

CAPS Markers TAO1 and TG105 in the Identification of I2 Resistant Gene in Nigerian Accessions of Tomato, *Solanum Lycopersicum* L.

Popoola, A. R.^{1,2}, Ercolano, M. R.², Ferriello, F.², Kaledzi, P. D.³, Kwoseh, C.³, Ganiyu, S. A.¹, Ojo, D. K.⁴, Adegbite, D. A.⁵ and Falana, Y.⁶

¹Department of Crop Protection, Federal University of Agriculture, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria. ²Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples, Via Universita, 100 80055 Portici, Italy. ³Department of Crop Science & Horticulture, Kwame Nkrumah University of Science and Technology, KNUST, Kumasi, Ghana. ⁴Department of Plant Breeding and Seed Technology, FUNAAB, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria. ⁵Department of Agricultural Economics and Farm Management, FUNAAB, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria. ⁶Agricultural Technical Services Department, Ekiti State Agricultural Development Programme, Ikole-Ekiti, Ekiti State, Nigeria.

(Received: 24:7:14; Accepted: 2:12:14)

Abstract

Fusarium oxysporum (f.sp.) *lycopersici* (Fol) is a soil-borne fungus that inhabits most tomato-growing regions of the world, causing vascular wilt disease. In Nigeria, the disease constitutes close to 40% loss in tomato yield annually. Cleaved Amplified Polymorphic Sequence (CAPS) markers TAO1 and TG105, developed elsewhere, were used to identify tomato accessions possessing the gene (I2) which confers resistance to Fol race 2. In this work, fifty Nigerian accessions of tomato, *Solanum lycopersicum* L., were screened with the two CAPS markers for resistant I2 gene. The primer pairs for TAO1 and TG105 produced amplification at 902 bp in 33 accessions and 450 bp in 38 accessions, respectively. The restriction enzymes *Fok1* and *Hinf1*, for TAO1 and TG105 respectively, produced fragments at base pairs indicative of susceptible, homozygous and heterozygous resistant accessions to Fol. Restriction fragments from the two markers indicated that 11 accessions were homozygous resistant to Fol. Four of these accessions (Delila, Gem Pride, K-Small and Oxheart) occurred in the two molecular markers as homozygous resistant to Fol. The combined effect of the two markers enhanced precision in the identification of tomato accessions with resistance status to *Fusarium* vascular wilt.

Keywords: CAPS markers, *Fusarium* wilt, I2 gene, tomato accessions,

Correspondence: popoolaar@funaab.edu.ng

Introduction

Fusarium oxysporum f.sp. *lycopersici* (Fol) is a soil-borne fungus which infects susceptible tomato plants through wounds or direct penetration of roots thereby colonizing the xylem vascular tissue. In resistant plants, the fungus is confined to the lower part of the roots, and additional symptoms do not develop (Ori et al., 1997). Entire plants or plant parts above the point of vascular invasion may die within few weeks after infection (Popoola et al., 2012). Three host-specific races (races 1, 2 and 3) of Fol have been described and named in their order of discovery (Beckman, 1987). The I (immunity to *Fusarium* wilt) gene for resistance to Fol race 1 was reported in the wild tomato *Lycopersicon pimpinellifolium* accession 160 (PI79532) (Bohn and Tucker, 1939). The I2 gene conferring resistance to Fol race 2 was discovered in PI126915, a *Lycopersicon esculentum* × *L. pimpinellifolium* hybrid (Stall and Walter, 1965). The I3 gene conferred resistance to race 3 Fol and was discovered in *L. pennellii* accessions PI414773 (McGrath et al., 1987) and in accessions LA716 (Scott and Jones, 1989). Ori et al. (1997) reported two members of a multigene family designated as

I2C-1 and *I2C-2*. They were only able to provide circumstantial evidence of the genes' involvement in Fusarium resistance (Simons et al., 1998).

In Nigeria and most parts of sub-Saharan Africa, *Fol* Race 2 has been implicated in the devastation of tomato field (Osunde and Ikediugwu, 2002). The disease had been rightly referred to as farmers' nightmare and total loss had been reported in endemic regions of Nigeria and Ghana (Popoola et al., 2011).

The control of *Fol* involves three strategies: husbandry practices, application of agrochemicals and use of resistant varieties (Barone and Fruscante, 2007). Resistant varieties are mostly produced by crossing resistant wild types and existing cultivars developed for their properties like good taste, shape and color. A molecular marker linked to resistance would be useful for tomato improvement programmes (Staniaszek et al., 2007) as it will improve precision and reduce time.

