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SHORT COMMUNICATION

MOLECULAR SELECTION OF TOMATO AND PEPPER BREEDING LINES POSSESSING RESISTANCE ALLELES AGAINST TOBAMOVIRUSES

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Molecular selection among newly created tomato and pepper lines was applied for identification of lines possessing DNA markers linked with the resistant loci against tobamoviruses *ToMV*, *TMV*, *PaMMV*, and *PMMoV*. Only four tomato lines among 184 had DNA marker linked with the resistant allele *Tm-2* conferring homozygosity at this locus. Resistance of these four lines was tested and confirmed also by virological testing by inoculation with *TMV* strain 0. Simultaneously tested lines heterozygous at this locus expressed full or unbalanced resistance. Fifty-eight out of 62 tested pepper lines had only marker linked to susceptible allele of the locus L. Three lines derived from tobamovirus resistant pepper cultivars Brill and Brilliant expressed marker linked to resistant allele L^3, and only one line derived from resistant cultivar Hurricane possessed both markers. Four selected pepper lines declared resistance also after artificial inoculation with the *TMV* P_0 pathotype. Molecular selection, both in tomato and pepper breeding lines, may be useful in breeding programs directed to tobamovirus resistance.

Key words: tomato, pepper, molecular marker, tobamovirus, *Tm-2*, *tm-2*, L^3 gene

Selection of advanced lines within the newly created breeding materials is a crucial operation in the plant breeding programs. Individuals possessing better resistance against different biotic and abiotic stresses can be efficiently selected by molecular markers linked with relevant loci. Also transfer of resistance genes from donor genotypes into elite cultivars is more effective using the marker assisted selection (MAS). This is particularly useful for incorporation of disease resistance genes that are difficult to detect in the same genotype and accumulate by gene pyramiding (Ordon *et al*. 1999). Molecular markers have been already widely used in genetic mapping and MAS related to resistance against different diseases in tomato and pepper (Foolad & Sharma 2005; Yang *et al*. 2012). *Tobamoviruses* such as pepper mild mottle virus (*PMMoV*), paprika mild mottle virus (*PaMMV*), tobacco mosaic virus (*TMV*), and tomato mosaic virus (*ToMV*) are easily transmitted between plants mechanically or in seeds.

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These viruses cause serious damages with systemic mosaic symptoms associated with yield and quality losses. The development of improved resistant genotypes is challenging especially if resistance genes originate from wild relatives. Phenotypic selection of resistant lines after pathogen inoculation is time-consuming, affected by environmental factors and plant developmental stage, moreover depends on availability of virus pathotypes. Therefore DNA markers in genetic linkage with resistance genes are very useful tools for effective breeding.

Response of commercial tomato (*Lycopersicon esculentum* L.) cultivars to *ToMV* infections is influenced by presence or absence of resistance genes *Tm-1, Tm-2*, and *Tm-2²* (Pelham 1966; Hall 1980) introgressed from the wild tomato species *Lycopersiocon hirsutum* f. *hirsutum* (*Tm-1*) and *Lycopersiocon peruvianum* L. (*Tm-2*, *Tm-2²*), respectively. The gene *Tm-1* provides resistance to *ToMV* strains 0 and 2 (Ohmori *et al*. 1996; Levesque *et al*. 1990). Genes *Tm-2* and *Tm-2²* are considered to be allelic and the allele *Tm-2* conferred resistance to *ToMV* strains 0 and 1, whereas allele *Tm-2²* resistance to *ToMV* strains 0, 1 and 2 (Hall 1989; Lanfermeijer *et al*. 2003). Three alleles *Tm-2, Tm-2²*, and *tm-2* (sensitive) have been cloned and sequenced (GenBank accessions AF536199, AF536200, AF536201) and cleaved amplified polymorphic sequences (CAPS) markers were developed to distinguish these alleles in tomato (Lanfermeijer *et al*. 2005). Shi *et al*. (2011) identified four allele-specific PCR-based markers for *Tm-2*, *Tm-2²*, and two for the susceptible allele *tm-2* as well as three allele-derived CAPS markers for distinguishing between them. Other three specific SNP markers developed for the locus *Tm-2* provide advanced tools for tomato breeders to detect *Tm-2* and *Tm-2²* resistance alleles.

