3-4-12. PASA-based Genotyping Techniques for the Determination of Knockdown Resistance Allele Frequencies in Field Populations of *Plutella xylostella* in Korea

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To investigate the status of pyrethroid resistance mediated by reduced neuronal sensitivity in the Korean populations of *Plutella xylostella*, we PCR-amplified and sequenced the genomic DNA fragment of sodium channel a subunit gene flanking the T929I and L1014F mutation sites, previously determined to be associated with pyrethroid resistance (Schuler et al., 1998). Sequence analysis of the genomic DNA fragments revealed the presence of the resistance-conferring two mutations in several laboratory and field populations of *P. xylostella*. In particular, the L1014F mutation, known to confer a moderate level of knockdown resistance, was found in all the samples examined, including a putative susceptible laboratory strain. Genotyping methods, PASA and bi-PASA, using individual genomic DNA samples were developed to detect the resistance-conferring mutations in a high throughput format from a number of field *P. xylostella* populations. Annealing temperature and primer concentration were determined to be the most critical factors to obtain optimized results. Genotyping by PASA and bi-PASA also confirmed that the L1014F mutation is present in all the populations examined and T929I mutation coexists along with the L1014F in many field populations. No haplotype with the single T929I mutation only, however, was found, suggesting that the resistance allele with double mutation arose by selection of a second T929I mutation from the allele containing the L1014F mutation. The high frequency of the resistance alleles of sodium channel indicates that pyrethroid resistance is wide spread in Korean populations of *P. xylostella*, demanding more intensive regulation of pyrethroid use for its control. Establishment of efficient genotyping techniques, such as PASA and bi-PASA, will greatly facilitate the development of an efficient *P. xylostella* resistance monitoring system.