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Assessment of resistance status of some tomato genotypes to bacterial wilt disease and evaluation of SNP marker (LEOH19) for selection of *BW* resistant gene

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Abstract

Marker assisted selection (MAS) has become very important and useful in selection of disease resistance genes in crop plants. Tomato, *Solanum lycopersicum*, is one of the most important vegetables worldwide but its production is being affected by pests and diseases, one of which is bacterial wilt caused by *Ralstonia solanacearum*. In endemic areas, the disease constitutes up to 100% yield loss. A-Two-season completely randomized design (CRD) experimental trial was conducted to assess the resistance status of forty (40) tomato genotypes. The results showed variations in resistance status, from highly resistance to highly susceptible. DNA concentrations from the assessed genotypes ranged between 14.46 and1430.52 ng/ul. Single Nucleotide Polymorphism (SNP) marker, LEOH19, was evaluated on the forty tomato genotypes for the identification of *bw*-gene that confers resistance to *R. solanacearum*. Genomic DNA was amplified using the primer sequence; (forward primer; 5´-AAGGCTCAGAAAGGGTCCAT-3´, reverse primer; 5´- GAGTTCATCAACACATCACACA-3´). The primer pairs produced amplification at 300 bp in 35 genotypes. After digestion, the product produced 300 bp in 33 genotypes. In screenhouse study, AVTO9803, AVTO0201, Tomachiva and Eyetom were found highly resistant and could be considered good materials in grafting and breeding programme for bacterial wilt resistance development. The results indicated that the primer amplified the specific sequence of the *bw*-gene locus in both the resistant and susceptible alleles. LEOH19 was found to be monomorphic and produced DNA fragments in both resistant and susceptible tomato genotypes and could, therefore, not be used as a molecular marker for marker assisted selection in tomato breeding programme.

Key words: Bacterial wilt, *Ralstonia solanacearum*, resistance, SNP, Tomato.

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Introduction

Tomato, *Solanum lycopersicum* L., is a crop of high economic importance in many countries. It is not only one of the world's most important vegetables, but also the most widely used vegetable crop for different purposes (Fernández-Marcos et al., 2011). It is an important source of vitamins A and C (Hanson et al., 2016). It can be eaten raw, cooked or processed to paste and canned before it is used. However, its production is being affected by many production constraints and one of which is bacterial wilt caused by *Ralstonia solanacearum*.

The pathogen causes diseases in tissues, vessels or ducts of plant systems (Bradbury, 1986). Aslam et al. (2017a) commiserated that the disease could cause serious yield loss. The disease can constitute up to 100% yield loss in endemic areas (Popoola et al., 2015) and control of diseases typically relies on frequent application of chemicals, which is becoming increasingly prohibitive due to their cost and adverse effects on human health and the environment (Teh et al., 2017). In Nigeria, a number of tomato plants tend to be diseased before flowering, leading to decrease in available fruits at harvest. This situation discourages the wide cultivation of tomato due to economic loss. Breeding for resistance against this destructive pathogen is very challenging.

The application of molecular markers helps in combining resistant genes to crop cultivars and lessens the time and cost constraints as well as inconvenience attached to conventional breeding (Zang et al., 2002). Molecular markers have gained favour in plant breeding as a powerful approach permitting construction of high density genetic maps, making it possible to locate genes more precisely (Stuber, 1992). Single nucleotide variations in genome sequence of individuals of a population are known as Single Nucleotide Polymorphisms (SNPs). They constitute the most abundant molecular markers in the genome and are widely distributed throughout genomes although their occurrence and distribution varies among species.

Maize has one SNP per 60120 bp (Ching et al., 2002). Teh et al. (2017) affirmed that the availability of high-quality SNP markers allowed development of haplotypes associated with the QTLs to identify grandparental origin of powdery mildew resistance. Majority of SNP genotyping assays are based on one or two of the following molecular mechanisms: allele specific hybridization, primer extension, oligonucleotide ligation and invasive cleavage (Sobrino et al., 2005). They are suitable for automation and are used for a range of purposes, including rapid identification of crop cultivars and construction of ultra high-density genetic maps. Single nucleotide polymorphism (SNP) is now a common molecular marker due to their large quantity and accessibility (Rafalski, 2002; Giancola et al., 2006; Caicedo et al., 2007; Choi et al., 2007; Jones et al., 2009; Valliyodan et al., 2016) and also demonstrated its good use in selection for disease resistance in tomato plant (Labate and Baldo, 2005; Yang et al., 2005). Kassa et al. (2017) developed and mapped forty-three SNP markers on chromosome 2BS tightly linked with the seedling leaf rust resistance gene Lr16 in wheat.

The prevalence of tomato bacterial wilt disease in Nigeria is so high and the identification of resistant genotype(s) will be of great benefit to tomato growers. This study was out to screen forty tomato genotypes for resistance to tomato bacterial wilt disease and to evaluate LEOH19 primer in identifying tomato genotypes with bw-gene which confers resistance to bacterial wilt disease.

Materials and Methods

Experimental Location and Source of Plant materials:

The experiment was carried out at Federal University of Agriculture, Abeokuta and Bioscience Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan. Nigeria. Forty tomato genotypes (local and exotic) were used. Some were sourced from Uganda, Italy, Kenya, AVRDC and Tanzania while some were from Nigeria: Institute of Agricultural Research and Training (IAR&T), Ibadan; Institute of Agricultural Research (IAR), Zaria; Ajara farm settlement, Lagos; Ile-ona, Ekiti State; Ilaro and Imeko, Ogun State; Ife-Odan, Osun State; Kano; Imo State; NACGRAB, Ibadan; Jos; Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State; and NIHORT, Ibadan.

