African Journal of Biotechnology Vol. 10(9), pp. 1511-1515, 28 February, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2095
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Molecular marker screening of tomato, (*solanum lycopersicum* L.) Germplasm for root-knot nematodes (*Meloidogyne* species) resistance

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Accepted 30 December, 2010

Tomato is one of the crops in which genetic resistance has specially been effective against root-knot nematodes. In this study, molecular screening was done on some tomato germplasm to detect markers for the gene that confers resistance (*Mi*) with specific primer (Mi23/F//Mi23/R). The cultivars; VFNT, FLA 505-BL 1172, 2641A, "Adwoa Deede" and Terminator FI showed the marker for the homozygous resistant genotypes (*Mi/Mi*). The cultivars, Tima and 2644A showed both markers, corresponding to heterozygous resistant genotypes (*Mi/mi*). Twenty one (21) of the cultivars did not show any of the markers presumably due to non-specificity at the primer-binding sites. Five (5) heterozygous individuals were determined out of 6 resistant cultivars following the Hardy-Weinberg principle in population genetics.

Keywords: Germplasm, *Meloidogyne incognita*, molecular marker, resistance, *Solanum lycopersicum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop in Ghana with many uses (Awuah, 2006). All the savanna and transitional zone soils in Ghana favour its production and total production in the country increased from 28,400 ha in 1996 to 37,000 ha in 2000 (MoFA, 1987). However, root-knot nematodes severely attack tomatoes resulting in severe yield losses and its infestation is the most common and serious problem associated with the crop's cultivation in Ghana (Addoh, 1971; Hemeng, 1980). The most effective nematicides have been restricted in agriculture because of high risks to human health and the environment (Thomas, 1996). Genetic resistance in tomato to the pest is efficient in reducing their populations, thereby reducing the need for pesticides application (Medina-Filho and Tanskley, 1983; Roberts et al., 1986). Host resistance is the most practical alternative to the use of nematicides (Da Conceicao et al., 2005).

The most important source of the resistance is conferred by the *Mi* family of genes from the wild tomato *Lycopersicon peruvianum*, providing effective resistance against *Meloidogyne* species (Hadisoeganda and Sasser, 1982).

In tomato breeding, the identification of the root-knot nematodes resistance gene, *Mi* is mainly by traditional screening bioassay. Results from field screening can be misleading because of variation in nematode populations and soil temperatures. If many recombinants or cultivars are to be screened, it will be time consuming, tedious and labour intensive. This situation can be overcomed by using molecular markers in a marker-assisted-selection (MAS) programme. MAS dictates that the selection of a trait of interest be conducted indirectly by selecting for markers that link to the trait (Melchinger, 1990; Dekkers, 2004); and it is most effective when there is a tight linkage between the marker and the trait of interest (Kelly, 1995).

The major advantages of DNA molecular markers are that, they are free of environmental effects on the phenotype, efficient and faster, less subjective, non-destructive, cheaper to run than standard bioassays. Molecular markers also have low negative selection pressure in populations and provide data that can be analyzed objectively. In addition, they are developmentally stable

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Table 1. Tomato cultivars evaluated.

Code	Cultivar	Source /Origin
1	FLA 505-BL1172	AVRDC, Taiwan
2	2641A	AVRDC, Taiwan
3	Wosowoso	Commercial, Ghana
4	FLA 496-11-6-0	AVRDC, Taiwan
5	Adwoa Deede	Commercial, Ghana
6	TLB111	AVRDC, Taiwan
7	Terminator F1	Green seeds, India
8	3008A	AVRDC, Taiwan
9	Roma-JAM VF	Commercial, U.S.A
10	Burkina Petomech	Commercial, France
11	Roma VF	Commercial, B. Fasso
12	Ventura F	Commercial, France
13	Slumac	Commercial, Holland
14	Red Cloud	Commercial, Holland
15	Rando	Commercial, Ghana
16	Akoma	Commercial, Ghana
17	Ghana Petomech	Commercial, France
18	Floradade	Commercial, U.S.A
19	FLA 478-6-3-0	AVRDC, Taiwan
20	Money maker	Comm. South Africa
21	Tima	Commercial, France
22	Rio grande	Commercial, Holland
23	Parona	Commercial, Ghana
24	Biemso	Commercial, Ghana
25	Power	Commercial, Ghana
26	2644A	AVRDC, Taiwan
R	VFNT(Resist. check)	TGRC, V. Williamson
S	UC82(Suscept. check)	TGRC, V. Williamson

