RELEVANCE OF THE MI23 MARKER AND THE POTATO
APHID BIOLOGY AS INDICATORS OF TOMATO PLANT
(SOLANUM Lycopersicum L.) RESISTANCE TO SOME PESTS

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Summary

Mi-1.2 gene, expressed in tomato plants, contributes to endogenous resistance against nematodes and some Hemiptera insects. The aim of this study was to screen the presence of dominant/recessive locus of the Mi-1.2 gene in tomato cultivars with different allelic combination using Mi23 SCAR method and to assess the capacity of the local potato aphid (Macrosiphum euphorbiae Thomas) population to develop on different tomato cultivars (dominant and recessive homozygotes in Mi-1.2 locus). The results showed that both Mi23 marker and potato aphid performance are relevant methods in screening tomato cultivars with a different allelic combination of Mi-1.2 gene. The assessment of biological potential of M. euphorbiae proved that, in comparison with control (tomato plants with recessive alleles of Mi-1.2 gene), the aphid mortality increased 9- and 4 – fold (in the first and second experimental series, respectively) and the female longevity decreased 3 – fold when fed on tomato cvs with dominant alleles of Mi-1.2 gene. Furthermore, the resistance against aphids manifests as an antibiosis mechanism in tomato plants carrying dominant alleles.

key words: Solanum lycopersicum L., Mi-1.2 gene, SCAR Mi23 marker, Macrosiphum euphorbiae Thomas

INTRODUCTION

Two species of phloem-feeding insects - the potato aphid (Macrosiphum euphorbiae Thomas) and the peach potato aphid (syn. the green peach aphid) (Myzus persicae Sulzer) (Hemiptera: Aphididae) can easily colonise tomato plants in both glasshouse and field conditions. Their population of high densities can cause considerable direct damage by feeding from the phloem and indirect damage by transmitting several plant viruses and also producing a large amount of honeydew (Dixon 1987).
Aphid-resistant tomato cultivars would be a valuable component of an integrated control strategies reducing costs resulting from chemical and/or biological protection. Therefore, the genes responsible for endogenous crop plant resistance to these herbivorous arthropods are the focus of many modern breeding programs. For example, single, dominant genes such as the \textit{Vat} gene in melon, \textit{Rag1} in soybean or \textit{AKR} in \textit{Medicago truncatula} Gaertner are associated with the resistance against the melon aphid (\textit{Aphis gossypii} Glover), \textit{Aphis glycines} Matsuura and the blue green aphid (\textit{Acyrthosiphon kondoi} Shinji and Kondo), respectively (Chen et al. 1997; Klingler et al. 2005; Li et al. 2007).

In tomatoes (\textit{Solanum lycopersicum} L.), a single, dominant gene \textit{Mi-1.2} confers resistance to the root-knot nematodes (\textit{Meloidogyne} spp.) (Milligan et al. 1998). From recent studies, it is known that the product of this gene also gives the resistance to some clones/isolates of \textit{M. euphorbiae}, the sweet potato whitefly (\textit{Bemisia tabaci}) and tomato psyllid (\textit{Bactericercus cockerelli}) (Kaloshian et al. 1995, 1997; Rossi et al. 1998; Goggin et al. 2001, 2004, 2006; Jiang et al. 2001; Nombela et al. 2001, 2003; Casteel et al. 2006). The level of transcript of \textit{Mi-1.2} gene is rather stable during the period of tomato plant growth and development, although the resistance to aphids does not appear until plants are six weeks old (Kaloshian et al. 1995). Plants expressing dominant alleles of \textit{Mi-1.2} gene but younger than six week-old are still susceptible to the potato aphid.

The first molecular marker of \textit{Mi} locus was \textit{Aps-1} gene encoding acid phosphatase-1 (Rick and Fobes 1974; Medina-Filho and Stevens 1980). Further studies have shown that there was a possibility of recombination in the region between the \textit{Aps-1} and \textit{Mi} locus in crosses segregating root-knot nematode resistance (Messeguer et al. 1991; Ho et al. 1992). Later, it was reported that the CAPS (Cleaved Amplified Polymorphic Sequence) REX-1 marker is more tightly linked to the \textit{Mi} locus (Williamson et al. 1994). The REX-1 marker is still widely used, however, it can give false positive results for the presence of dominant \textit{Mi} locus in some of the begomovirus-resistant tomatoes (El Mehrach et al. 2005). Recently, a 5 kb-long sequence (not predicted to encode any protein), located between the \textit{Mi-1.2} gene and the \textit{Mi-1.3} pseudogene, was discovered (Seah et al. 2007). Since there is a strong similarity between dominant and recessive \textit{Mi} loci, except for a short sequence (57 nt long), which differentiates resistant and susceptible genotypes, it seems that the short sequence could serve as a new, effective marker. Primers that flanked this distinct sequence were designed. This allowed a highly specific co-dominant SCAR (Sequence-Characterized Amplified Region) marker of the \textit{Mi} gene to be defined (Seah et al. 2007).