Screening of accessions under field conditions based on phenotype is time-consuming and challenging. Tightly linked molecular markers associated with resistance genes can improve selection efficiency and avoid associated field problems. Cleaved Amplified Polymorphic Sequence, CAPS, is a PCR-based marker system that could identify the resistant and susceptible genotypes and such findings have been consistent with phenotypic data (Dilip and Feng, 2010). TAO1 and TG105 are co-dominant CAPS markers known to be closely linked to the *I2* gene and could easily be used to identify genotypes possessing the *I2* gene, which confers resistance to *Fol* race 2 (Dilip and Feng, 2010; Staniaszek et al., 2007).

Popoola et al. (2012) identified some Nigerian accessions of tomato, *Solanum lycopersicum* L., containing homozygous resistant *I2* gene using Cleaved Amplified Polymorphic Sequence (CAPS) marker TAO1. In this paper, we report a more precise identification of tomato accessions that were resistant to Fusarium wilt using two CAPS markers – TAO1 and TG105.

Materials and Methods

Plant material: Fifty accessions of tomato (*Solanum lycopersicum* L.) were used in the amplification experiment with two CAPS markers – TAO1 and TG105. The accessions (Table 1) were mostly indigenous to Nigerian villages and settlements with little information on presence or absence of *I2* resistant gene. Few accessions were sourced from Ghana, Kenya and Italy. Accessions which produced amplicons with either primer were used in the restriction enzyme digestion.

DNA extraction: Fresh young leaves of 50 varieties/lines of tomato were subjected to DNA extraction, using the procedure described by Doyle and Doyle (1987).

Primer sets for TAO1 and TG105 markers: The forward and reverse primers for TAO1 (Staniaszek et al., 2007) were:-

f: 5'-GGGCTCCTAATCCGTGCTTCA-3'

r: 5'-GGTGGAGGATCGGGTTTGTTC-3'

For TG105, the set of primers as provided by Dr. M. R. Ercolano, Department of Soil, Plant, Environmental and Animal Production Sciences, DISPAPA, University of Naples, Via Universita, 100 80055 Portici, Italy were:-

f: 5'-CTTCAGAATTCCTGTTTTAGTCAGTTGAAC-3'

r: 5'-ATGTCACATTTGTTGCTTGACCATCC-3'

CAPS reaction (Akkale and Tanyolac, 2009): The CAPS markers were amplified in 25 µl reaction mixture comprising 20 mM Tris-HCl buffer pH 8.4, 50 mM KCl, 0.8 mM MgCl₂, 0.1 mM of each deoxynucleotide, 0.12 mM of each primer, 1 U Taq DNA Polymerase and 30 ng genomic DNA sample as template. The PCR parameters were: 94°C for 5 mins, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final extension time of 7 min at 72°C, using a Biometra THERMAL CYCLER T1.

Restriction enzyme digestion and analysis of amplified products (Staniaszek et al., 2007): PCR amplicons were digested with 5 units of *FokI* and *HinfI* restriction endonucleases (New England BioLabs Inc®) for TAO1 and TG105 primers, respectively. The digestion was performed in a 20 µl reaction volume containing recommended buffer (NEBuffer 4) for 1 h at 37°C and an enzyme inactivation step was performed for 20 min at 70°C.

Gel electrophoresis and imaging (Popoola et al., 2012): Amplification products were resolved by electrophoresis in 2% agarose gels under 3 V/cm potential and 100 mA current in TAE buffer for 3 h. Gels were stained with cybergreen for 30 min and visualized under UV light using Kodak 2D Imaging System. Resolution was against 1 kb DNA ladder.

Bioassay for Fusarium wilt in tomato accessions: Fusarium inocula were prepared according to a technique recorded in Popoola et al. (2012). Fusarium wilt tests were conducted on eleven

