Bul. Agron. 27(3) 10-14(1999)

IDENTIFICATION OF CO-DOMINANT DNA MARKER TIGHTLY LINKED TO *Tm-2* LOCUS IN TOMATO

Sobir *)

ABSTRAK

Tm-2 adalah gen yang mengendalikan ketahanan terhadap tomato mosaic virus (ToMV) pada tomat (*Lycopersicon esculentum*) dan berasal dari *Lycopersicon peruvianum.* Pada penelitian ini digunakan dua galur tomat yang mempunyai latar belakang genetik yang sama GCR26 dan GCR36, akan tetapi keduanya dapat dibedakan oleh adanya gen *Tm-2* pada GCR36. Melalui *analisis random amplified-polymorphic DNA* (RAPD) terhadap dua galur tomat tersebut di atas diketahui adanya primer RAPD yang menghasilkan fragmen spesifik untuk GCR236. Selanjutnya fragmen tersebut sekuennya dianalisis, dan dari hasil analisis sekuen tersebut dibuat sepasang primer *sequence charactrized amplified region* (SCAR) yang merupakan marka DNA kodominan yang bisa membedakan baik genotipe homozigot gen *Tm-2.* Oleh karena fragmen dihasilkan terkait dengan *Tm-2*, maka primer SCAR tersebut merupakan sarana yang dapat digunakan untuk pemulian tanaman tomat bagi pembentukan varietas tahan virus.

ABSTRACT

Tm-2 is a gene conferring resistance to tomato mosaic virus (ToMV) in *Lycopersicon esculentum*, and originated from *Lycopersicon peruvianum,* a wild relatives of tomato. A random amplified-polymorphc DNA (RAPD) screening was employed with genomic DNA from ToMV-susceptible (GCR26) and resistant tomato (GCR236) near isogenic lines (NILs). A polymorphic band links to ToMV resistant line was detected; furthermore the fragment was cloned and sequenced. A pair of primer was designed from the most-end of fragment sequence. PCR ampilification of genomic DNA with these primers resulted in single polymorphic band between susceptible and resistant tomato lines. This sequence characterized amplified region (SCAR) primer proved as codominant marker that enables to distinguish a homozygous and a heterozygous individual plant in segregating populations, and proveded a comvenient and rapid assay for selection during breeding programs and hybrid seed production, respectively.

Key word: DNA marker, TM-2 gene, Resistance to ToMV

INTRODUCTION

Tomato mosaic, tobamovirus (ToMV) is the most common mosaic disease in tomato (*Lycopersicon esculentum* (L) Mill.). This virus systemically infects tomato, and causes light and dark green mosaic, bright yellow mottling, fern-leaf growth, or necrosis of leaves, stems, and fruit, which seriously affect fruit yield quality (Broadbent, 1964). A breeding program from early 1940s started in Hawaii (Frazier *et al.*, 1964) was introduced ToMV-resistance of wild-relatives including *L. hirsutum* and *L. peruvianum* into *L. esculentum.* In the backcross generations, *Tm-1* originated from *L hirsutum* and *Tm-2* originated

*) Plant Breeding and Genetics Laboratory, Faculty of Agriculture, Bogor Agricultural University

from *Lycopersicon peruvianum* were identified as ToMV-resistancegenes (Pelham, 1966). The *Tm-2* gene was found to tightly link to a recessive gene, netted-virescent (*nv*), which causes stunding and yellowing of the leaves in homozygous. Clayberg (1960) found that *Tm-2* to confer higher level of resistance than *Tm-1*. *Tm-2* confers resistance to more isolates of ToMV *Tm-1* does (Pelham, 1966).