Bacterial isolation and inoculum preparation:

One gram of soil was weighed into test tube containing 10 ml sterile distilled water. Agitated for about 30-60 sec. Bacterial suspension was streaked on Kelman's tetrazolium chloride (TZC: Casamino acid, Peptone, Glucose, Agar and 1% solution of 2, 3, 5-triphenyl tetrazolium chloride) agar medium, incubated at room temperature for 24-48 hr and purified by subculturing. Bacterial mass was suspended in sterilized distilled water and concentration was adjusted to 10^8 cfu/ml.

Pathogenicity test of Ralstonia solanacearum on tomato:

Beske (susceptible) tomato was raised on sterilized soil. Tomato seedlings (a month-old) were inoculated (soil drenching) in screenhouse two weeks after transplanting with bacterial suspension (20 ml of 10^8 cfu/ml) until the development of wilt symptom. Sterile distilled water served as a negative control. Before inoculation, tomato seedlings were not watered for 24 hrs. Watering resumed after inoculation every other day according to the technique of Popoola et al. (2015). Incubation was done for two weeks at 25 - 28°C with relative humidity between 85 and 90%.

(a) *Screenhouse resistance evaluation*

Treatment and Experimental Design:

Seedlings of 40 tomato genotypes (Table 1) were raised in nursery trays containing steam-sterilized sandy-loam topsoil (30 min at 120° C). Seedlings were transplanted after a month into pots containing sterilized soil. The design was a completely randomized design (CRD) with three replications. The first trial was conducted between March and June while the second trial was carried out between August and November of year 2012.

Inoculation of tomato and evaluation of resistance status:

Five millilitre (5 ml) of the suspension of the pathogen, *Ralstonia solanacearum*, was inoculated into each plastic pot two weeks after tomato transplanting by soil drenching. Evaluation of resistance status of tomato accessions was determined following 0-5 scale according to Zakir et al. (2005) with some modifications where 0= 0% plant wilted (Highly Resistant); 1=1-10% plant wilted (Resistant); 2=10.01-25% plant wilted (Moderately Resistant); 3=25.01-50% plant wilted (Moderately Susceptible); 4=50.01-75% plant wilted (Susceptible); 5=75.01-100% plant wilted (Highly susceptible).

Data collection and analysis:

Data on incidence and severity were collected. Data were subjected to ANOVA and means separated by Duncan's Multiple Range Test (DMRT) using SAS 9.1 for windows.

(b) *SNP marker analysis*

DNA Extraction, PCR and restriction enzyme digestion:

Genomic DNA was extracted from a two-week old fresh leaves of screenhouse-grown plants using protocol according to Zymo Research Plant/Seed DNA MiniPrep Kit (commercial kit) and the Dellaporta et al. (1983) method with little modification made on the amount of reagents used. For commercial kit, fresh tomato leaf sample of 150 mg was weighed into a ZR BashingBead™ Lysis Tube and 750 µl Lysis Solution was added. Disruption of the cells was done at high speed for 10 min. The Lysis Tube was centrifuged in a microcentrifuge at 10,000 x g for 1 min. Supernatant (400 µl) was transferred to Zymo-Spin™ IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000 rpm (≏ 7,000 x g) for 1 min. Thereafter, 1,200 µl of Plant/Seed DNA Binding Buffer (diluted with beta-mercaptoethanol to a final solution of 0.5% v/v) was added to the filtrate in the collection tube and mixed with micropipette. From the collection tube, 800 µl of the mixture was transferred to Zymo-Spin™ IIC Column in a collection tube and centrifuged at 10,000 x g for 1 min. The flow through was discarded and 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection tube and centrifuged at 10,000 x g for 1 min. Plant/Seed DNA Wash Buffer, 500 µl, was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 min. Zymo-Spin™ IIC Column was transferred to clean 1.5 ml microcentrfuge tube and 100 µl DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 x g for 30 sec to elute the DNA. The eluted DNA from was transferred to a centrifuged Zymo-spin™ IV-HRC Spin Filter (green top) in a

clean 1.5 ml microcentrifuge tube and centrifuged at exactly 8,000 x g for 1 min.

Tomato leaf sample of 150 mg was also used for DNA extraction in Dellaporta et al. protocol. The PCR-based DNA marker used was LEOH19 (forward primer; 5´-AAGGCTCAGAAAGGGTCCAT-3´, reverse primer; 5´- TGAGTTCATCAACACATCACACA-3´), custom synthesized by Inqaba Biotec West Africa Limited, which amplifies at 300 bp. Twenty-five microlitre (25 ul) each PCR reactions mixture consisted of 9.5 µl sterilized ddH_2O, 1 ul Dimethyl sulphure oxide, 5 ul 10xPCR buffer (100mM Tris-HCl, pH 9.0; 500 mM KCl), 1.2 ul $MgCl_2$ (15 mM), 1 ul dNTPs (20 mM each), 1 ul each primer (20 uM), 0.3 ul Taq DNA polymerase (2 U/ul) (Violet, Taiwan), and 5 ul template DNA (15 ng/ul). The amplification procedures consisted of an initial Denaturation step for 5 minutes at 94°C and 32 cycles of 30 seconds at 94°C, 30 seconds primer annealing at 60°C, 45 seconds extension step at 72°C, followed by a final extension at 72°C for 5 minutes. The PCR fragments were separated by gel electrophoresis with 2% agarose gel in 0.5 x

TBE buffer, stained with ethidium bromide and visualized with UV light. Thereafter, 5 ul of PCR product was digested with BsaB I restriction enzyme in a 10 ul cocktail (8 ul sterile ddH_20, 1 ul 10x buffer, 1 ul restriction enzyme) by using GeneAmp$^{(R)}$PCR system 9700 thermocycler; version 3.12. The digested product (15 ul) was separated in 1.5% agarose gels stained with ethidium bromide and 0.5 x TBE buffer for 2 hours at 96-100V. A 100 bp ladder was used as molecular weight marker. It was photographed under UV light after electrophoresis.