Table 1: Sources of tomato accessions

Accessions	Source	S/N	Accessions	Source
1 Roma round	IARoT, Ibadan, Nigeria	27	K-Small	Nairobi, Kenya
2 Beske	IARoT, Ibadan, Nigeria	28	FUNAAB BO1	FUNAAB, Nigeria
3 NG/MR/MAY/09/005	NACGRAB, Ibadan, Nigeria	29	FUNAAB BO2	FUNAAB, Nigeria
4 NG/MR/MAY/09/019	NACGRAB, Ibadan, Nigeria	30	FUNAAB SR1	FUNAAB, Nigeria
5 NG/OE/MAY/09/011	NACGRAB, Ibadan, Nigeria	31	FUNAAB SR2	FUNAAB, Nigeria
6 NG/AO/MAY/09/030	NACGRAB, Ibadan, Nigeria	32	K-Kwung	Nairobi, Kenya
7 GH/11/41	Kumasi, Ghana	33	Tomachiva	NIHORT, Nigeria
8 NG/AA/SEP/09/037	NACGRAB, Ibadan, Nigeria	34	Derica Opa	Imo State, Nigeria
9 Delila	JG Bodunde, FUNAAB, Nigeria.	35.	FUNAAB CG2	FUNAAB, Nigeria
10. Markis	JG Bodunde, FUNAAB, Nigeria.	36	Derica	Imo State Nigeria
11. Hypee 45	JG Bodunde, FUNAAB, Nigeria.	37.	Roma VF	IAR, Zaria Nigeria
12 Gem Pride	JG Bodunde, FUNAAB, Nigeria.	38	Gboko	Benue State, Nigeria
13 Perfect Pee	Ajara farm settlement, Lagos	39	Mbase local	Imo State, Nigeria
14 F1 Mongal	Ajara farm settlement, Lagos	40	Omo mola	Olorunda Iwoye Nigeria
15 Pure water	Ile-ona, Ekiti State, Nigeria	41	Ibadan Local	Ibadan market, Nigeria
16. Santana	Ilaro, Imeko, Ogun State	42	Totem	Ibadan market, Nigeria
17. Tyre type	Ife-Odan, Osun State	43	Lilly of the Valley	Markurdi, Benue State
18. Hausa type	Ife-Odan, Osun State	44	Oxheart	FUNAAB, Nigeria
19 UC 82 B	Kano market, Nigeria	45	Zeukertruabe	NIHORT, Ibadan
20 Dankukumi	Kano market, Nigeria	46	NHGB/09/111	FUNAAB, Nigeria
21. UTC18	Kano market, Nigeria	47	NHGB/09/116	FUNAAB, Nigeria
22 Funavto 98	FUNAAB, Nigeria	48	Brandy wine	Gwagwalada, FCT
23 Ontario	Napoli, Italy	49	Roman FN	JG Bodunde, FUNAAB, Nigeria
24 FUNAAB Oblong	FUNAAB, Nigeria	50	Floradade	JG Bodunde, FUNAAB, Nigeria
25 K-Kibirigwi	Nairobi, Kenya			
26 FUNAAB Small	FUNAAB, Nigeria			

* FUNAA B =Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.; NIHORT = National Horticultural Research Institute, Ibadan; NACGRAB = National Center for Genetic Resources and Biotechnology, Ibadan

accessions that showed homozygous resistant polymorphic bands. Control resistant and susceptible accessions were also included as positive and negative checks, respectively. Sandy-loam top soil (60% sand, 25% silt and 15% clay) was heat sterilized with the aid of electric soil sterilizer at 65°C

for 90 minutes. Thirty to thirty-five tomato seeds, surface sterilized with sodium hypochlorite, were sown in plastic buckets measuring 28 cm diameter and 30 cm deep, and containing 8 kg sterilized soil.

The roots of 13 to 14-day-old tomato seedlings were washed in tap water and trimmed. The seedlings were inoculated by dipping the roots of each accession for 10 minutes in the fungal spore suspension. Those dipped in sterile water served as control. Seedlings were then transplanted into pots filled with sterilized soil, kept in the screenhouse, and examined for three weeks for visual disease symptoms - wilt and desiccation. At 21-days post inoculation, seedlings were cut at 1 cm above soil level to determine vascular discoloration. A seedling was determined to be susceptible if, according to El-Mohtar et al. (2006), wilt symptoms or vascular discoloration were apparent.

Results

Amplification with CAPS Primer TAO1: Amplification with TAO1 primers showed the presence of 902 bp fragment in thirty-three out of 50 tomato accessions studied (Fig. 1). Advantage of this primer came from the possibility of further subjection of amplicons to restriction enzyme digestion to observe polymorphisms that are characteristic of susceptible and resistant accessions. CAPS markers could also differentiate homozygous resistant accessions from heterozygous resistant ones.

Amplification with CAPS Primer TG105: A 450-bp fragment was produced during amplification with TG105. This was observed in 38 accessions (Fig. 2). Most of the accessions produced bands at both 902 and 450 bp, suggesting an agreement between the two molecular markers. Eight accessions did not produce amplicons at either 902 or 450 bp indicating these eight accessions did not possess *I2* gene at all, either at recessive or dominant level.