Genetic resistance against *Tobamovirus* spp. in pepper (genus *Capsicum*) provides the *L* locus. Five alleles at this locus *L* were revealed: L^0 – related to susceptibility; L^1 – resistance to P_0 pathotypes of TMV, L^2 – resistance to P_0 and P_1 pathotypes of PaMMV that overcome L^1 resistance; L^3 – resistance to P_0, P_1, and $P_{1,2}$ pathotypes of PMMoV that overcome L^2 resistance; and L^4 – resistance to P_0, P_1, $P_{1,2}$, and $P_{1,2,3}$ pathotypes of PMMoV that overcome L^3 resistance (Tomita *et al*. 2011). Also other alleles as L^{1a}, L^{1c}, and L^{2b} with different temperature sensitivity were identified (Tomita *et al*. 2011). Recently pathotypes $P_{1,2,3,4}$ of the *PMMoV* braking the L^4 resistance were identified (Antignus *et al*. 2008; Genda *et al*. 2007). Alleles L^1, L^2, L^3, and L^4 were identified in *Capsicum annuum* L. (e.g. cv. Bruinsma Wonder and Verbeterde Glas), *Capsicum frutescens* L. (cv. Tabasco), *Capsicum chinense* Jacq. (PI159236), and *Capsicum chacoense* Hunz. (PI260429), respectively. Different molecular markers linked to the *L* locus were developed, e.g. RAPDs (Sugita *et al*. 2004), AFLPs (Tomita *et al*. 2008), SCARs converted from RAPD and AFLP markers (Kim *et al*. 2008; Matsunaga *et al*. 2003), and SNPs (Yang *et al*. 2009; Yang *et al*. 2011).

The goal of this study was to test the applicability of the molecular selection by DNA markers linked to alleles of resistance against tobamoviruses ToMV, TMV, PaMMV, and PMMoV within tomato and pepper breeding lines.

Altogether 184 tomato and 62 pepper lines developed within the breeding programs of the company Zelseed Ltd. (Horná Potôň, Slovakia) were screened with the aim to select lines possessing the desired resistance alleles. Total plant DNA was isolated from young leaves by the DNeasy Plant Mini kit (Qiagen). Polymerase chain reactions (PCR)

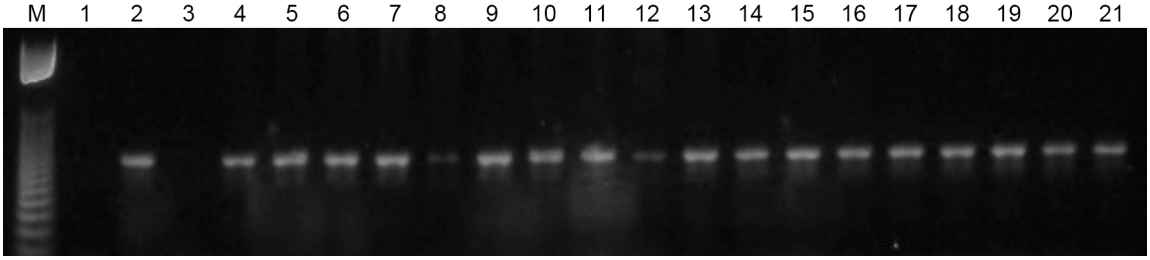

Figure 1. Analysis of the amplified 393 bp DNA fragment linked to the susceptible allele *tm-2* in tomato (M – size marker, 1 – negative control, 2 – cv. Monalbo, 3 – cv. Moperou, 4-21 – breeding lines)

were carried out using specific primers. Analysed tomato and pepper lines were tested simultaneously by artificial inoculation with relevant virus isolates – ToMV pathotype P_0 and TMV pathotype P_0, both obtained from the Netherlands Inspection Service for Horticulture (Naktiunbouw, Roelofarendsveen, The Netherlands). Symptoms manifested on the pepper and tomato plants were evaluated according to official guidelines of the European Union Community Plant Variety Office (CPVO) – Protocol for Distinctness Uniformity and Stability Tests for *Capsicum annuum* L. (Technical Protocol 076) and Protocol for Distinctness Uniformity and Stability Tests for *Lycopersicon esculentum* Mill. (Technical Protocol 044/3).

