia or directly used to evaluate for desirable agronomic traits. Because of the high genotype × environment interaction for bacterial wilt resistance, a multi-location testing of genotypes and inoculation with the different aggressive strains is essential for the identification of stable resistant lines. All commonly grown tomato cultivars in Ethiopia lack resistance against the bacterial wilt strains tested. Naser *et al* (2007 reported similar result, therefore, development of bacterial wilt resistant varieties are highly required under Ethiopian condition. Furthermore, future release of varieties with desirable agronomic traits, testing for their reaction to bacterial wilt need to be carried to confirm resistance. The study recommends a breeding program incorporating resistant sources into existing tomato cultivars or evaluating the identified resistant sources for desirable agronomic traits. In this study, only a limited number of *R. solanacearum* strains were used for the aggressiveness test and a limited number of tomato genotypes were evaluated for resistance. In studies carried out elsewhere sources of resistance and resistance genes or quantitative trait loci to numerous pathogens were identified from wild *Lycopersicon* (now *Solanum)* spp. (Grube et al. 2000; Carmeille et al. 2006; Hong Hai et al. 2008). These could be useful for resistance breeding in tomato in Ethiopia.

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