Digestion pattern of TAO1 amplicons using Fok1 restriction enzyme: Amplicons were subjected to restriction enzyme digestion to determine susceptibility and nature of resistance - homozygous or heterozygous for *I-2* resistance gene. PCR products from primers of TAO1 were digested using restriction enzymes *Fok1*. Restriction fragments, 410 and 390 bp, specific to *I2* homozygous resistance were detected in Accession No. 9 (Delila), 12 (Gem Pride), 27 (K-Small) and 44 (Oxheart) (Fig. 3). No heterozygous resistant fragments (bands at 800, 410 and 390 bp) were detected. Other accessions produced restriction fragments at 800 bp, indicating susceptibility to *Fusarium oxysporum* f.sp. *lycopersici*.

Digestion pattern of TG105 amplicons using Hinf1 restriction enzyme: Restriction enzyme *Hinf1* produced more polymorphic bands than *Fok1*. Ten accessions produced fragments at 350 bp only to indicate homozygous resistance to the pathogen (Fig. 4a). The enzyme also produced fragments at 500 and 150 bp for susceptible accession (Fig. 4b). Fragments at base pairs 500, 350 and 150 would indicate heterozygous resistance, but no accession was found with this trait.

Bioassay for Fusarium wilt in tomato accessions: Four accessions (Delila, Gem Pride, K-Small and Oxheart) showed no symptom of wilt at 21 days after inoculation, although they had some manifestation of vascular discoloration. All other accessions had between 7 and 14% of the seedlings showing visual wilt symptoms. The overall performances of the eleven accessions tallied largely with the results of restriction enzyme digestion (Table 2).

Discussion

Molecular markers have been used successfully to identify tomato accessions with resistant genes to *Fusarium* wilt (Barone and Frusciante, 2007; El Mohtar et al., 2007; Akkale and Tanyolac, 2009; Popoola et al., 2012). This process of identification is crucial in the development of tomato varieties that are resistant to the highly destructive pathogen, *Fusarium oxysporum* f.sp. *lycopersici*, responsible for the vascular wilt of tomato. Developing resistant varieties is the most environmentally sound and effective strategy for the management of the disease (El-Mohtar et al., 2007).

Marker-assisted screening had reduced a bulk of genetic material to manageable size which could be taken to the field for phenotypic or bioassay confirmation (Popoola et al., 2012). Past workers had observed close correlation between the genotypic and phenotypic determination of resistance to disease, and it will not be out of place in the future to settle only for marker-assisted trait determination. Difficulties encountered during bioassay are pointing to that direction. As observed by Alon et al. (1974) and Sarfatti et al. (1989), bioassays for resistance to *Fusarium* wilt are time-consuming and complicated by variations in host genotype, inoculum concentration, stage of seedling development, soil temperature and soil contamination by other pathogens or races. Out of fifty accessions studied in this work, seventeen produced no fragment at 902 bp when subjected to

pcr amplification with *TAO1* marker. Thirty-three accessions produced amplicons at 902 bp on this marker.

TABLE 2. Bioassay and restriction enzyme analyses for the identification of tomato accessions resistant to Fusarium wilt

Accession	Bioassay (no. out of 30 seedlings)			Lane number and base pairs of restriction fragments		Genotypic classification based on restriction fragments
	Healthy	Vascular discoloration	Wilted	<i>FokI</i>	<i>HinfI</i>	
GH/11/41	22	5	3	-	7(350bp)	HR
Delila	27	3	0	9(410,390 bp)	9(350bp)	HR
Gem Pride	26	4	0	12(410,390bp)	12(350bp)	HR
UTC18	24	4	2	-	21(350bp)	HR
Funavto 98	25	4	1	-	22(350bp)	HR
Ontario	20	6	4	-	23(350bp)	HR
K-Small	27	3	0	27(410,390bp)	27(350bp)	HR
FUNAAB BO1	24	4	2	-	28(350bp)	HR
FUNAAB BO2	26	3	1	-	29(350bp)	HR
Derica Opa	24	4	2	-	34(350bp)	HR
Oxheart	30	0	0	44(410,390bp)	-	HR

