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Absence of DNA Polymorphisms in *Myzus persicae* (Homoptera: Aphididae) in Relation to their Host Plants

기주식물 종류에 따른 복숭아혹진딧물(*Myzus persicae*)의 DNA Polymorphism 비교

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ABSTRACT DNA polymorphisms were analyzed for 8 clones of the green peach aphid, *Myzus persicae* Sulzer, by random amplified polymorphic DNA-polymerase chain reaction(RAPD-PCR). The insect has different host preferences and was even classified into two different species, *M. persicae* Sulzer and *Myzus nicotinae* Blackman by their morphological characters, but this point is still in arguement. To identify the differences between two types of the green peach aphid by RAPD-PCR, the template DNA was extracted from 4 clones each of tobacco-feeding and non-tobacco-feeding forms and one hundred primers of 10-nucleotides-long were tested in PCR. The amplified DNAs were analyzed by agarose gel electrophoresis. Eighty-three primers gave amplified DNA fragments with 1 to 22 in number and 500 to 20,000 base pairs in length, but no amplification was observed in the other 17 primers. The average number of fragment per each amplification was about 13. In the case of 82 out of 83 random primers, band patterns of amplified DNA were identical among 8 clones, even though some differences were noticed in the intensity of specific bands. Polymorphism was detected by only one primer within the tobacco-feeding forms, but not between the two host types. The results did not detect any relationship between RAPD polymorphism and their host preference.

KEY WORDS *Myzus persicae, Myzus nicotinae*, host preference, RAPD-PCR, DNA polymorphism

초 록 복숭아혹진딧물(*Myzus persicae* Sulzer)은 두가지 서로 다른 기주선호성을 가지는데 이 기주선호성과 형태적 특징에 기초하여 담배진딧물(*Myzus nicotinae* Blackman)과 담배 이외의 다른 채소류에 서식하는 복숭아혹진딧물(*M. persicae*)로 분류하였지만(Blachmean, 1987) 이 분류 방법에 동의하지 않는 학자들도 많다. 이런 이유로 RAPD-PCR 기법을 이용하여 한국에 서식하는 복숭아혹진딧물에 대하여 그들의 2차 숙주선호성에 따른 DNA의 변이 정도를 살펴보았다. 실험곤충으로는 담배와 배추에서 채집하여 사육한 진딧물 각 4 clones 씩을 사용하였다. 각 clone은 한 개체를 사육하여 얻은 자손들과 그들의 후손으로 이루어 졌으며, 사육한 진딧물에서 핵 DNA를 추출하고, 10개 nucleotide 길이의 random primer 100가지를 사용하여 PCR한 후 1% agarose gel 전기영동법으로 분석하였다. 사용한 100종류의 random primer 중 83가지에서 DNA 단편이 합성되었다. 증폭된 1개의 primer당 단편의 수는 1개에서 22개였고 평균 단편 수는 약 13개였으며, 각각 단편의 길이는 500에서 20,000 base pair사이에 분포하였다. 82가지 primer의 경우에 일부 단편의 짙기에는 차이가 있었으나 단편종류의 분포는 동일하게 나타났다. 한가지 primer경우에만 담배섭식형 1개 clone에서 다른 7가지 clones에 없는 band가 1개 나타났다. 이때 나머지 7 clones의 단편 분포 형태는 모두 동일하였다. 따라서 이 band는 숙주 선호성과는 무관한 것으로 보인다. 결국 이 실험에 사용한 100종류의 primer에 기초하여 RAPD-PCR기법으로 DNA를 증폭한 결과 복숭아혹진딧물의 숙주선호성이 개체군간의 유전적인 차이점에 기인한다는 가설을 뒷받침할 만한 증거를 찾지 못하였다.

검색어 *Myzus persicae, Myzus.nicotinae*, 숙주선호성, RAPD-PCR, DNA polymorphism

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The green peach aphid, *Myzus persicae* Sulzer, is an important, host-alternating vector which is able to transmit over 100 plant virus diseases to many different plant species including many major crops such as beans, sugar beet, sugar cane, brassica, potato and tobacco. It uses the peach tree, *Prunus persicae*, as its primary host plant and has an exceedingly wide range of secondary host plants, including over 40 different plant families (Dixon 1987). Several hundred host species over 22 different plant families have been known in Korea (Paik 1972). Most aphids, even among the heteroecious species, show a very high degree of host specificity. In this sense, *M. persicae* is a very rare case (Dixon 1987).