Results

Bacterial isolation and Pathogenicity test:

After isolation as described above, the inoculum was streaked on Kelman's tetrazolium chloride medium, incubated for 24-48 hrs at room temperature; typical fluid white colonies with a pink center were observed. Typical bacterial wilt symptom was observed on tomato plants inoculated with *R. solanacearum* suspension. The bacterium was re-isolated from infected plants by streaming test technique (Popoola et al., 2015). The ooze was streaked on tetrazolium chloride agar and typical colonies of *Ralstonia solanacearum* were observed.

Screenhouse resistance evaluation:

A total of 40 genotypes of tomato were screened for resistance to bacterial wilt (Table 2). In the first trial, AVTO9803, AVTO0201, Tomachiva and Eyetom were classified as highly resistant (HR). Delila, Gempride and Derica had incidence of 13.90%, 12.10% and 27.80% respectively and each with severity score of 1.00 while NG/MR/MAY/09/006 had incidence of 40.57% with the severity score of 1.33. They were characterized as resistant (R). However, Roman round and AVTO0102 had 100% disease incidence each while Beske and Pure-water had 70.80% and 85.60% incidence respectively, with severity score of 5 and were therefore categorised as highly susceptible (HS) genotypes. Reactions were consistent during second trial except in Santana, Tyre-type, UC82-B, AVTO1016 and NG/OE/MAY/09/019 with a breakdown of resistance. The disease incidence ranged from 0-100% and severity score from 0-5.

Molecular marker assisted selection of tomato bacterial wilt resistance:

Table 1 showed the DNA concentrations of 40 tomato genotypes sourced from different locations. Concentrations ranged from 14.46-1430.52 ng/ul. Kerewa yielded the highest concentration (1430.52 ng/ul) while Omomola produced the least concentration (14.46 ng/ul). It was observed that F1 Mongal, Pure-water, Santana, Kerewa and Tyre-type produced outstanding nanodrop yields in the following order (871.50, 687.51, 798.18, 1430.52 and 924.65 ng/ul). Forty (40) tomato

plants were subjected to SNP marker, LEOH19, analysis. Target DNA fragment at 300-base pair (bp) linked to *bw*-gene from tomato plants were amplified 35 (87.50%) of the total number of tomato genotypes with the primer (Plate 1a and b). After digestion with BsaB 1, the digested product produced 300 bp in 33 (82.50%) genotypes with 4 polymorphic bands of unknown resistant status (Plate 2). The result indicated that the primer amplified the specific sequence of the gene *bw* locus and both the resistant and susceptible alleles at the *bw* locus were amplified by the primer pair.

Table 1: Concentrations of genomic DNA in tomato leaves using Nanodrop spectrophotometry.

S/N	Tomato Genotype	Source	Concentration (ng/ul)
1	Roma round	IARcT, Ibada Nigeria.	23.40
2	Beske	IARcT, Ibada Nigeria.	48.11
3	Delila	IAR, Zaria, Nigeria.	27.33
4	Gem pride	,,	26.25
5	Perfect pee	,,	35.08
6	F1-Mongal	Ajara farm settlement, Lagos, Nigeri	871.50
7	Purewater	Ileona, Ekiti State, Nigeria	687.51
8	Santana	Ilaro, Imeko, Ogun State, Nigeria.	798.18
9	Kerewa	IfeOdan, Osu State, Nigeria	1430.52
10	Tyretype	IfeOdan, Osu State, Nigeria	924.65
11	UC82B	Kano	15.54
12	Dankukumi	Kano	21.61
13	Derica	ImoState	15.17
14	Roma VF	Imo State an FUNAAB.	17.47
15	Gboko	Imo State	15.30
16	Omo mola	Iwoye, Ogun State, Nigeria	14.46
17	AVTO1016(CLN2514A)	AVRDC, Tanzania	18.36
18	AVTO9803(CLN1621F)	,,	16.68
19	AVTO0102(CLN2366B)	,,	46.94
20	AVTO0201(CLN1466EA	,,	20.50
21	NG/MR/MAY/09/005	NACGRAB, Ibadan, Niger	14.66
22	NG/MR/MAY/09/006	,,	21.30
23	NG/OE/MAY/09/019	,,	20.58
24	NG/AO/MAY/09/011	,,	33.86
25	NG/AA/SEP/09/037	,,	20.17
26	Hausa type	IfeOdan, Osu State, Nigeria	15.98
27	UTC18	Kano	18.00
28	Uganda round	Uganda	21.22
29	Uganda serated	Uganda	23.19
30	Oval	Nairobi, Keny	25.95
31	Kibirigwi	,,	17.74
32	Cholenaria	,,	18.49
33	Small oblong	,,	17.78
34	Okitsu	Italy	16.44
35	Ontario	,,	16.27
36	Momor	,,	24.61
37	Danjos	Jos	19.03
38	Ibadan local	FUNAAB	20.38

39	Tomachiva	NIHORT, Ibadan, Nigeria.	17.76
40	Eyetom	Oyo, Nigeria.	16.93

Table 2: Incidence and severity of forty tomato genotypes and their resistance status to bacterial wilt.