The polymerase chain reaction (PCR) based RAPD method has applied to identify DNA markers linked to *Tm-2* locus (Ohmori *et al.*, 1995). However, user of RAPD markers is restricted because they are sensitive to variations in reaction conditions Gu *et al.* (1995). To increase fidelity, the RAPD markers can be converted into specific, stable sequence characterized amplified region (SCAR) markers. Since SCAR primers are longer than RAPD primers, highly stringent annealing temperature can be employed to prevent mismatching in the priming site during DNA amplification (Paran and Michelmore, 1993). SCAR markers linked to disease-resistant genes in tomato have also been developed for *Tm-1* (Ohmori *et al.*, 1996) and *Ve* (Kawchuk *et al.*, 1998) loci.

In this report, the RAPD marker linked to *Tm-2* locus was characterized, and based on the polymorphic fragment specific to near isogenic lines (NILs) carryng *Tm-2* gene, a pair of SCAR marker was designated, and the SCAR primer was generated a single band of different size in both NILs.

MATERIALS AND METHODS

Plant Materials

Two tomato near isogenic lines, GCR26 which is susceptible to ToMV and GCR236 in which has a common genetic background of GCR26 but carries *Tm-2* and *nv* genes (Smith and Ritchie 1983).

F2 population was developed from those NILs at Molecular Genetics Laboratory, Research Institute for Bioresources, Okayama University, Japan, from October 1997 to October 1998.

Cloning and sequencing of the RAPD fragments

Total DNA was extracted from approximately 0.1 g of fresh leaf tissue of 40 days old plants by the method of Murray and Thompson (1980) with slight modifications. The tissue was frozen in liquid nitrogen and pulverized with plastic pipette tip in a 1.5 Eppendorf tube and 400 µ of extraction buffer [100 mM- Tris-HCl pH 8.0, 0.35 M sorbitol, 5 mM EDTA and 0.1% (v/v) 2-mercaptoethol] was added to each of the powdered samples. The samples were centrifuged for 15 min at 4000 rpm in microcentrifuge. The pellet was resuspended 200 µl of extraction buffer containing 1.0% (w.v) CTAB (hexadecyltrime-thylammonium bromide), 1 M NaCl, 25 mM EDTA and 1.0% (w/v) lauroylsarcosine at final concentrations, and incubated at 60%C for 20 min. The lysate was extracted once with chloroform/isoamylalcohol and the aqueous phase was mixed with an equal volume of 2-isopropanol to precipitate DNA. The pelle twas washed with 70% ethanol and dissolved in the appropriate TE-Rnase.

PCR-ampification of DNA fragments and separation of the PCR products to detect polymorphisms 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 100 ml each of dATP, dCTP, dGTP and dTTP, 0.5 pmol of an oligonucleotide primer, 10 ng of DNA, and 0.5 units of Taq DNA polymerase (Perkin Elmer Cetus). One drop of pure mineral oil was overlaid onto the reaction mixture prior to the ampification. DNA was amplified in a Perkin Elmer Cetus DNA Thermal Cycler 480 for 45 cycles of 1 min at 94° for denaturing, 1 min at 37°C for annealing, and 2 min at 72° for primer extension. Ampification products were resolved by electrophoresis in 1.4% (w/v) agarose gels and stained with ethidium bromide.

The RAPD band specific to GCR236 was excised from agarose gels, and purified using a Sephaglas™ Band Perp kit (Pharmacia). Purified DNA was inserted into *Sma* I site of Bluescript II SK vector (Stratagene). Sequences of the fragment determined by an automated DNA sequencer (377-18 Sequencer, Applied Boisystems).

SCAR analysis

A pair SCAR primer was designed to have sequences identical to the 5'-endmost 24 bases of the cloned RAPD amplified fragment. PCR reaction for SCAR primers was carried out in 25 µl reaction containing 2 U of AmpliTaq DNA polymerase (Perkin Elmer), 1Xbuffer, 1.5 mM $MgCL_2$, 100mM of each DTP, 0.4 pM of each primer and 100 ng of DNA template. The PCR procedure consisted 2 min at 94°C of pre-PCR, 25 cycles of 30 seconds at 94°C, 15 min at 65°C and 1 min at 72°C, followed by one cycle of 7 min at 72°C. Amplified products were resolved by electrophoresis in a 1.2 % agarose gel.

