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Monitoring of Genetic Variability in Dicofol-susceptible, Dicofol-resistant, and its Reverse-selected Strains of *Tetranychus urticae* by RAPD-PCR

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Abstract Genetic variability was monitored by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) in dicofol-susceptible (S), dicofol-resistant (R) and its reverse-selected (RS) strains of two-spotted spider mite, of *Tetranychus urticae*. Before the reverse-selection, RS strain, selected reversely from R strain, was 23-fold resistance ratio at LC_{50} to S strain. The resistance was started to in incline slowly to the resistance level of S strain after one year, and the resistance ratio was 4-fold in the 7 years after then. PCR-amplification of *T. urticae* DNA showed polymorphism in the amplifications with 12 primers in 100 kinds of arbitrary DNA sequences. RAPD amplification with primer OPR-12 (5'-ACAGGTGCGT-3') showed amplified bands at 1,000 base pair in the S- and RS-strain, and at 350 base pair in R-strain. The results of polymorphism are genetic variabilities derived from development and selection of resistance in each strain. The peculiarly amplified fragments were guessed to participate in dicofol resistance. From the analysis of genetic similarity, it is inferred the gene composition of S- and RS-strain is much closer than that of R-strain.

Key words: *Tetranychus urticae*, RAPD-PCR, genetic variability, resistance, polymorphism

Introduction

Two-spotted spider mite, *Tetranychus urticae* Koch, is a major pest damaging of numerous crops worldwide. Many acaricides have long been used to control the mite, however whose resistance has rapidly developed to various acaricides having different chemical structures [1]. The development of the resistance is known to be accelerated under confined environmental conditions such as greenhouse [2]. Various aspects of insecticide selection have already been described in several comprehensive studies. Since the mite has a very short life cycle and is prolific, furthermore, its resistance to acaricides has more readily emerged compared to the resistance of other pests [3]. In addition, the mite resistant to certain acaricide has revealed to have cross resistance to other acaricides [4,5]. Thus, the acaricides have often lost their activity to control the mite. However, at present the mechanisms of resistance and its inheritance to acaricides are largely unknown.

Recently, a technique of random amplified polymoirphic DNA-polymerase chain reaction (RAPD-PCR) has widely been used to assess genetic variability in a wide range of organisms including sibling species of spider mite, Tetranychidae [6-10], since its use was described initially by Welsh and McClelland and Williams et al [11,12]. PCR includes the use of specific primers that flank the region of interest and the amplification of the specific DNA fragments. Multiple DNA fragments may be produced and used as markers for genome mapping and identification of individuals, populations, or species. In this study, we used RAPD-PCR technique to investigate genetic variability and genetic relationships among the populations of dicofol-susceptible (S), dicofol-resistant (R), and reverse-selected (RS) strains of *T. urticae* which have been selected for several years in our laboratory.

Materials and Methods

Mites, three strains of *Tetranychus urticae*, S, R and RS, were used in this study. S strain has been maintained as a susceptible reference since 1986 in our laboratory. R strain has been exposed for seven years to dicofol [2,2,2,-trichloro-1, 1-bis-(4-chlorophenyl)-ethanol, 96%]. Some of R strain has not been treated with dicofol since its 60 generations and then maintained as a RS strain. All strains have been reared on green bean leaves in a growth chamber at 25℃ and 70 ± 5% RH with a photoperiod of 16 hours.

Genomic DNA was separately extracted from each strain of *Tetranychus urticae* according to the method of Jowett with minor modification [16]. The purity of the collected

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genomic DNA and its concentration were determined by measuring the A_{260} and A_{280} with a Beckman spectrophotometer. For RAPD analysis, commercially available 100 different 10-mer oligodeoxynucleotide primers were purchased from Operon Technologies, Inc. The nucleotide sequence of each primer was generated randomly. PCR was performed by the procedure of Williams et al [12]. Three-step amplifications in a Perkin Elmer DNA thermal cycler were performed as follows; an initial denaturation step for 2 min at 94℃ followed by 45 cycles of 15 sec at 94℃, 30 sec at 37℃, and 1 min at 60℃, and followed by last elongation for 5 min at 60℃. PCR products were separated on a 2% agarose gel containing 0.5 μl/ml ethidium bromide. Genetic variability among the *Tetranychus urticae* strains was analysed using a NTSYS-PC program. Dendrogram was made based on the RAPD data.

Results and Discussion

Figure 1 shows the changes in susceptibility of S, R and RS strains of *Tetranychus urticae* to dicofol. LC_{50} of S strain was sustained definitely (28-30 ppm). R strain was supported to be high resistant. LC_{50} of R strain picked up to 1,200 ppm and maintained at 1,000 ppm. Its resistance ratio at LC_{50} was 35-fold to S strain. Before reverse-selection, RS strain showed the 23-fold resistance at LC_{50} to S strain. The resistance of RS strain was started to incline slowly. In seven years ofter then, it inclined almost to the resistance of S strain (4-fold to S strain). It is because the frequency of resistance gene is not so high in a closedown environment such as laboratory and the adaptation of resistant strain is weaker than that of susceptible strain [13,14].