		Trial 1†			Trial 2†		
S/N	Tomato genotype	Incidence (%)	Severity	Resistance status	Incidence (%)	Severity	Resistance status
1	Roma round	100.00 ^{a}	5.00 ^{a}	HS	99.55 ^{a}	4.67 ^{ab}	HS
2	Beske	70.76 ^{abc}	4.67 ^{a}	HS	99.00 ^{ab}	4.67 ^{ab}	HS
3	Delila	13.89 ^{cd}	1.00 ^{cde}	R	17.07 ^{de}	1.33 ^{cde}	R
4	Gem pride	12.08 ^{cd}	1.00 ^{cde}	R	22.08 ^{cde}	1.33 ^{cde}	R
5	Perfect pee	72.22 ^{abc}	3.67 ^{abcd}	S	72.22 ^{abcd}	4.00 ^{abc}	S
6	F1-Mongal	66.67 ^{abc}	3.33 ^{abcd}	MS	66.67 ^{abcd}	3.33 ^{abcd}	MS
7	Pure-water	85.63 ^{ab}	4.67 ^{a}	HS	98.41 ^{ab}	4.67 ^{ab}	HS
8	Santana	44.45 ^{abcd}	2.33 ^{abcde}	MR	44.45 ^{abcde}	2.67 ^{abcde}	MS
9	Kerewa	33.50 ^{bcd}	1.67 ^{bcde}	MR	33.50 ^{cde}	1.67 ^{bcde}	MR
10	Tyre-type	72.22 ^{abc}	3.67 ^{abcd}	S	72.22 ^{abcd}	1.67 ^{bcde}	MR
11	UC82-B	66.67 ^{abc}	3.33 ^{abcd}	MS	72.17 ^{abcd}	3.67 ^{abcd}	S
12	Dankukumi	50.00 ^{abcd}	2.00 ^{abcde}	MR	50.00 ^{abcde}	2.00 ^{abcde}	MR
13	Derica	27.78 ^{bcd}	0.67 ^{de}	R	26.83 ^{cde}	0.67 ^{de}	R
14	Roma VF	38.89 ^{abcd}	2.00 ^{abcde}	MR	39.37 ^{abcde}	2.00 ^{abcde}	MR
15	Gboko	38.89 ^{abcd}	2.00 ^{abcde}	MR	35.50 ^{cde}	2.00 ^{abcde}	MR
16	Omo mola	72.22 ^{abc}	3.67 ^{abcd}	S	68.97 ^{abcd}	3.67 ^{abcd}	S
17	AVTO1016	33.33 ^{bcd}	1.67 ^{bcde}	MR	62.00 ^{abcd}	3.00 ^{abcde}	MS
18	AVTO9803	0.00 ^{d}	0.00 ^{e}	HR	0.00 ^{e}	0.00 ^{e}	HR
19	AVTO0102	100.00 ^{a}	5.00 ^{a}	HS	100.00 ^{a}	5.00 ^{a}	HS
20	AVTO0201	0.00 ^{d}	0.00 ^{e}	HR	0.00 ^{e}	0.00 ^{e}	HR
21	NG/MR/MAY/09/005	45.00 ^{abcd}	2.67 ^{abcde}	MS	57.38 ^{abcde}	3.00 ^{abcde}	MS
22	NG/MR/MAY/09/006	40.57 ^{abcd}	1.33 ^{cde}	R	20.57 ^{cde}	1.33 ^{cde}	R
23	NG/OE/MAY/09/019	51.27 ^{abcd}	3.33 ^{abcd}	MS	71.27 ^{abcd}	3.67 ^{abcd}	S
24	NG/AO/MAY/09/011	56.67 ^{abcd}	2.67 ^{abcde}	MS	50.00 ^{abcde}	2.67 ^{abcde}	MS
25	NG/AA/SEP/09/037	44.83 ^{abcd}	3.00 ^{abcde}	MS	47.23 ^{abcde}	2.67 ^{abcde}	MS
26	Hausa type	52.13 ^{abcd}	3.33 ^{abcd}	MS	52.13 ^{abcde}	3.33 ^{abcd}	MS
27	UTC18	38.07 ^{abcd}	2.33 ^{abcde}	MR	38.07 ^{bcde}	2.33 ^{abcde}	MR
28	Uganda round	46.00 ^{abcd}	3.00 ^{abcde}	MS	46.00 ^{abcde}	3.00 ^{abcde}	MS
29	Uganda serated	44.10 ^{abcd}	2.67 ^{abcde}	MS	50.00 ^{abcde}	2.67 ^{abcde}	MS
30	Oval	36.77 ^{bcd}	2.33 ^{abcde}	MR	59.43 ^{abcde}	2.33 ^{abcde}	MR
31	Kibirigwi	38.00 ^{abcd}	1.67 ^{bcde}	MR	41.33 ^{abcde}	1.67 ^{bcde}	MR
32	Cholenaria	63.03 ^{abc}	4.00 ^{abc}	S	80.33 ^{abc}	4.00 ^{abc}	S
33	Small oblong	41.10 ^{abcd}	2.67 ^{abcde}	MS	41.66 ^{abcde}	2.67 ^{abcde}	MS
34	Okitsu	46.80 ^{abcd}	2.33 ^{abcde}	MR	52.48 ^{abcde}	2.33 ^{abcde}	MR