There are many biotypes in *M. persicae* based on the differences in their morphology (Eastop and Russell 1967), color (Takada 1979b), biology (Sylverster 1954, Blackman 1972 1974), ability to transmit virus (Tamaki *et al.* 1982a), resistance to insecticide (Takada 1979a, Bunting and Van Emden 1980), reaction to photoperiod (Blackman 1971), reaction to photoperiod and/ or temperature (Whalon and Smilowitz 1979, Takada 1982a 1982b, Tamaki *et al.* 1982b), and host preference (Annis *et al.* 1982).

Tobacco-feeding form and non-tobacco-feeding form were even classified into two separate species, *Myzus nicotinae* Blackman and *M. persicae* Sulzer (Blackman 1987). Morphological studies of numerous samples from four continents showed that samples from tobacco can be distinguished by canonical variates analysis. These two taxa were believed to be genetically isolated from each other because *M. nicotinae* was thought to be anholocyclic, therefore, lacking the ability to produce sexual morphs and interbreed with *M. persicae*. It appears that the morphological homogeneity of anholocyclic strains implies that their reproduction may be continuously parthenogenetic. Blackman (1987) also noticed that the situation is different in Japan, central Asia and Southern Kazakhstan; populations of *M. persicae* group aphids on tobacco are holocyclic and the samples on tobacco from these area did not conform with *M. nicotinae*. However, anholocyclic clones were reported in Japan (Ueda and Takada 1977, Tamaki *et al.* 1982a 1982b). In addition, different results or situations were reported in some other areas. Therefore, many taxonomists do not agree with this classification scheme. Previous surveys of allozyme variability also revealed little or no genetic variability among or within biotypes or individuals (Brookes and Loxdale 1987, Mark and Smilowitz 1980, May and Holbrook 1978, Suomalainen *et al.* 1980). In the case of an analysis of soluble enzymes to estimate heterozygosity in *M. persicae* on tomato, potato and tobacco from 11 different locations in the US, there was neither genetic variability within a population nor among populations (May and Holbrook 1980). The taxonomic status of *M. persicae* populations on tobacco is not resolved clearly.

That is why this experiment was carried out with random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. RAPD-PCR is a recently developed technique (Williams *et al.* 1990, Welsh and McClelland 1990) as a simple, rapid and sensitive mean for studying individual identity, family relationship, population genetics, genetic mapping, molecular taxonomy, differentiation of cryptic species and so on. RAPD-PCR is useful for rapid identification of genetic polymorphisms in aphids (Black *et al.* 1992).

MATERIALS AND METHODS

Experimental insect

The tobacco-feeding form of green peach aphid larvae and adults were collected from a green house (clone No. 1 & 2) in January and a tobacco field (clone No. 3 & 4) in June in Suwon, 1995. The non-tobacco-feeding forms were collected from Chinese cabbage fields in Suwon (clone Nos. 5 & 6) and Daejon (clone Nos. 7 & 8) in August, 1995.

Clonal-cultures were established starting from a single apterous virginopara, and the progenies were born by parthenogenic reproduction. All the clones were maintained on radish (leaf). Aphids were grown in acryl cages in an insectary at 16L: 8D photoperiod, 26±1°C and R.H. 60~80%. The experimental insects

comprise 4 clones for each form.

Total DNA isolation

Template DNA isolation procedure was modified slightly from that described by Wilkerson *et al.* (1993). Five adults were ground with a glass pestle in an 1.5 ml microcentrifuge tube with 100 μl of an extraction buffer [100 mM Tris-HCl, pH 8.0; 100 mM EDTA; 100 mM NaCl; 200 μg/ml proteinase K; 0.5% SDS; 1% Nonidet P-40]. After incubation at 55°C for 4 hours, the extract was added with RNase A to a final concentration of 100 μg/ml and incubated at room temperature for 30 min. The solution was extracted once with an equal volume of saturated phenol by heating to 55°C for 10 min with periodic mixings. After centrifugation (at 12,000 rpm for 3 min) in a microcentrifuge, the supernatant was extracted with phenol/chloroform/isoamyl alcohol (25: 24: 1) and then with chloroform/isoamyl alcohol (24: 1) as above. The supernatant was collected, 2 volumes of absolute ethanol were added to it, and the solution was stored at -20°C for 30 min to precipitate the DNA. The DNA was pelleted by centrifugation (at 12,000 rpm for 30 min), washed with 500 μl of 70% ethanol and dried at room temperature. The DNA pellet was dissolved in 100 μl of TE buffer/mg of sample weight [TE buffer : 10 mM Tris-HCl, pH 8.0; 1 mM EDTA].