Two tomato cultivars Moperou and Monalbo were used as the control genotypes carrying resistant allele *Tm-2* and susceptible allele *tm-2*, respectively. Presence or absence of the susceptible allele *tm-2* in all tomato lines was determined by dominant allele-specific PCR markers amplified using two primer pairs – Tm2S-f1/Tm2S-r1 and Tm2S-f2/Tm2S-r2, designed based on the nucleotide sequences of the Genbank accession AF536199 (Shi *et al*. 2011). These primers amplified DNA fragment in genotypes containing the allele *tm-2* in homozygous or heterozygous status. Presence or absence of the resistant allele *Tm-2* in 184 tomato lines were determined by the dominant allele-specific PCR marker using primer pair Tm2R-f1c/Tm2R-r3 designed from the sequences of the GenBank accessions AF536200 and AF536201 (Shi *et al*. 2011). Parameters of the PCRs for all three primer pairs were set according to Shi *et al*. (2011). Amplified products were separated by gel electrophoresis in 1.5% agarose gels and stained with ethidium bromide. These dominant allele-specific markers are not able to distinguish homozygous and heterozygous genotypes at the locus *Tm-2* individually, but combination of these markers can do it. Similar to Shi *et al*. (2011), three types of fragments were amplified. The 393 bp fragment relates to the susceptible allele *tm-2* (amplified by primers Tm2S-f1/Tm2S-r1, Figure 1), 284 bp fragment for the susceptible allele *tm-2* (using primers Tm2S-f2/Tm2S-r2), and 444 bp fragment for the resistant allele *Tm-2* (using primers Tm2R-f1c/Tm2R-r3, Figure 2). Altogether 133 tomato lines out of 184 analysed possessed only fragment for the susceptible allele *tm-2* (homozygotes) and 33 lines both fragments for susceptible and resistant alleles (heterozygotes). The remaining 4 lines expressed only fragment related to the resistant allele *Tm-2* (homozygotes) and they were also tested by artificial inoculation with *TMV* strain 0. All of them showed full resistance (9 points), while heterozygotes showed full or unbalanced resistance. These lines were selected as the most promising for further exploitation in tomato virus resistance breeding program.

The pepper cultivars Hurricane and Century were used as positive controls carrying resistance genes L^3 and L^4, respectively. Others two – Piperade and Yolo Wonder were used as negative controls. Presence or absence of the L^3 gene in 62 pepper lines was determined using the codominant SCAR markers $PMFR11_{269}$ and $PMFR11_{283}$ derived from RAPD markers $E18_{272}$ and $E18_{286}$ (Sugita *et al*. 2004). Also PCR reactions with primer pair PMF1 and PMR1 were according to Sugita *et al*. (2004). Amplified products were separated in 6% polyacrylamide gel and stained by the silver staining method (Bassam *et al*. 1991). Similar to Sugita *et al*. (2004) two types

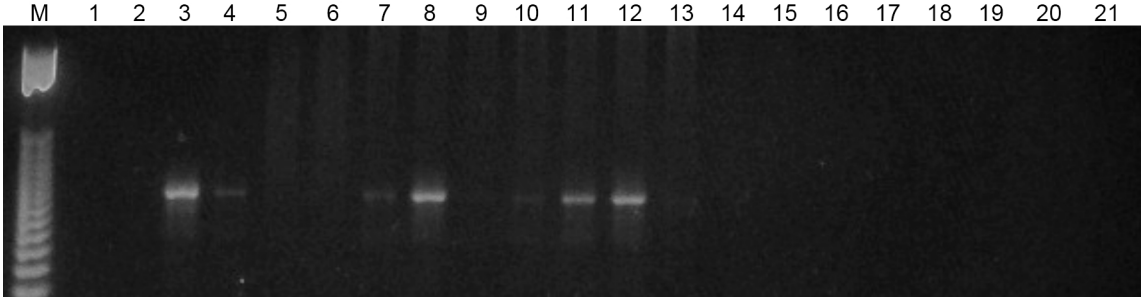

Figure 2. Analysis of the amplified 444 bp DNA fragment linked to the resistant allele *Tm-2* in tomato (M – size marker, 1 – negative control, 2 – cv. Monalbo, 3 – cv. Moperou, 4-21 – breeding lines)

of fragments were amplified – the 283 bp fragment ($PMFR11_{283}$) corresponding to the susceptible allele and 269 bp fragment ($PMFR11_{269}$) to the resistant allele (Figure 3). Fifty-eight from 62 analysed pepper lines expressed only fragment for the susceptible allele. Three genotypes expressed only the fragment for the resistant allele. All were derived from the Hungarian cultivars Brill and Brillant, both declared as resistant to tobamoviruses. Only one line possessed both fragments. It was derived from the Hungarian cultivar Hurricane declared as resistant to tobamoviruses. Above mentioned four lines were tested by artificial inoculation with the TMV pathotype P_0 and all confirmed resistance. Molecular selection process thus enabled to select for a number of resistant materials suitable for subsequent crossing with elite pepper cultivars. However, Sugita *et al*. (2004) found out that the fragment $PMFR11_{269}$ was not detected in some genotypes regardless of the presence of the L^3gene. The reason can be either the presence of different sources of the L^3gene or recombination between $PMFR11_{269}$ and the L^3gene.