35	Ontario	42.87^{abcd}	2.33^{abcde}	MR	44.67^{abcde}	2.33^{abcde}	MR
36	Momor	35.77^{bcd}	2.00^{abcde}	MR	44.43^{abcde}	2.00^{abcde}	MR
37	Danjos	35.83^{bcd}	2.33^{abcde}	MR	35.83^{cde}	2.33^{abcde}	MR
38	Ibadan local	64.07^{abc}	4.00^{abc}	S	80.57^{abc}	4.00^{abc}	S
39	Tomachiva	0.00^{d}	0.00^{e}	HR	0.00^{e}	0.00^{e}	HR
40	Eyetom	0.00^{d}	0.00^{e}	HR	0.00^{e}	0.00^{e}	HR
	Control	0.00^{d}	0.00^{e}		0.00^{e}	0.00^{e}	
	SEM	45.55	2.48		49.84	2.55	

†Means followed by a common letter within a column are not significantly different (P = 0.05) according to DMRT. **HR:** Highly Resistance; **R:** Resistant; **MR:** Moderately Resistant; **MS:** Moderately Susceptible**; S:** Susceptible; **HS:** Highly Susceptible.

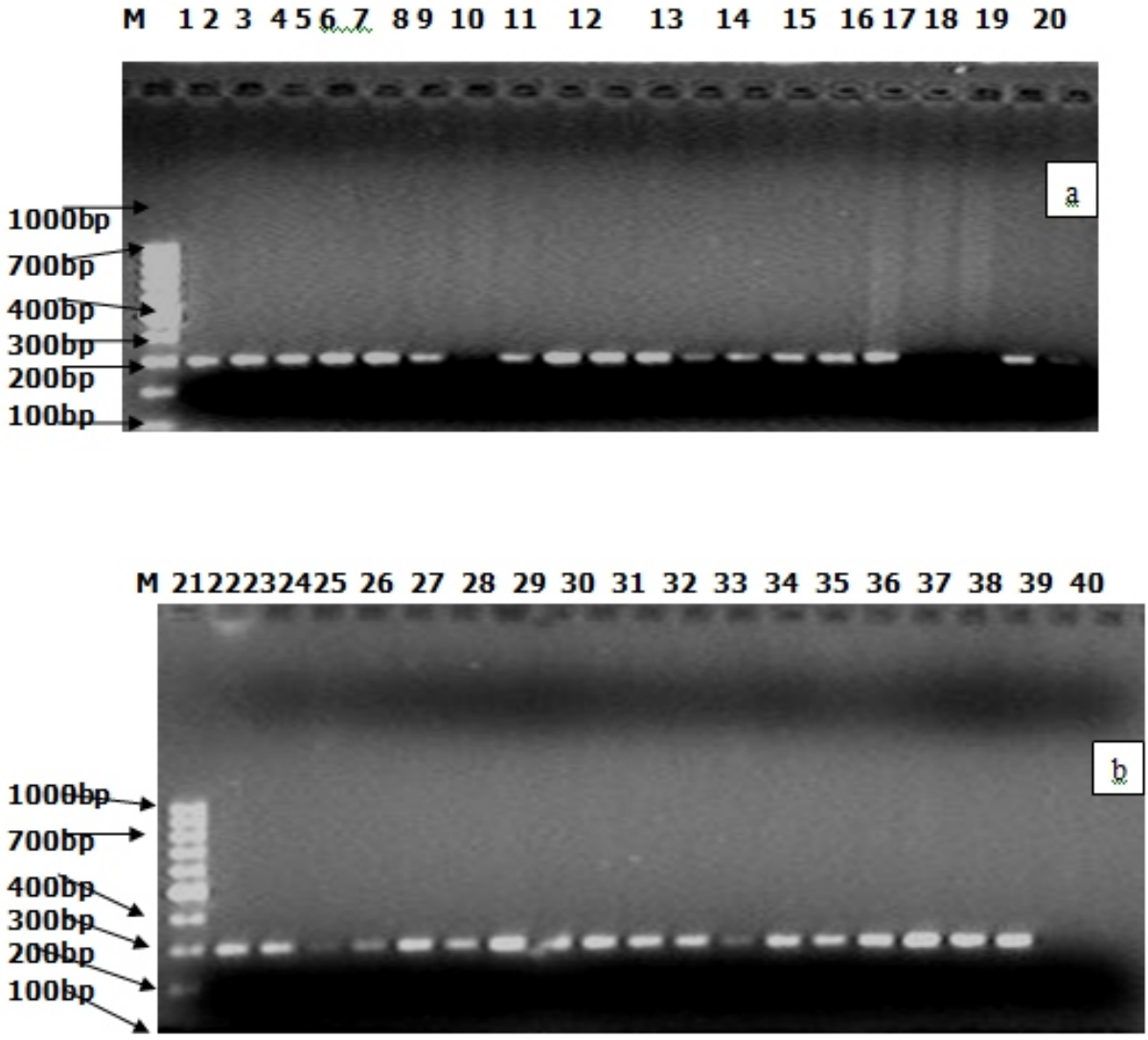

Plate 1: Gel electrophoresis of Polymerase Chain Reaction (PCR)-amplified products in 2% agarose (a and b) with LEOH 19 primer (300 bp). **M;** 100 bp ladder, Lane 1-40;**1**:Roma round, **2:**Beske,**3**:Delila, **4**:Gem pride, **5:**Perfect pee, **6**:F1-Mongal, **7**:Pure-water, **8**:Santana, **9:**Kerewa, **10:**Tyre-type, **11:**UC82-B, **12:**Dankukumi, **13:**Derica, 14:Roma VF, **15**:Gboko, **16**:Omo