We intended to elucidate genetic variability derived from development and selection of resistance. PCR amplification was carried out in genomic DNA of each strain (showed in Fig. 1). In the case of 9 kinds of primers, the same banding patterns were obtained in S, R and RS strains. And 12 kinds

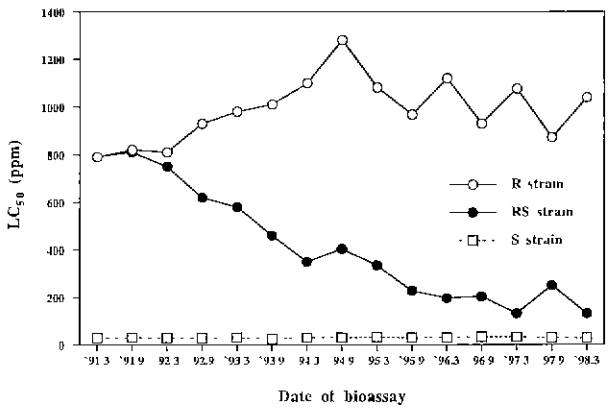

Fig. 1. Changes in susceptibility of S, R and RS strains of *Tetranychus urticae* to dicofol.

of primers showed polymorphic band patterns. It is natural that the same band patterns should be amplified, because R strain is originated from S and RS strain is also derived from R strain. The polymorphic band patterns were shown in Fig. 2. Amplification with OPR-12 (5'-ACAGGTGCGT-3') showed bands at 1.5 kbp in S and RS strain, and at 0.85 kbp in R strain. These polymorphic bands imply the difference of DNA sequence. They are genetic variabilities derived from development and selection of resistance to dicofol in each strain. Especially, it is guessed that amplified band (at 0.35 kbp) with OPR-12 only in R strain is the gene to participate in dicofol resistance.

Table is nucleotide sequences showing polymorphism in S, R and RS strains. GC (guanidine+cytocine) contents are normally 60-70%. They amplified about 2-5 bands peculiarly between 100 and 2,642 bp. It is reported that amplified degree of random primer for RAPD-PCR is influenced considerably by GC contents [15]. In this study, as GC contents of primers increased, it showed better amplified degree.

The 25 DNA markers were acquired form PCR products amplified with 12 random primers showing polymorphism.

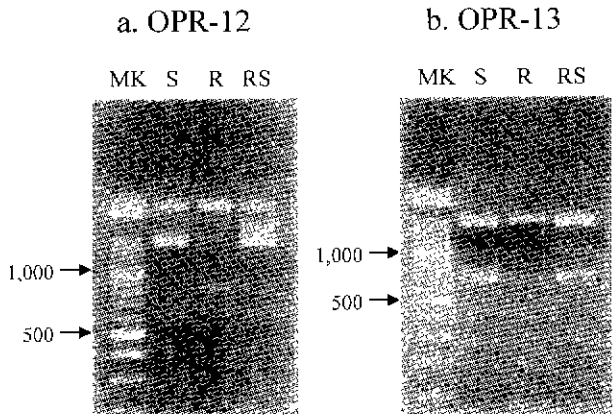

Fig. 2. Representative RAPD amplifications showing polymorphism. Panel a, OPR-12 (5'-ACAGGTGCGT-3'); panel b, OPR-13 (5'-GGACGACAAG-3').

Table Nucleotide sequences showing polymorphism in S, R, and RS strains of *Tetranychus urticae*

Primer	Sequence (5'→3')	GC content (%)	Amplified fragments
N-05	GAGACGCACA	60	2
N-07	CAGCCCAGAG	70	4
N-11	TCGCCGCAAA	60	3
N-12	CACAGACACC	60	2
N-6	AAGCGACCTG	60	2
N-18	GGTGAGGTCA	60	4
R-02	CACAGCTGCC	70	3
R-08	CCCGTTGCCT	70	3
R-12	ACAGGTGCGT	70	3
R-13	GGACGACAAG	60	5

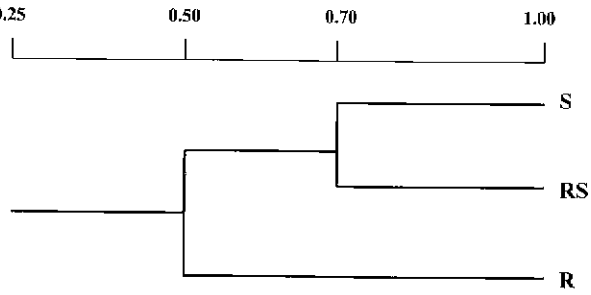

Fig. 3. Genetic relationships among S, R and RS strains of *Tetranychus urticae*. The dendrogram was based on the RAPD data.

We coded these markers following, if the bands exist, it is coded as "1" ; if the bands isn't, it is coded as "0" (data not shown). We analyzed these markers with a NTSYS-PC program and derived the dendrogram, the resulting is shown in Fig. 3. Genetic similarity coefficient was 0.5 between S and R strain, 0.5 between RS and R, and 0.7 between S and RS strain, respectively. We may infer that the gene composition of S and RS strain is much nearer than that of R strain. These results prove the degree of susceptibility in each strain (Fig. 1). The genetic variability caused polymorphism and derived genetic coefficients in S, RS, and R strains. It is inferred that polymorphism bands may participate in the resistant mechanism. Next, we intend to clone bands amplified in only R strain and confirm it to influence the resistance.

Our results suggest that the differential susceptibility among dicofol-susceptible, dicofol-resistant and its reverse-selected strains is at least associated with genetic variability at DNA level. To understand fully dicofol-susceptible and/or dicofol-resistant of *Tetranychus urticae*, it is prerequisite step that unique DNA fragments shown in the R and/or RS strain are isolated and sequenced.

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