Random primer

One hundred different 10-nucleotides-long primers (Table 1) were used (purchased from Nucleic Acid-Protein Serve Unit, the Biotechnology Laboratory at University of British Columbia).

The nucleotide sequence of each primer is generated randomly. The mean % G+C content for the primers is 60.3% with a range of 50~80%.

Polymerase chain reaction

PCR was carried out by the procedure of Bowditch *et al.* (1993); 100 μl with 1 μl of template DNA solution, 50 ng of each primer, 2.5 units of *Taq* DNA polymerase (Boehringer Manheim) and a reaction buffer [10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM

Table 1. Oligonucleotide sequences and number of amplified DNA fragments for each random primer. Primer numbers are taken from the manufacturer (Nucleic Acid-Protein Serve Unit, the Biotechnology Laboratory at University of British Columbia)

primer number	sequence (5' to 3')	GC content (%)	No. of amplified DNA fragments
501	CGG ATA TAC C	50	0
502	GCA TGG TAG C	60	19
503	ATC GTC CAA C	50	0
504	ACC GTG CGT C	70	13
505	CCC TTT ACA C	50	17
506	CCT TTC CCG A	60	4
507	AGA CGT ACT C	50	4
508	CGG GGC GGA A	80	11
509	ACA GAG ACT G	50	0
510	CGC ATC TCT T	50	0
511	GAA TGG TGA G	50	14
512	GGG TGG ACA T	60	17
513	TAT ACG ACC C	50	0
514	CGG TTA GAC G	60	22
515	GGG GGC CTC A	80	20
516	AGC GCC GAC G	80	17
517	GGT CGC AGC T	70	11
518	TGC TGG TCC A	60	15
519	ACC GGA CAC T	60	13
520	TGC GCA GCC C	80	0
521	CCG CCC CAC T	80	16
522	TCG TCT AGC A	50	16
523	ACA GGC AGA C	60	14
524	CGG TTA CTA G	50	0
525	GCT GGT TGG A	60	16
526	AAC GGG CAC C	70	12
527	CTT CAA CGT G	50	12
528	GGA TCT ATG C	50	17
529	CAC TCC TAC A	50	6
530	AAT AAC CGC C	50	16
531	GCT CAC TGT T	50	17
532	TTG AGA CAG G	50	21
533	GCA TCT ACG C	60	16
534	CAC CCC CTG C	80	21
535	CCA CCA ACA G	60	20
536	GCC CCT CGT C	80	18
537	CGA AAG GAC T	50	12
538	TGA CCT CTC C	60	11
539	CTT ACG TCA C	50	1
540	CGG ACC GCG T	80	16
541	GCC CCT TTA C	60	4
542	CCC ATG GCC C	80	12
543	CGC TTC GGG T	70	5
544	TAG AGA CTC C	50	7
545	ACG TTG AGA C	50	1
546	CCC GCA GAG T	70	9

Table 1. (Continued)