HR = Homozygous Resistant

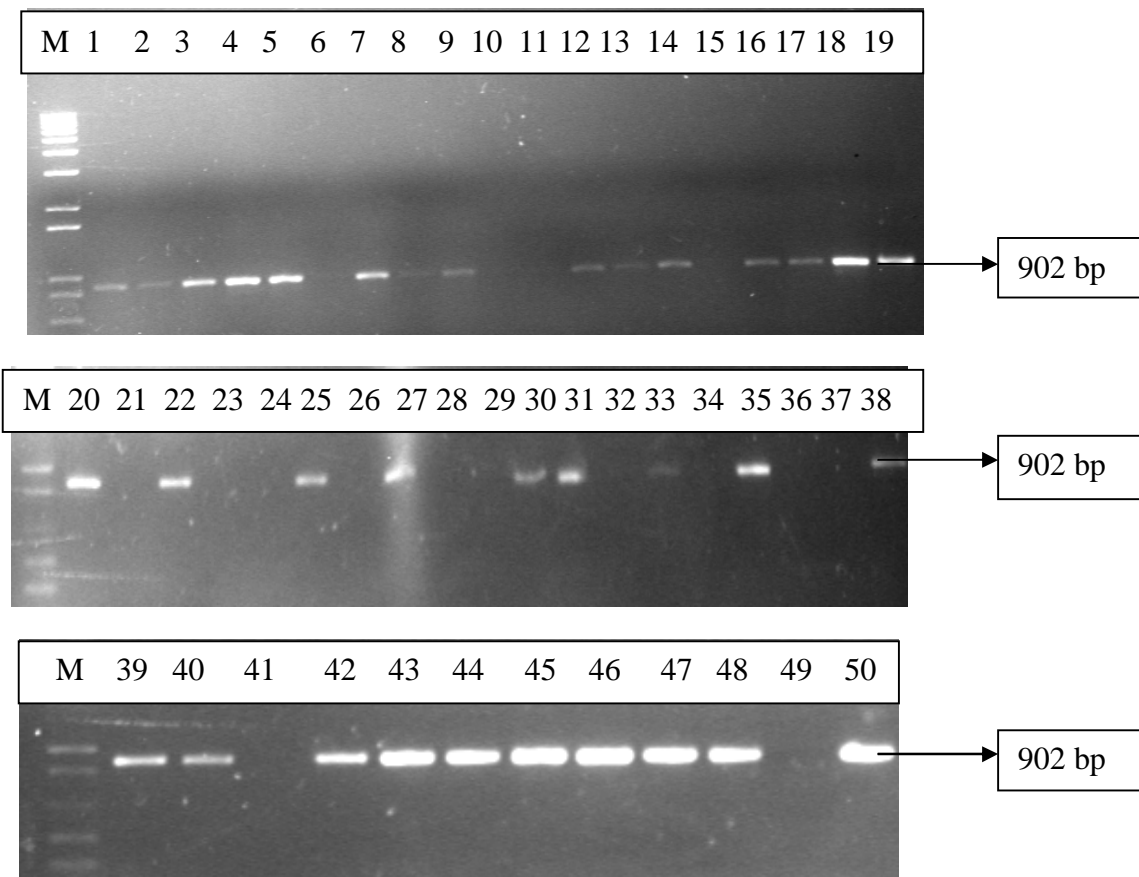


Figure 1. Gel electrophoresis of polymerase chain reaction (PCR)-amplified products of CAPS primer *TAO1* in 2% agarose. Lanes 1 – 50 contained genomic DNA of fifty accessions of tomato and lanes M contained the 1 kb DNA ladder. Thirty-three accessions produced amplicons at 902 bp characteristics of *TAO1*-primed *I2* gene. Seventeen accessions did not produce amplicon.

With the use of *TG105* marker, thirty-eight accessions were amplified at the characteristic 450 bp. The 902- and 450-bp-long fragments in the two markers were found to be polymorphic in ten and four tomato accessions, respectively. Staniazsek et al. (2007) reported that a size of 902-bp-long fragment of *TAO1* marker was polymorphic in both susceptible and resistant tomato plants after further digestion of the 902-bp-amplicon by appropriate restriction enzyme. With restriction enzyme *Fok1*, *I2*-specific restriction fragments were detected in four accessions namely Delila, Gem Pride, K-Small and Oxheart. The fragments were at 410 and 390 bp, suggesting homozygous resistant status. Three of these accessions were equally detected by *Hinf1* in addition to seven more accessions giving a total of ten accessions which produced *I2*-specific restriction fragments at 350 bp corresponding to homozygous resistant genotypes. These fragments showed that both alleles that were related to the *I-2* gene were present in the accessions.

The two markers (*TAO1* and *TG105*) are complementary and have produced a wider polymorphism, leading to identification of dominant allele of the *I2* gene for resistance to *Fol*. Huang et al. (2005) observed that *TAO1* resided very closely to *TG105*, which was about 0.1 centimorgan (cM) away from *I2*. The homozygous genotypes identified in this work were GH/11/41, Delila, Gem Pride, UTC18, Funavto 98, Ontario, K- Small, FUNAAB BO1, FUNAAB BO2, Derica Opa and Oxheart. Identification of homozygotes for the *I-2* gene is especially important when searching for parent lines for backward integration of the resistant gene to other farmer-preferred varieties.

