Genetic study on acaricide resistance in the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

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The two-spotted spider mite, *Tetranychus urticae* Koch, is an economically important pest for various agricultural crops, since it has developed resistance rapidly to newly developed acaricides. Recent issues surrounding the management of acaricide resistance in *T. urticae* has become complicated due to cross-resistance among acaricides with different mode of action and chemical structure. However, developmental mechanisms of such resistance have not been well understood.

This study firstly focused on cross-resistance between cyenopyrafen (25A: mitochondrial complex II inhibitor) and pyridaben (21: mitochondrial complex I inhibitor). Laboratory selection revealed partial cross resistance between cyenopyrafen and pyridaben. Synergism tests and crossing experiments revealed that different resistance profiles; pyridaben resistance showed partial maternal effect which is not observed in cyenopyrafen resistance. Furthermore, both cytochrome P450s and carboxyl esterases played a major role in cyenopyrafen-resistance, whereas cytochrome P450s (but the effects by carboxyl esterases were not included) and target site insensitivity caused pyridaben resistance. Therefore, the observed cross-resistance was not caused by identical detoxification system or target site insensitivity, but rather by the secondary effects of detoxification enzymes individually selected by each chemical.

Cross-resistance between acequinocyl (20B: mitochondrial complex III

inhibitor) and bifenazate (20C) has also been investigated. I performed laboratory selection with acequinocyl and characterized resistance traits by testing synergism and maternal effect, and by comparing DNA sequencing of target site (cytochrome b). Consequently, acequinocyl laboratory selection resulted in increased from 19.8 to 38.1-folds acequinocyl resistance ratio, and simultaneously, from 15.9-fold to 37.5-fold in bifenazate resistance. Moreover, acequinocyl resistance showed maternal inheritance and strong synergism by piperonyl butoxide (cytochrome P450s inhibitor). Cytochrome b sequencing detected G126S mutation, indicating target site mutation and detoxification by cytochrome P450 play an essential role in the acequinocyl resistance. In contrast, maternal effects were not clear in bifenazate resistance, suggesting that G126S is not likely a major resistance factor of bifenazate resistance in the acequinocyl selected strain. Therefore, cytochrome P450 genes selected by acequinocyl are likely to confer cross resistance against bifenazate.

The genetic basis of resistance to the three acaricidal complex II (succinate dehydrogenase; SDH) inhibitors, cyflumetofen, cyenopyrafen, and pyflubumide was also investigated. Japanese field collected strains of *T. urticae* selected with these acaricides under laboratory condition showed extremely high levels of resistance (LC_{50} values further exceeded 10,000 mg L^{-1}) than those reported in previous studies. Synergism study showed lack of significant synergism, and crossing experiment showed non-maternal inheritance in these resistance traits. These resistance profile clearly different from previous studies, which reported metabolic resistance plays major role in resistance. To further analyze resistance mechanisms, I performed genome wide QTL mapping to identify resistance

associated loci for the three complex II inhibiters.

Prior to perform QTL mapping, I constructed a microsatellite linkage map as a new experimental tool for acaricide resistance study. I newly designed primer sets for 63 loci of microsatellite markers by using *T. urticae* genome database. Then I performed crossing experiments and linkage analysis by using these newly designed markers and previously reported ones (11 loci). Consequently, I constructed a microsatellite linkage map comprising 64 markers assembled into three linkage groups (LGs).Consequently, I constructed a microsatellite linkage map comprising 64 markers assembled into three linkage groups (LGs). The total length of the map was 683.8 centimorgan (cM), with an average marker spacing of 11.03 cM. The map covers 86.6 % of the total genome sequence. Therefore, the map seemed to be useful for genome wide gene mapping for resistance associated genes in *T. urticae*.

Then, I performed QTL mapping by using the microsatellite linkage map for resistance to high levels of resistance to cyflumetofen, cyenopyrafen, and pyflubumide. As a result, I identified significant QTLs contributing to resistance to cyflumetofen (one QTL on LG1), cyenopyrafen (one QTL on LG3), and pyflubumide (two QTLs on LG1 and LG3). The QTL peaks on LG1 for cyflumetofen and pyflubumide overlapped and included the SdhB locus. For cyenopyrafen resistance, the QTLs on LG3 included the SdhC locus. For cyflumetofen resistance, I found an I260T mutation in SdhB. For pyflubumide and cyenopyrafen resistance, I detected I260V and S56L substitutions in SdhB and SdhC, respectively, by direct sequencing. Both I260 in SdhB and S56 in SdhC were present in highly conserved regions of the ubiquinone binding site formed

at the interface among SdhB, SdhC, and SdhD. Mutations at these positions have been implicated in resistance against fungicides that act as Sdh inhibitors in various pathogens. Therefore, I consider these mutations to be target-site resistance mutations for these acaricidal SDH inhibitors.

The present study provided better understanding of acaricide resistance in *T. urticae* and new genetic tool for resistance study. This will contribute to improve resistance management through future establishment of resistance monitoring using genetic diagnosis and development of new acaricide.