RESULTS AND DISCUSSION

Results

By comparing amplification products from NILs GCR26 (+/+) and GCR236 (*Tm-2/Tm-2; nv/nv*), using random primer OPG 10 (Operon), it has found that a fragment of 800 bp lengths was specific to GCR236 (data on shown). The fragment was cloned and sequenced. The ten terminal bases of the fragment were exactly matched to the OPG10 primer sequences. Based on the 5'-endomst 24 bases of the sequences, a pair of primer with 24 bp of size was designate. The nucleotide sequences of primers used for SCAR locus are (sequence of corresponding RAPD marker is represented in italic):

SCG10800M :
*AGGGCCGTC*TACGTGTGTATGCTT

SCG10800R :
*AGGGCCGTC*TTGCACATTTGTCAT

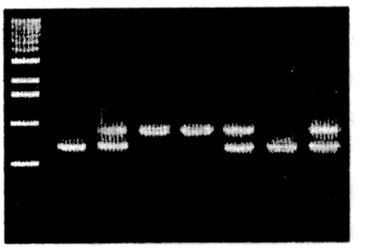

Figure . Ethidium-bromide-stained electrophoretic pattern of co-dominant SCAR marker SCG10800 linked to *Tm-2* and *nv loci in tomato.* P1 (GCR26, +/+), F1 *(Tm-2/+)*, P2 (GCR236, *Tm-2/Tm-2*), R (F2, *Tm-2/Tm-2*), H (F2, *Tm-2/+*), S (F2, +/+) and M (1 kb DNA ladder).

PCR amplification of DNAs from GCR26 and GCR236 with a pair primer of above, produce single band of different size between the NILs was presented in Figure 1. Amplified band from GCR236 (*Tm-2/Tm-2*) was matched to the size those of RAPD fragment specific to GCR236 (data not shown). However, the SCAR primer also amplified a fragment of different size in GCR26 (+/+). These results were implied that the SCAR primer could be used as co-dominant marker to differentiate either of recessive genotype and dominant genotype.

The pair of primers was also used to amplify the DNAs from F1 cross GCR26xGCR236, and found to be produced fragments of same size to both produced from DNAs from GCR26 and GCR236. Analysis of PCR amplification of SCAR marker SCG10800 to DNAs from F2 segregation populations revealed that the pair of primer amplifies single band of different size in homozygous genotypes and two bands of same size to each parents in heterozygous genotypes (Figure 1).

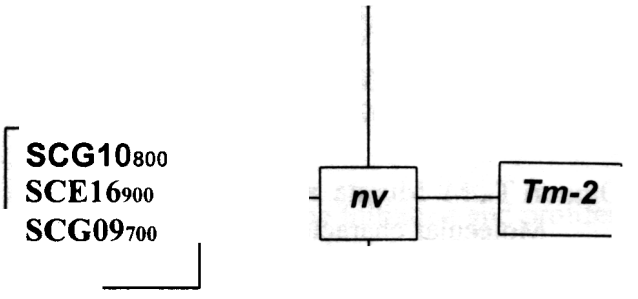

Figure 2 A lingkage map of SCG10800 SCAR marker to the *nv* locus, *Tm-2* locus and two dominant SCAR markers, SCE16900 and SCG09700

For further comfirmation that the SCG10800 fragment was originated from the introgressed region surrounding the *Tm-2* locus, a lingkage analysis was employed to the F_2 population consisting of 108 plants derived from a cross between GCR236 (*Tm-2/Tm-2, nv/nv*) and GCR26 (+/+, +/+). The fragment coresponding to the SCG10800 SCAR marker was proved to be tightly linked to the *nv* locus and the two dominant SCAR markers $SCE16_{900}$ and $SCG09_{700}$ (Sobir et al., 2000), suggesting that the locus corresponding to the SCG10800 SCAR markar is resides in the introgessed region surrounding the *Tm-2* locus (Figure 2).