mola, **17:**AVTO1016, **18**:AVTO9803, **19**:AVTO0102, **20**:AVTO0201, **21**:NG/MR/MAY/09/005, **22**:NG/MR/MAY/09/006, **23:**NG/OE/MAY/09/019, **24:**NG/AO/MAY/09/011, **25**:NG/AA/SEP/09/037, **26**:Hausa type**, 27:**UTC18, **28**:Uganda round, **29**:Uganda serated, **30**:Oval, **31**:Kibirigwi, **32**:Cholenaria, **33:**Small oblong, **34**:Okitsu, **35**:Ontario, **36**:Momor, **37**:Danjos, **38**:Ibadan local, **39**:Tomachiva and **40**:Eyetom.

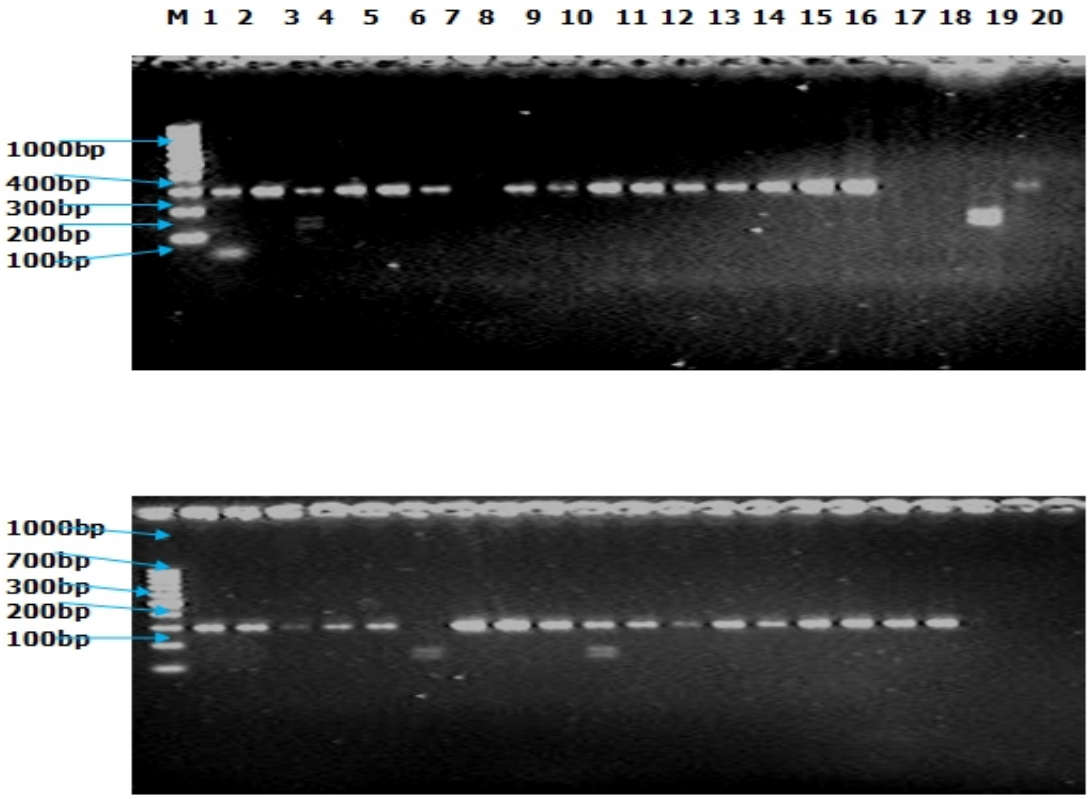

Plate 2: Restriction pattern of LEOH19 digested with BsaB I restriction enzyme in tomato genotypes 1-40. M; 100 bp ladder, Lane 1-40; **1**:Roma round, **2**:Beske,**3**:Delila, **4**:Gem pride, 5:Perfect pee, **6**:F1-Mongal, **7**:Pure-water, **8**:Santana, **9**:Kerewa, **10**:Tyre-type, **11**:UC82-B, **12**:Dankukumi, **13**:Derica, **14**:RomaVF,**15**:Gboko,**16**:Omo mola,**17**:AVTO1016,**18**:AVTO9803, **19:**AVTO0102,**20**:AVTO0201,**21**:NG/MR/MAY/09/005,**22:**NG/MR/MAY/09/006, **23**:NG/OE/MAY/09/019, **24**:NG/AO/MAY/09/011, **25**:NG/AA/SEP/09/037, **26**:Hausa type, **27:**UTC18, **28**:Uganda round, **29**:Uganda serated, **30**:Oval, **31:**Kibirigwi, **32**:Cholenaria, **33:**Small oblong, **34**:Okitsu, **35**:Ontario, **36**:Momor, **37**:Danjos, **38**:Ibadan local, **39**:Tomachiva and **40**:Eyetom.