The present study aims to report on the relevance of the \textit{Mi23} marker to screen the presence of dominant or recessive locus of \textit{Mi-1.2} gene in tomato cultivars with a different allelic combination. Simultaneously, the capacity of the local potato aphid to develop on tomato dominant and recessive homozygotes in \textit{Mi-1.2} locus was
evaluated to verify the antibiotic effect of the Mi-1.2 gene.

MATERIALS AND METHODS

Plant material and extraction of total DNA
Four cultivars of tomato plants with a different allelic combination were assessed by SCAR method. There were homozygous Motelle and heterozygous Faustine F₁ cvs that contain dominant Mi allele introgressed from S. peruvianum, and Moneymaker and Brooklyn F₁ with both recessive alleles of Mi-1.2 gene. Seeds of Motelle and Moneymaker cvs were supplied by The Gene Bank (Centre de Recherches Agronomiques D’Avignon INRA, France). Seeds of conventional tomato cultivars (Faustine F₁ and Brooklyn F₁) were obtained from Syngenta (Netherlands).

Ten plants of each cultivar were grown in a peat substrate (Karaska, Łomianki, Poland), in individual pots, under glasshouse conditions. Leaf samples were collected from 10 week-old plants and genomic DNA was extracted from each leaf sample using DNeasy Plant Mini Kit according to the producer’s manual (Qiagen, Germany). Each of 10 plants of each cultivar was treated as one replication. DNA concentration and purity were determined spectrophotometrically. Average purity of nucleic acids equaled 1.7 (A280/A260).

SCAR amplification conditions
Primer sequences and PCR conditions were carried out as described previously by Garcia et al. (2007) with a modified reaction mixture. PCR reaction was performed in 20 μl volume containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTP, 1 U Taq polymerase, 1 μM of each primer (Mi23F: 5’-TGG AAA AAT GTT GAA TTT CTT TTG-3’; Mi23R: 5’-GCA TAC TAT ATG GCT TGT TTA CCC-3’) and 30 ng of template DNA. The amplification reaction was carried out in GeneAmp 9700 thermocycler (Applied Biosystems, USA). The cycle parameters were: 94°C for 3 min. followed by 35 cycles of 30 sec. at 94°C, 1 min. at 57°C, 1 min. at 72°C, and a final extension time of 7 min. at 72°C. PCR products were analyzed by electrophoresis in 1.8% (w/v) agarose gel in 1 x TBE buffer after staining with ethidium bromide.

Insects
Aphids ( Macrosiphum euphor -biae Thomas) were derived from individuals collected from eggplant (Solanum melongena L.) growing in the glasshouse of Warsaw University of Life Sciences – SGGW, Warsaw, Poland. The aphid population was reared on tomato plants cv. Robin F₁ (PNOS, Ożarów Mazowiecki, Poland). To assess the M. euphorbiae capacity to develop on Motelle and Money- maker cvs, wingless aphid females were placed individually on cut leaflets of each cultivar and placed in Petri dishes filled with wet cotton wool. Two experiments were carried out: (experiment I: n=20; experiment II: n=10). In each, single female was treated as one replication. Females were maintained in environmentally controlled growth chamber (L/D=16/8 h; temp: D/N=23/20°C; RH 70%) (Sanyo, Japan) and checked every day for longevity and mortality. Results were compared with Mann-Whitney U test at P=0.05.
RESULTS AND DISCUSSION

Figures 1 a-b show the representative electrophoretic profiles of PCR products for four cultivars of tomato plants with a different allelic combination. The results indicate that all examined tomato cultivars were positive for the presence of Mi/mi locus. Since the Mi23 marker is tightly linked to Mi locus and is codominant (Seah et al. 2007), it allows to distinguish effectively homo- and heterozygotes. It is clear that both cultivars Moneymaker and Brooklyn F1, as homozygotes, gave one specific product of amplification at the 430 bp size (Fig. 1 a, line 2,3,9). In the case of homozygous Motelle cultivar that contains dominant Mi alleles, DNA band of 380 bp size was well visible (Fig. 1 b, line 1,2,4,7,9). In contrast, two bands of 430 and 380 bp size appeared simultaneously for heterozygous plants of Faustine F1 cultivar (Fig. 1 a, line 4-8).

From the comparison of CAPS and SCAR procedures described by Williamson et al. (1994) and Garcia et al. (2007), respectively, it is clear that the SCAR method is easier and faster than the CAPS method, however both methods are effective. In our study, SCAR Mi23 marker method has been explored alternatively to CAPS, since PCR products had different length and they could be separated by electrophoresis. CAPS method is used when PCR products cannot be separated by electrophoresis because of similar length (despite different sequence). That is why they have to be digested by restriction enzymes to identify polymorphism (Masojć 2005). According to Garcia et al. (2007), the process of digestion complicates and prolong the procedure. Therefore, it seems reasonable to use SCAR instead of CAPS system in the case of the assessment of the Mi-1.2 gene presence.