and young seedlings can be screened very early for the presence or absence of a particular trait (Luc et al., 1999; Hussey and Janssen, 2002). Standard bioassays used for the screening of tomato germplasm for resistance to root-knot nematodes require at least 1-3 months (Bost and Triantaphyllou, 1982; Hussey and Barker, 1973) and involves a considerable amount of labour. Molecular markers represent a precise and efficient tool that tomato breeders and researchers will find inexpensive and easy to use as a screening tool in large populations (Milligan et al., 1998).

A successful plant breeding programme for plant parasitic nematodes resistance depends on the identification of effective resistant sources (Niu et al., 2007). There is a limited application of MAS in tomato breeding in Ghana. In this work, a co-dominant SCAR marker (Mi-23) that is tightly linked to the *Mi-1.2* gene was employed to amplify polymerase chain reaction (PCR) fragments of twenty-eight (28) tomato germplasm in a marker-assisted selection programme.

MATERIALS AND METHODS

A total of 28 tomato cultivars including a resistant check cv. VFNT (*Mi*/*Mi*) and a susceptible check cv. UC82 (*mi*/*mi*) were used for this study. They were collected from local and international sources; and different regions (Burkina Fasso, France, Holland, India, Taiwan, South Africa and U.S.A) (Table 1). DNA was extracted from fresh leaves of tomato plants following the protocol of Egnin et al. (1998). PCR was carried out in 10x µl reaction mix containing 6.07 µl PCR water, 1.00 µl 10x PCR buffer, 0.90 µl $MgCl_2$ (25 mM), 0.40 µl dNTPs (10 mM), 0.25 µl (10 µM) each for the forward and reverse primers and 0.125 µl Taq DNA polymerase. 1 µl DNA was added to 9 µl of each reaction mix PCR tube.

The tubes were then covered and placed in the thermocycler (Mycycler-BIO-RAD) using the following cycles; an initial denaturation cycle of 94°C for 3 min, followed by 35 cycles of 94°C for 3 min, annealing at 57°C for 1 min, then a final extension at 72°C for 10 min and held at 4°C. Amplified fragments were separated by electrophoresis through 1.5% agarose gel in a 1x TAE (Tris-acetate-EDTA) buffer, after gel resolution had been stained with 4 µl ethidium bromide and visualized under UV light and a photograph taken (Figure 1).

RESULTS AND DISCUSSION

PCR products of the cultivars; FLA 505-BL 1172, 2641A, "Adwoa Deede", Terminator FI, Tima and 2644A in the lanes 1, 2, 5, 7, 21, and 26 respectively amplified a 380-bp fragment expected of resistant tomato genotype linked to the *Mi-1* locus with primers M23/F//M23/R. The products also amplified the same locus with the resistant check (VFNT). In conjunction with traditional screening, *Mi* gene specific primers (C1/2 and C2S4) were used to differentiate between resistant and susceptible plants with a 1.6 kb DNA band being detected in resistant plants but absent in susceptible plants. The primers could distinguish between resistant and susceptible plants whereas resistant heterozygote and homozygote individuals were not distinguished (Devran and Elekçioğlu, 2004). In an earlier study with the same primers (C1/2 and C2S4), resistant and susceptible plants were distinguished from each other whereas resistant heterozygous individuals were not distinguished (Williamson et al., 1994).