Discussion

Dangerous effects of chemical pesticides can be altered with the use of non-chemical strategies and resistant cultivars, in endemic areas, can be a substitute to the shocking effect of *R. solanacearum* (Aslam et al., 2017a; Aslam et al., 2017b). In this present study, forty (40) tomato genotypes were screened for their resistance to *R. solanacearum* in sreenhouse. Techawongstien et al. (2007) identified some tomato genotypes resistant to *Ralstonia solanacearum* when conducted experiments over two seasons. Lawson and Summers (1984) identified tomato lines with significant resistance. Consistency in reaction of highly resistant tomato genotypes used in this study could be a confirmation for recommendation to plant breeders in breeding programme for resistant varieties. However, about 50% of the genotypes were either mean susceptible and susceptible to *Ralstonia solanacearum*. This was in concordance with the report of Parker et al. (2001) that tomato plants are highly susceptible to disease attack at all stages of their growth. Meanwhile, marker-assisted breeding can expedite cultivar improvement for disease resistance by predicting the presence of resistance alleles when selecting parents or offspring, even researchers had used markers for the identification of resistant genes to Fusarium wilt in tomato (Teh et al., 2017). In this study, the primer pair, LEOH19 was found to be monomorphic and produced DNA fragments in both resistant and susceptible, it could not, therefore, be used as a molecular marker for marker assisted selection in tomato breeding.

Restriction patterns of 300 bp were corresponded to the presence or absence of *bw*-gene as reported by Truong (2007). Sodium Dodecyl Sulphate (SDS) is an ionic detergent that binds to and denatures proteins and helps cell lysis (Mirmohammadsadeghi et al., 2013). The results showed that the inclusion of a chemical lysis (SDS) in Dellaporta et al. method might be the essence of producing higher DNA concentrations in some of the genotypes used. This was in agreement with the results of Ganiyu et al. (2017) when comparing the levels of yield and purity of genomic DNA from some tomato cultivars using two different extraction methods.

In Dellaporta et al. method, the plant cell wall was broken using mechanical force in the presence of the extraction buffer and SDS in the buffer liberated DNA by lysing cell and nuclei with subsequent centrifugation which precipitated cell debris, polysaccharides and protein complexes that interfere with the quality of the DNA. The amount of DNA concentrations obtained from the five tomato leaf samples using the Dellaporta et al. method were higher than those obtained using the ZR method.

Parmar et al. (2013) reported non-amplification of some alleles when identifying SSR markers linked to Fusarium wilt resistance in *Solanum lycopersicum*. The present study also showed the presence of "Null alleles" in some cultivars as a result of non-amplification which might be because of mutations at a primer binding site or insertion or deletion of the genetic segment. However, four of the genotypes were found to be polymorphic, though, of unknown resistant status. This was not in agreement with the report of Truong (2007) in which there were no polymorphic bands when BsaB I was used as restriction enzyme for amplicons from LEOH19 marker. In screenhouse study, tomato genotypes that were found highly resistant could be considered good materials in grafting and breeding programme for bacterial wilt resistance development. LEOH19 linked to *bw*-gene could not be executed for the discrimination of resistant and susceptible amongst the tomato population and therefore, could not be used as a molecular marker for marker assisted selection in tomato breeding programme.

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References

Aslam, M. N., Mukhtar, T., Hussain, M. A. and Raheel, M. (2017a) Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. J. Plant Dis. Prot. pp1-6 https://doi.org/10.1007/s41348-017-0100-1

Aslam, M. N., Mukhtar, T., Ashfaq, M. and Hussain, M. A. (2017b). Evaluation of chili germplasm for resistance to bacterial wilt caused by *Ralstonia solanacearum* Austr. Plant Pathol. 46(3): 289292. https://doi.org/10.1007/s13313-017-0491-2

Bradbury, J. F. (1986). Guide to Plant Pathogenic Bacteria. Commonwealth Agricultura Bureaux (CAB) International, Wallingford. Oxford university press, USA. 332 pages.

Caicedo, A. L., Williamson, S.H. and Hernandez, R. D. (2007). Genome-wide patterns of nucleotide polymorphism in domesticated rice. PLoS

Ching, A., Caldwell, K. S., Jung, M., Dolan, M., Smith, O. S., Tingey, S., Morgante, M. and Rafalski, A. J. (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet. 3 (1):19.

Choi, I. Y., Hyten, D. L., Matukumalli, L.K. (2007). A soybean transcript map: gene distribution, haplotype and single nucleotide polymorphism analysis. Genet. 176: 685 - 696.

Dellaporta, S. L., Wood, J. and Hicks, J. B. (1983). A Plant DNA Minipreparation: Version II. Plant Mol. Biol. Rep., 1:19-21. http://dx.doi.org/10.1007/BF02712670

Fernández-Marcos, M., Sanz, L., Lewis, D. R., Muday, G. K. and Lorenzo, O. (2011) Nitric oxide causes root apical meristem defects and growth inhibition while reducing PIN-FORMED 1 (PIN1)-dependent acropetal auxin transport. Proc. Natl. Acad. Sci. *USA* 108: 18506 - 18511

Ganiyu, S. A., Yusuf, S. M., Agbolade, J. O. and Imonmion, J. E. (2017). The levels of yield and purity of genomic DNA from five different tomato cultivars using two different extraction methods. Nig. J. Biotech. Vol. 33: 131-135. http://www.ajol.info/index.php/njb/index and www.biotechsocietynigeria.org

Giancola, S., McKhann, H. I., Berard, A., Camilleri, C., Durand, S., Libeau, P., Roux, F., Rebound, X. and Gut Brunel, D. (2006) Utilization of three highthroughput SNP genotyping methods, the good assay, Amplifluor and Taqman, in diploid and polyploidy plants. Theor. Appl. Genet.112: 1115 - 1124.