Discussion

The capability of SCG10800 SCAR marker to generate single fragment size from NILs GCR26 and GCR236 was showed that the SCAR marker be able to differentianting homozygous genotype as well as heterozygous genotype. Hence the SCG10800 SCAR marker is the first PCR based co-dominant marker links to *Tm-2* locus, so fair. The DNA co-dominant marker links to *Tm-2* locus is advantageous to distinguishing the homozygous resistant genotype in breeding program for ToMV-resistant varieties, since the homozygous *Tm-2* (*Tm-2/Tm-2*) is confers stronger resistance than the heterozygous *Tm-2* (*Tm-2/+*).

PCR based SCAR marker have advantageous to RFLP markers, because the SCAR analysis is easy to generate, no blotting required and no need high amount of genomic DNAs. More over SCAR marker also advantageous RAPD marker, because SCAR primers longer than RAPD primers, therefore highly stringent annealing temperature can be employed and SCAR primers also amplify single easily scored band. The availability of co-dominan $SCAG10_{800}$ SCAR marker links to *Tm-2* locus is prove a convenient DNA marker to facilitating ToMV resistant varieties breeding in tomato.

Availability of DNA markers tightly linked to target locus is undoubtedly very important for plant breeding, since DNA markers have advantageous in reliability over visible and isoxymes markers. Their characters are not affected by environmental changes and detectable in various organs in any stage of growth. DNA markers affer advantages for breeding, not only for monogenic traits but for quantitative traits in selecting of desirable loci from the donor parent, maintaining useful loci in the recurrent parent, and shorting the introgressed region reduce undesirable traits from donor parent near. In addition to these advantages, the number of backcross generation to breed a pure line can be reduced by the use of co-dominant DNA markers.

ACKNOWLEDGEMENTS

The author wishes to express his great appreciations to Prof. F. Motoyoshi and Prof. M. Murata of Research Institute for Bioresources, Okayama University, for providing plant materials and facilities used in the experiment.

REFFERENCES

Broadbent L.H. 1964. The epidemiology of tomato mosaic. VII. The effect of TMV on tomato fruit yield and quality under glass. Ann Appl Biol. 52: 233-241.

Clayberg C.D. 1960. Relative resistance of *Tm-1* and *Tm-2* to tobacco mosaic virus. TGC Rep. 10: 13-14.

Frazier W., A K Kikuta ., J.S. Mc Farlane and J.W. Hendrix. 1946. Tomato improvement in Hawaii. Proc Am Soc Sci. 47: 277-284.

Gu K.W., N.F. Weeden , Yu J., and D.H. Wallace. 1995. Large scale, cost-effective screening of PCR products in marker-assisted selection applications. Theor Appl Genet. 91: 465-470.

Kawchuk L.M., J. Hachey , and D.R. Lynch. 1998. Development of sequence characterized DNA markers linked to a dominant Verticillium wilt resistance gene in tomato. Genome. 41: 91-95.

Murray M. and W.F. Thompson. 1980. Rapid isolation of high-molecular-weight plant DNA. Nucleic Acid Res. 8: 4321-4325.

Ohmori T., M. Murata and F. Motoyoshi 1995. Identification of RAPD markers linked to the *Tm-2* locus in tomato. Theor Appl Genet. 90: 307-311.

Ohmori T., M. Murata and F. Motoyoshi 1996. Molecular characterization of RAPD and SCAR markers linked to the *Tm-l* locus in tomato. Theor Appl Genet. 92: 151-156.

Paran I. and R. Michelmore. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet. 85: 985-993.

Pelham J. 1966. resistance in tomato to tobacco mosaic virus. Euphytica. 15: 258-267.

Smith J.M.W. and D.B. Ritchie. 1983. Acollection of near isogenic lines of tomato : research tool of the future? Plant Mol Biol Rep. 1: 41-45.

Sobir, T. Ohmori , M. Murata and F. Motoyoshi. 2000. Molecular characterization of the SCAR markers tightly linked to the *Tm-2* locus. Theor Appl Genet (in press).