Presence or absence of the L^4 gene in all screened pepper lines were determined by the dominant SCAR marker L4SC340 derived from the AFLP marker L4-b closely linked to the locus L^4 (Kim *et al*. 2008). PCR reactions with primers L4SC340F and L4SC340R were similar to Kim *et al*. (2008), nevertheless optimized by the mix FailSafe PCR PreMix Selection kit (Epicentre). The amplified products were separated by electrophoresis on a 6% polyacrylamide gel using the silver staining method (Bassam *et al*. 1991). Unfortunately this marker did not show any polymorphism in our group of pepper lines. The reason can be similar as described Kim *et al*. (2008), that in pepper differentials for the *Tobamovirus* pathotype this marker showed band for plants harbouring loci L^+, L^2, L^3 and L^4 and did not show band only for the L^1 locus. Thus the applicability of this marker depends on the genetic background of tested breeding material.

Obtained results showed that DNA markers used for detection of resistance genes against tobamoviruses in pepper and tomato can be successfully used in breeding programs. They enabled to identify genotypes which could be used as donors of the genes conferring effective resistance against tobamoviruses. These donors of resistance genes could be transferred into elite pepper or tomato cultivars and DNA markers can be used for screening within hundreds of off-springs in selection process.

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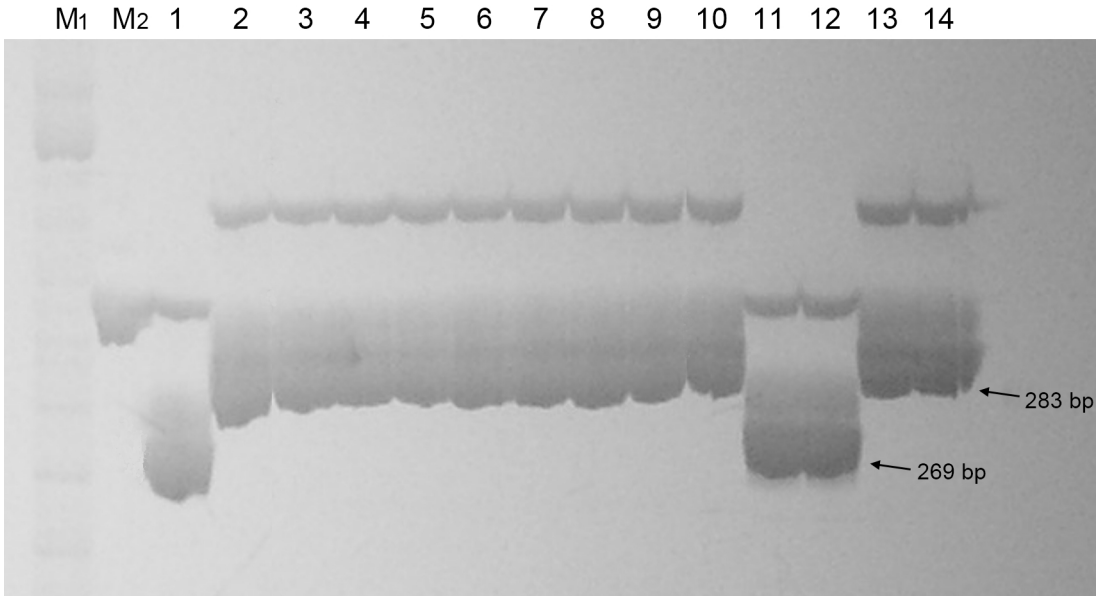

Figure 3. Analysis of two DNA fragments amplified by the primer pair PMF1 and PMR1 in pepper – 283 bp fragment linked to the susceptible allele and 269 bp fragment linked to the resistant allele (M_1 – marker 1, M_2 – marker 2, 1-11 – pepper lines, 12 – cv. Hurricane, 13 – cv. Yolo Wonder, 14 – cv. Piperade)

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