In the current work, the resistant (VFNT) and susceptible (UC82) checks were distinguished from each other whilst heterozygous (*Mi/mi*) and homozygous (*Mi/Mi*) resistant cultivars were not distinguished. When data analysis was done via Binary Table following the Hardy-Weinberg principle in population genetics, (1 = present and 0 = absent) 5 heterozygous cultivars were determined out of the 6 resistant cultivars identified (Table 2 and 3).

According to Seah et al. (2007), the susceptible genotypes, M82-1-8 and Gh13 (*mi*/*mi*) and the resistant genotypes, Motelle and Gh2 (*Mi*/*Mi*) were characterized by PCR fragments 430-bp and 380-bp respectively, as expected of susceptible and resistant genotypes. In the

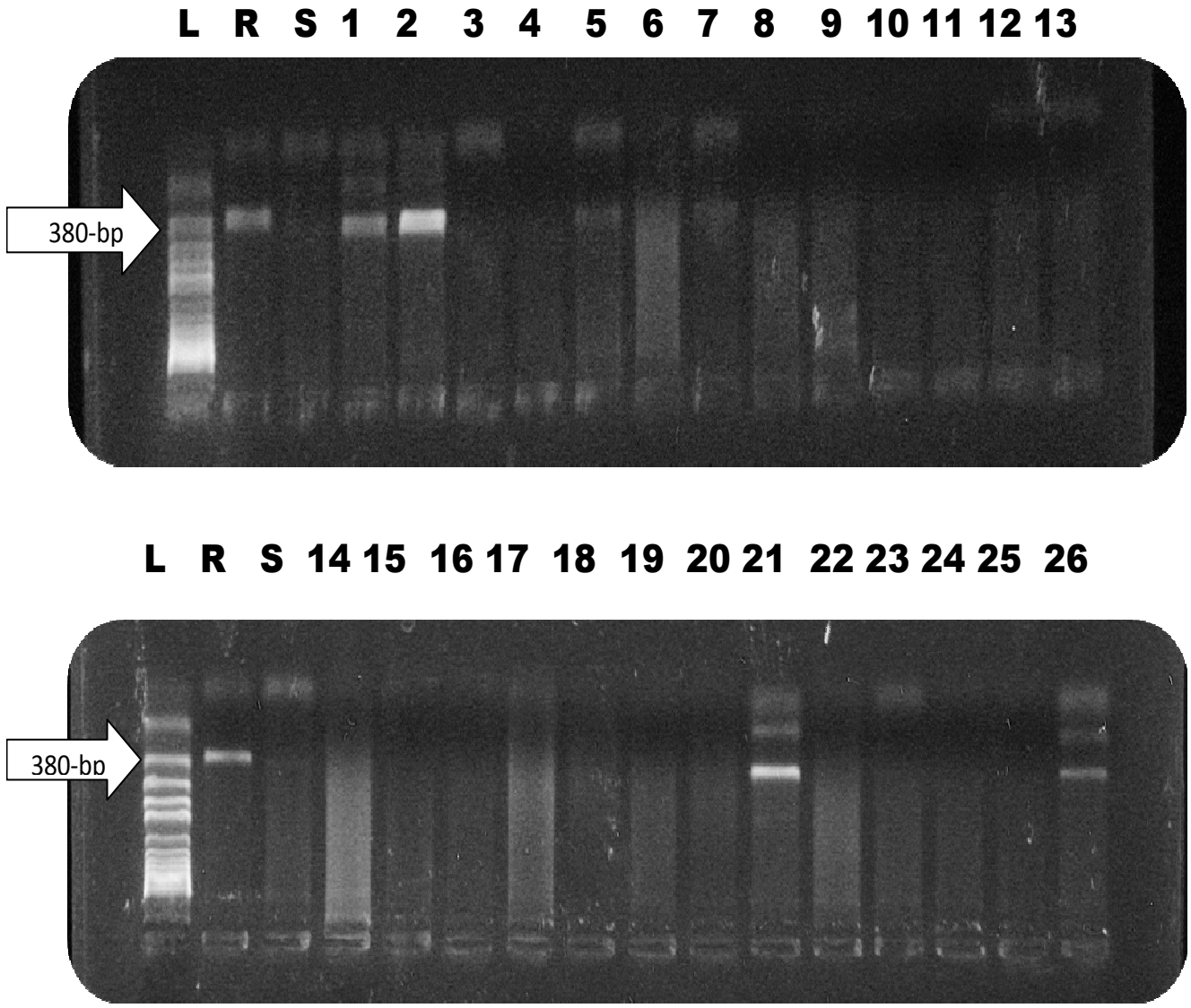

Figure 1.0. PCR amplification to detect tomato *Mi* gene using Egnin et al., 1998 DNA extraction method and primers Mi23F and Mi23R. From left: L (200-bp ladder), R (resistant cv. VFNT), S (susceptible cv. UC82), 1 (FLA 505-BL 1172), 2 (2641A), 3 (Wosowoso), 4 (FLA 496-11-6-0), 5 (Adwoa deede), 6 (TLB111), 7 (Terminator FI), 8 (3008A), 9 (Roma-JAM VF), 10 (BK Petomech), 11(Roma VF), 12 (Ventura F), 13 (Slumac), 14 (Red Cloud), 15 (Rando), 16 (Akoma), 17 (GH Petomech), 18 (Floradade), 19 (FLA 478-6-3-0), 20 (Money maker), 21 (Tima), 22 (Rio grande), 23 (Parona), 24 (Biemso), 25 (Power), 26 (2644A).

current work, the resistant check (VFNT) amplified the same PCR fragment of 380-bp. The susceptible check (UC82) however, did not show any amplification, presumably due to non-specificity at the primer-binding sites (Palumbi et al., 1991). When six commercial hybrids (Celebrity, Charanta, Crista, Dominique, Tequila and Viva Italia) with reported resistance to root-knot nematodes were tested with the primers Mi23/F//Mi23/R, all