primer number	sequence (5' to 3')	GC content (%)	No. of amplified DNA fragments
547	TAT GAC CTG G	50	0
548	GTA CAT GGG C	60	17
549	CCG GCT TAT G	60	8
550	GTC GCC TGA G	70	11
551	GGA AGT CCA C	60	12
552	CTA AAT GGC G	50	16
553	TTC GAG ATC G	50	0
554	TCA TCC AGG G	60	0
555	GTG AAC AGC A	50	10
556	ATG GAT GAC G	50	16
557	GTG TAG AGC C	60	18
558	CGA TAT CCG G	60	6
559	GAG AAC TGG C	60	21
560	CAC TGC TGT C	60	16
561	CAT AAC GAC C	50	0
562	CAA AGT AGC C	50	2
563	CGC CGC TCC T	80	16
564	CGG CGT TAC G	70	15
565	GGT CGA TTT C	50	0
566	CCA CAT GCG A	60	14
567	AGA CAC CTG A	50	12
568	ACC TGT TCT C	50	0
569	CGA ATT GCT G	50	17
570	GGC CGC TAA T	60	11
571	GCG CGG CAC T	80	16
572	TTC GAC CAT C	50	15
573	CCC TAA TCA G	50	0
574	GCC AGA CAA G	60	14
575	GGA GAT GTA C	50	14
576	CAC CTA ATG G	50	0
577	GTC TGA TGT G	50	12
578	GGT GTC CAC T	60	16
579	TGG AAT CGT G	50	13
580	GCG ATA GTC C	60	11
581	CCC GTT AAG G	60	11
582	GGT ATA GAC G	50	15
583	GTA TTT GCG C	50	17
584	GCG GGC AGG A	80	16
585	CCC GCG AGT C	80	8
586	CCG GTT CCA G	70	11
587	GCT ACT AAC C	50	0
588	CAG AGG TTG G	60	18
589	GAC GGA GGT C	70	0
590	CCG GCA TGT T	60	13
591	TCC CTC GTG G	70	3
592	GGG CGA GTG C	80	15
593	CGA GCT TTG A	50	5
594	AGG AGC TGG C	70	14
595	GTC ACC GCG C	80	13
596	CCC CTC GAA T	60	2
597	TGG TTC CCG A	60	3
598	ACG GGC GCT C	80	16
599	CAA GAA CCG C	60	7
600	GAA GAA CCG C	60	22

$MgCl_2$; 200 mM of each dNTP (dGTP, dATP, dTTP, dCTP)]. Before PCR reaction, each sample was overlaid with two drops of mineral oil to prevent evaporation of the sample.

Amplifications were carried out in a Perkin Elmer DNA Thermal Cycler TC-1 with the following cycle parameters. An initial denaturation step for 5 min at 94°C, an annealing step for 2 min at 35°C, and an elongation step for 3 min at 72°C followed by 39 cycles of 1 min at 94°C, 2 min at 35°C, 3 min at 72°C, followed by the last elongation for 10 min at 72°C.

Agarose gel electrophoresis

Mineral oil was removed from the samples obtained from PCR reaction and the samples were extracted with phenol/chloroform/isoamyl alcohol (25: 24: 1), vortexed for 3 min and centrifuged at 12,000 rpm for 3 min. The supernatant was collected and NaCl was added to a final concentration 200 mM. Two volumes of absolute ethanol were added to it and it was stored at -20°C for 30 min to be followed by centrifugation at 12, 000 rpm for 30 min. The pellet was washed with 70% ethanol, dried at room temperature and resuspended in 10 μl of TE buffer.

The samples were loaded onto the 1% agarose gel containing 0.5 μl/ml EtBr. Electrophoresis chamber contained a Tris-borate EDTA buffer [89 mM Tris-borate, 2 mM EDTA]. Electrophoresis was performed at 80 V for 4~5 hours. The results were photographed under UV light.

RESULT AND DISCUSSION

Eighty three out of 100 primers provided discrete PCR products when visualized using agarose gel elec-

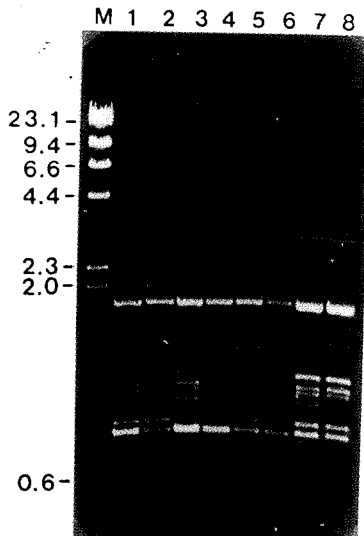

Fig. 1. RAPD profile showing the same band patterns from 8 clones with the primer # 511 (5'-GAATGGTGAG-3'). M: /Hind III size marker, lanes 1 & 2: tobacco-feeding clone No. 1 & 2 from a green house, lanes 3 & 4: tobacco-feeding clone No. 3 & 4 from field tobacco plants, lanes 5 & 6: non-tobacco-feeding clone No. 5 & 6 in Suwon, lanes 7 & 8: non-tobacco-feeding clone No. 7 & 8 in Daejon