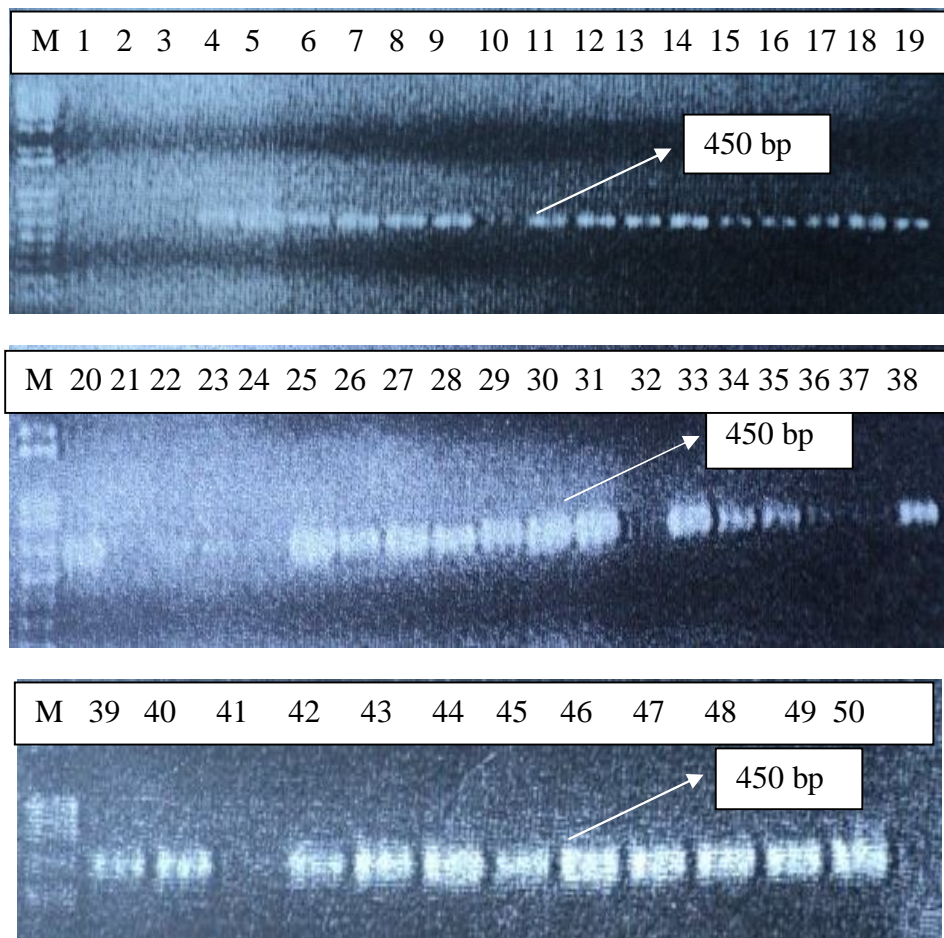


Fig. 2. Gel electrophoresis of PCR-amplified products in 2% Agarose using *TG105* primers. Amplification was at 450 bp. Twelve accessions were not amplified. Lanes 1 – 50 contained genomic DNA of fifty accessions of tomato and lanes M contained the 1 kb DNA ladder.