Hanson, P., Lu, S., Wang, J., Chen, W., Kenyon, L, Tan, C., Tee, K. L., Wang, Y., Hsu, Y., Schafleitner, R., Ledesma, D., Yang, R. (2016) Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. Sci. Hort. 201: 346354, http://dx.doi.org/10.1016/j.scienta.2016.02.020

Jones, E., Chu, W., Ayele, M., Ho, J., Bruggeman, E., Yourstone, K., Rafalski, A., Smith, O. S., McMullen, M. D., Bezawada, C., Warren, J., Babayew, J., Basu, S. and Smith, S. (2009) Development of Single Nucleotide Polymorphism (SNP) Markers for Use in Commercial Maize (*Zea Mays* L.) Germplasm. Mol. Breed. 24:165 - 176.

Kassa, M. T, Frank, M. Y., Colin, W. H., Curtis, J. P., Pierre, R. F., Andrew, G. S., James, G. M., Humphreys, D. G., Nicole, R. H., John, P. F., Brent, D. M. and Curt, A. M. (2017). Highly predictive SNP markers for efficient selection of the wheat leaf rust resistance gene Lr16. BMC Plant Biol. 17:45, DOI 10.1186/s12870-017-0993-7

Labate, J. A. and Baldo, A. M., (2005) Tomato SNP discovery by EST mining and resequencing. Mol. Breed. 16: 343 - 349.

Lawson, V.F. and Summers W.L. (1984) Disease reaction of diverse sources of *Lycopersicon* to *Xanthomonas campestris* pv. *vesicatoria* pepper strain race 2. Plant Dis. 68:117 - 119.

Mirmohammadsadeghi, H., Abedi, D., Mohmoudpour, H. R. and Akbari, V. (2013) Comparison of five methods for extraction of genomic DNA from a marine Archaea, *Pyrococcus furiosus*. Pak. J. Med. Sci. 29(1):390-394. doi: http://dx.doi.org/10.12669/pjms.291(Suppl).3540

Parker, S.K., Nutter Jr, F.W. and Gleason, M. (2001) Directional spread of septoria leaf spot in tomato rows. Plant Dis. 81:272 - 276

Parmar, P., Sudhir, A., Preethi, R., Dave, B., Panchal, K., Subramanian, R. B., Arvind Patel, A. and Kathiria, K. B. (2013) Identification of a SSR marker (TOM-144) linked to Fusarium wilt resistance in *Solanum lycopersicum*. Am. J Mol. Biol., 2013, 3, 241-247, http://dx.doi.org/10.4236/ajmb.2013.34031

Popoola, A. R., Ganiyu, S. A., Enikuomehin, O. A., Bodunde, J. G., Adedibu, O. B., Durosomo, H. A. and Karunwi, O. A. (2015) Isolation and Characterization of *Ralstonia solanacearum* Causing Bacterial Wilt of Tomato in Nigeria. Nig. J. Biotech., 29: 1-10. Published by Biotechnology Society of Nigeria. http://www.ajol.info/index.php/njb/index and www.biotechsocietynigeria.org, DOI: http://dx.doi.org/10.4314/njb.v29i1.1

Rafalski, J. A. (2002) Application of single nucleotide polymorphisms in crop genetics. Curr. Opin. Plant Biol. 5: 94 - 100.

Sobrino, B., Briona, M. and Carracedoa, A. (2005) SNPs in forensic genetics: a review on SNP typing methodologies. Foren. Sci. Intl. 154:181 - 194

Stuber, C.W. (1992) Biochemical and molecular markers in plant breeding. Plant Breed. Rev. 9:37 - 61.

Techawongstien, S., Thummabenjapone, P. and Bolkan, H. (2007) Screening tomato varieties for their bacterial wilt resistance over seasons in north-east Thailand. II Intl. Symp. Tom. Dis. ISHS.263268

Teh, S. L., Fresnedo-Ramírez, J., Clark, M. D., Gadoury, D. M. , Sun, Q., Cadle-Davidson, L. and Luby, J. J. (2017) Genetic dissection of powdery mildew resistance in interspecific half-sib grapevine families using SNP-based maps. Mol. Breed. 37: 1-16 doi:10.1007/s11032-016-0586-4

Truong, T. H. (2007) Characterization and mapping of bacterial wilt resistance in the tomato (*Solanum lycopersicum*) cultivar Hawii7996 and wild tomato germplasm. Master Dissertation. Gottfried Wilhelm Leibniz Universitat Hannover. pp 169.

Valliyodan, B., Dan, Q. and Patil, G. (2016) Landscape of genomic diversity and trait discovery in soybean. Sci. Rep., vol. 6, article 23598.

Yang, W., Miller, S. A., Scott, J. W., Jones, J. B. and Francis, D. M. (2005) Ming tomato genome sequence databases for molecular markers: application to bacterial resistance and marker assisted selection. Acta Hort. (ISHS) 695: 24 - 250.

Zakir Hussain M., M.A. Rahman and M.A. Bashar. 2005. Screening of brinjal accessions for bacterial wilt caused by *Ralstonia solanacearum*. Bangladesh J. Bot. 34(1): 53 - 58

Zhang, L. P., Khan, A., Nino-Liu, D. and Foolad, M. R. (2002) A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a L*ycopersicon esculentum* x *Lycopersicon hirsutum* cross. Genom. 45:133 - 146