of them had the three banded pattern associated with heterozygous plants for the *Mi-1* locus (Seah et al., 2007). When the same primers (Mi23/F//Mi23/R) were used in the current study, two (Tima and 2644A) out of the six resistant cultivars amplified the three banded pattern associated with heterozygous plants for the *Mi-1* locus while the resistant check (VFNT) amplified the expected single major band of 380-bp (Figure 1). This study clearly demonstrates the use of an *Mi* gene specific marker in screening for root-knot nematodes resistant tomato genotypes that is reliable, timely and efficient.

ACKNOWLEDGEMENTS

This work was supported by a grant from the WECARD/ CORAF/SCARDA. The authors are grateful to Dr V.M. Williamson, University of California, Davies, who donated

Table 2. Binary Table.

Cultivar	Score
2644A	1
FLA 505 (BL 1172)	1
Wosowoso	0
FLA 496-11-6-1-0	0
Adwoa Deede	1
TLB 111	0
Terminator FI	1
3008A	0
Roma (JAM) VF	0
Burkina petomech	0
Roma VF	0
Ventura F	0
Slumac	0
Red cloud	0
Rando	0
Akoma	0
Ghana petomech	0
Floradade	0
FLA 478-6-3-0	0
Money maker	0
Tima	1
Rio grande	0
Parona	0
Biemso	0
Power	0
2641A	1

Absent = 0; Present = 1.

Table 3. Estimation of heterozygous cultivars.

Genotype	(*RR*), (*Rr*)	*rr*	Total	Allele frequency
Genotype **frequency** (expected)	$p^2 + 2pq$	q^2	1	p = 0.12
Number of individuals	6	20	26	q = 0.88
Genotype **frequency** (observed)		$q^2 = 0.77$		1

Genotype frequency of susceptible cultivars (rr) $q^2 = 0.77$
Frequency of recessive allele $q = \sqrt{0.77} = 0.88$
$p + q = 1$, therefore $p = 1 - 0.88 = 0.12$
Expected number of heterozygous (*Rr*) can be estimated as follows:
2pqN, where N = sample size, 2(0.12) (0.88) (26) = 5.49 = 5.

molecular markers for this work.

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