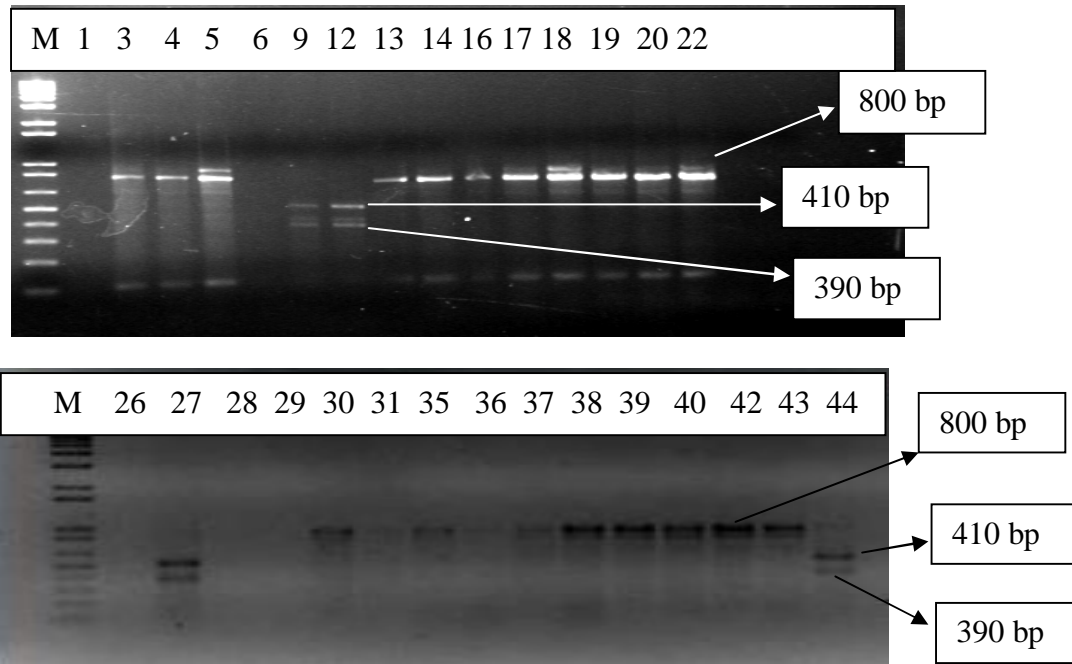


Fig. 3. Restriction enzyme (*FokI*) patterns of amplicons obtained with CAPS marker *TAO1*. Susceptible accessions amplified at 800 bp while homozygous resistant ones (9, 12, 27 and 44) amplified at 410 and 390 bp. Numbered lanes contained genomic DNA of tomato accessions while lane M contained the 1 kb DNA ladder.

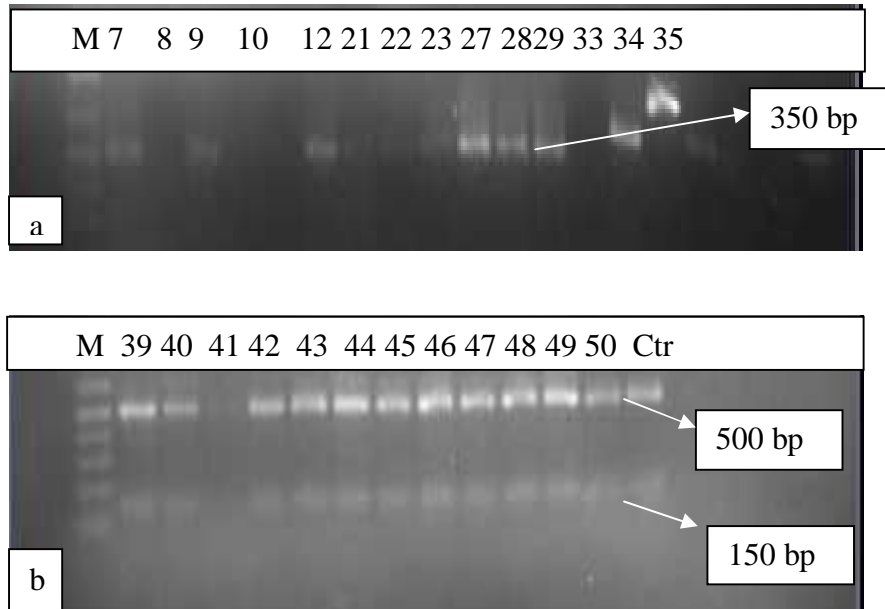


Fig. 4. Gel electrophoresis of *HinI1* restriction enzyme patterns of CAPS marker *TG105* amplicons with homozygous resistant tomato accessions (**a**) showing bands at 350 bp and susceptible ones having bands at 500 and 150 bp (**b**). Numbered lanes contained genomic DNA of tomato accessions while lane M contained 1 kb DNA ladder.

Conclusion

This work had combined the diagnostic potentials of two sets of molecular markers in a process that provided additional advantage of identifying co-dominance through the use of Cleaved Amplified Polymorphic Sequence. This is an advantage over our earlier use of a single molecular marker which was able to identify only four accessions with homozygous resistance to Fusarium wilt. With the two markers, a further identification of seven more accessions had been achieved. With this, we have a wider choice of genotypes in marker-assisted selection of wilt-resistant tomato genotypes for breeding purposes.

Acknowledgement

We acknowledge the Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples, Portici, Italy for providing the facilities used in the molecular aspect. This research was jointly supported by United Kingdom's Department for International Development (DFID) under the Development Partnerships in Higher Education programme DELPHE-5 programme, the British Council, Abuja Office, Nigeria and Agricultural Research Council of Nigeria under the Competitive Agricultural Research Grants Scheme, ARCN-CARGS.