Effect of the tomato plant cultivar on the elements of bionomy of the potato aphid was strong and clearly visible (Fig. 2 a-b). When aphids fed on tomato plants with dominant alleles of Mi-1.2 gene, aphid mortality equaled 86 and 90% and longevity equaled 4.45±2.89 and 6.50±2.07 days in the first and second experiment, respectively (Fig. 2 a-b). When aphids fed on plants with recessive alleles of Mi-1.2 gene their mortality was lower (10 and 25%) and longevity was longer (14.95±4.17 and 17.10±4.36 days) (Fig. 2 a-b). In the first and second experiments, M. euphorbiae female longevity differs significantly between treatments (experiment I: W=388.5; P<0.001; experiment II: W=97.0; P<0.001). Markedly higher mortality (9- and 4-fold) and significantly lower longevity (3-fold) of the potato aphids on Motelle then on Moneymaker plants demonstrate that tomatoes equipped in dominant alleles of the Mi-1.2 gene are resistant against potato aphids and the resistance is manifested as an antibiosis mechanism. The results obtained in the present study are in agreement with the data presented previously on Mi gene mediated resistance to M. euphorbiae (Kaloshian et al. 1995,1997; Rossi 1998; Goggin 2001; Cooper & Goggin 2005). However, the two spotted spider mite (Tetranychus urticae Koch, Acari: Tetranychidae) underwent full developmental cycle on tomato plants regardless of allelic combination of the Mi-1.2 gene (Godzina et al. 2010).
Fig. 1 a-b. Representative electrophoretic profile of PCR products for Mi gene presence. M – 100 bp DNA ladder.
Fig. 2 a-b. Average mortality (a) and female longevity (X±SD) (b) of *M. euphorbiae* when fed on tomato cultivars. *M. euphorbiae* female longevity differs significantly between treatments (experiment I: W=388.5; P< 0.001***; experiment II: W=97.0; P< 0.001***).

In the future studies, the effect of heterozygous tomato cvs on the biography of Polish local potato aphid population should be analysed. Results of a pilot experiments (Godzina, unpublished data) on the performance of *M. euphorbiae* on cut leaflets of the dominant and recessive homozygotes (Motelle and Moneymaker cvs) and heterozygote (Dasher F₁ cv, DeRuiter, Switzerland) showed twice lower female longevity of aphids when they
fed on Motelle cv (7.40±2.22 days) then on Moneymaker cv (17.00±4.62 days) whereas female longevity on Dasher F₁ cv. was intermediate (14.00±4.22 days). Similarly, Martinez de Iarduya & Kaloshian (2001) showed that density of aphids per leaflet fed on heterozygous tomato cvs reached intermediate number compared to the insect density on dominant and recessive homozygous cvs. The relevance of these results for commercially available cultivars of tomato plants grown in glasshouse/field condition needs further study.

CONCLUSIONS

1. The SCAR method with specific primers Mi23 is an effective marker of resistance against *M. euphorbiae* to screen tomato plants with different allelic combination.

2. In tomato plants, dominant *Mi-1.2* gene is the source of resistance against the local population of *M. euphorbiae* and the resistance is manifested as an antibiosis mechanism.

3. Due to the strong response to dominant *Mi-1.2* gene presence, the elements of bionomy of *M. euphorbiae* seems a good biological marker of tomato plant resistance to the root-knot nematode.

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**MARKER MI23 I BIOLOGIA MSZYCY ZIEMNIACZANEJ SMUGOWEJ JAKO MOLEKULARNY I BIOLOGICZNY WSKAŹNIK ODPORNOŚCI ROŚLIN POMIDORA NA NIEKTÓRE NICJENIE I PLUSKWIAKI**

**Streszczenie**

Gen *Mi-1.2* w roślinach pomidora zwyczajnego (*Solanum lycopersicum* L.), warunkuje odporność na nicienie (*Meloidogyne* spp.) i niektóre owady z rzędu Pluskwiaki (Hemiptera). Celem badań była ocena markera MI23 w diagnostyce roślin pomidora, o zróżnicowanych składach alleli. Równocześnie analizowano potencjał rozrodczy mszycy ziemniaczanej smugowej (*Macrosiphum euphorbiae* Thomas), której ograniczona bionomia na roślinach z dominującymi allelami genu *Mi-1.2* powinna być dobrym wskaźnikiem ich odporności i na nicienie, i na pluskwiaki. Wśród analizowanych odmian i mieszańców F₁ pomidora obecność locus Mi/mi stwierdzono we wszystkich badanych genotypach. O antybiotycznym oddziaływaniu roślin pomidora z dominującymi alleleм genu *Mi-1.2* na mszycę ziemniaczaną smugową świadczy wysoka śmiertelność osobników dorosłych (86 i 90%) oraz bardzo krótki czas życia samiec (4,45±2,89 i 6,50±2,07 dni). Na homozigotach z recesywnymi alleleм genu *Mi-1.2* śmiertelność była 9- i 4-krotnie niższa (10 i 25%), a długość życia około 3-krotnie dłuższa (14,95±4,17 i 17,10±4,36 dni), co potwierdza odporność testowanych roślin nie tylko na nicienie, ale i na lokalną populację *M. euphorbiae.*