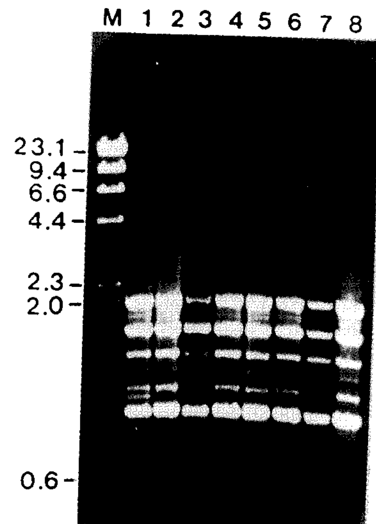

Fig. 2. RAPD polymorphic patterns with the primer # 579 (5'-TGGAATCGTG-3'). M: /Hind III size marker, lanes 1 & 2 : tobacco-feeding clone Nos. 1 & 2 from a green house, lanes 3 & 4: tobacco-feeding clone Nos. 3 & 4 from field tobacco plants, lanes 5 & 6: non-tobacco-feeding clone Nos. 5 & 6 in Suwon, lanes 7 & 8: non-tobacco-feeding clone Nos. 7 & 8 in Daejon

trophoresis (Table 1). Most of the bands typically varied in intensity and were 500 and 20,000 bp in size with a given primer and DNA template. A group of 1 to 5 intense bands appeared and a group of additional, weaker bands were also present. The number of amplified DNA fragments was 1 to 22 with the average about 13. The primer number, sequence, GC content and the number of amplified fragments were shown in Table 1.

For 82 primers, banding patterns were identical from 8 clones of aphids. Polymorphism was detected with the only one primer, No. 579; 5'-TGGAATCGTG-3' are shown in Fig. 2. Clone 1 of tobacco-feeding form showed an extra band of about 800 base pairs long fragment. In this case, however, polymorphisms was not between tobacco-feeding forms and non-tobacco-feeding forms.

The RAPD-PCR technique is useful for a rapid identification of genetic polymorphisms in aphids. Even the limited survey of individuals or primers could detect enormous amount of genetic variations (Black *et al.* 1992). The RAPD-PCR results show the feasibility in an identification of several aphid species (Cenis *et al.* 1993). The differences were obvious not only when comparing different genera but also comparing species within the genus.

There are various body colours in *M. persicae*. The body colors have been used for biotype classification as the host plants have been used for the research. But the body color was not considered in this study simply because the color pattern was not consistent even among the progenies of a clone (unpublished observation). Color variation can also be influenced by aphid age, photoperiod, temperature, host plant species and plant age. For example, there is a tendency that adults have deeper colour than larvae. Eearly instars of the green variant are yellowish-green and the adults aptera are mid-green or dark-green. The pink variant is yellowish-red in early instar and adults are rather uniformly rose pink (Blackman 1987).

M. persicae is probably originated from Asia, because its principal primary host, *Prunus persicae*, is

also from Asia (Blackman and Eastop 1984). Although tobacco is native to America, the ability of *M. persicae* to colonize tobacco is likely to have arisen in East or South-East Asia. Because the insect was reported as a tobacco pest since 1881 in this area, long before in other parts of the world (Takada and Tamura 1987). Though a tobacco-adapted genotype may have spread from eastern Asia to all over the world producing the severe outbreaks on tobacco, it is thought that the populations of each continent or area have some different characters.

To understand population genetics of the *M. persicae* group aphids, it is necessary to study further for the populations in East, South-East Asia and Central Asia compared with European and American populations.

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LIST OF ABBREVIATION

dATP : Deoxyadenosine triphosphate
dCTP : Deoxycytidine triphosphate
dGTP : Deoxyguanosine triphosphate
dTTP : Deoxythymidine triphosphate
DNA : Deoxyribonucleic acid
EDTA : Ethylendiaminetetraacetic acid
EtBr : Ethidium bromide
PCR : Polymerase chain reaction
R.H. : Relative humidity
RAPD : Random amplified polymorphic DNA
RFLP : Retriction fragment length polymorphism
SDS : Sodium dodecyl sulfate
TE : Tris-EDTA
UV : Ultra-Violet

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