References

- Akkale, C and Tanyolac, B. (2009). Screening of resistance genes to Fusarium Root Rot and Fusarium wilt diseases in F3 family lines of tomato using RAPD and CAPS markers. *Inter. J. Nat. & Eng.Sci.* 3(3): 144 - 148
- Alon, H., Katan, J. and Kedar, N. (1974). Factors affecting penetrance of resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomatoes. *Phytopathol.* 64: 461- 467.
- Barone, A and Frusciante, L (2007). Molecular marker-assisted selection for resistance to pathogens in tomato. In: Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A., and Dargie, J.D ed. *Marker-Assisted Selection: Current status and future perspectives in crops, livestock, forestry and fish*, pp. 151-164, Electronic Publishing and Support Branch, Communication Division, FAO, Rome.
- Beckman, C. H. (1987). The nature of wilt diseases of plants. Publ. American Phytopathological Society, St. Paul, MN, USA. Pp. 75
- Bohn, G. W and Tucker, C. M. (1939). Immunity to Fusarium wilt in the tomato. *Science* 89: 603–604.
- Dilip, R. P. and Feng, C. (2010). Genomics of fungal disease resistance in tomato. *Curr. Genomics* 11: 30-39
- Doyle, J. J. and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11-15.
- El Mohtar, C. A., Atamian, H.S., Dagher, R.B., Abou-Jawdah, Y., Salus, M.S., and Maxwell, D.P. (2007). Marker-assisted selection of tomato genotypes with the I-2 gene for resistance to *Fusarium oxysporum* f.sp. *lycopersici* race 2. *Plant Dis.* 91:758 – 762
- Huang, S., van der Vossen, E.A.G., Kuang, H., Zhang, N., Borm, T.J.A., van Eck, H.J., Baker, B., Jacobsen, E and Visser, R.G.F. (2005). Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J.* 42: 251 – 261
- McGrath, D. J., Gillespie, D. and Vawdrey, L. (1987). Inheritance of resistance to *Fusarium oxysporum* f. sp. *lycopersici* races 2 and 3 in *Lycopersicon pennellii*. *Aus. J. Agric. Res.* 38:729–733.
- Ori, N., Eshed, Y., Oaran, I., Presting, G., Aviv, D., Tanksley, S., Zamir, D. and Fluhr, R. (1997). The *I2C* family from wilt disease resistance locus *I2* belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. *The Plant Cell* 9: 521 – 532

- Osuinde, M. I. and Ikediugwu, F. E. O. (2002). Occurrence of *Fusarium* and *Ralstonia (Pseudomonas) solanacearum* on root surface of tomato (*Lycopersicum esculentum*). Afr. J. Sc. Techn. 3:18 – 21.
- Popoola, A.R., Ercolano, M. R., Kaledzi, P.D., Ferriello, F., Ganiyu, S.A., Dapaah, H.K., Ojo, D.K., Adegbite, D.A., Falana, Y. and Adedibu, O. B. (2012). Molecular and phenotypic screening of tomato genotypes for resistance to *Fusarium* wilt. Ghana J. Hort. 10: 61 – 67.
- Popoola, A.R., Ercolano, M. R., Kaledzi, P.D., Ferriello, F., Ganiyu, S.A., Dapaah, H.K., Ojo, D.K., Adegbite, D.A., Falana, Y. and Adedibu, O. B. (2011). Building capacity in agrobiotechnology to improve tomato yield in wilt-endemic areas of Nigeria and Ghana – A report of DfID-sponsored bilateral research cooperation. Presented at the Nigerian Society for Plant Protection Conference, Federal University of Technology, Akure, Nigeria. 4 – 8 September, 2011
- Sarfatti, M., Katan, J., and Zamir, D. (1989). An RFLP marker in tomato linked to the *Fusarium oxysporum* resistance gene *I2*. Theoret. Appl. Genetics 78: 755 - 759
- Scott, J. W. and Jones, J. P. (1989). Monogenic resistance in tomato to *Fusarium oxysporum* f. sp. *lycopersici* race 3. Euphyt. 40:49–53.
- Simons, G., Groenendijk, J., Wijbrandi, J., Reijans, M., Groenen, J., Van der Lee, T., Bleeker, M., Zabeau, M., Onstenk, J., and Vos, P. (1998). Dissection of the *Fusarium* gene cluster in tomato reveals six homologs and one active gene copy. The Plant Cell 10:1055–1068
- Stall, R. E. and Walter, J. M. (1965). Selection and inheritance of resistance in tomato to isolates of races 1 and 2 of the *Fusarium* wilt organism. Phytopathol. 55:1213–1215.
- Staniasek, M., Kozik, E. U. and Marczewski, W. (2007). A CAPS marker TAO1₉₀₂ diagnostic for the I-2 gene conferring resistance to *Fusarium oxysporum* f. sp. *lycopersici* race 2 in tomato. Plant Breeding 126(3), 331-333.