

Uptake of Double-stranded RNA Molecules by Plant Insects and Mites upon their Exogenous Application on Tomato Leaves

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Abstract—Main conclusion: Exogenously applied double-stranded RNA (dsRNA) molecules on tomato leaves, moved rapidly from local to systemic leaves and were uptaken by agricultural pests viz. aphids, whiteflies, and mites. Four small interfering RNAs, deriving from the applied dsRNA, were molecularly detected in plants, aphids and mites but not in whiteflies.

Double-stranded RNA (dsRNA) acts as the elicitor molecule of the RNA silencing (RNA interference, RNAi), the endogenous and evolutionary conserved surveillance system present in all eukaryotes. DsRNAs and their subsequent degradation products, namely the small interfering RNAs (siRNAs), act in a sequence-specific manner to control gene expression. Exogenous application of dsRNAs onto plants elicits resistance against plant viruses. In the present work, exogenously applied dsRNA molecules, deriving from Zucchini yellow mosaic virus (ZYMV) HC-Pro region, onto tomato plants were detected in aphids (*Myzus persicae*), whiteflies (*Trialeurodes vaporariorum*) and mites (*Tetranychus urticae*) that were fed on treated as well as systemic tomato leaves. Furthermore, four

siRNAs, deriving from the dsRNA applied, were detected in tomato and the agricultural pests fed on treated tomato plants. More specifically, dsRNA was detected in agricultural pests at 3 and 10 dpi in dsRNA-treated leaves and at 14 dpi in systemic leaves. In addition, using stem-loop RT-PCR, siRNAs were detected in agricultural pests at 3 and 10 dpi in aphids and mites. Surprisingly, in whiteflies carrying the applied dsRNA, siRNAs were not molecularly detected. Our results showed that exogenous application of dsRNAs moves rapidly in tomato and were uptaken by agricultural pests fed on tomato. As a result, this non-transgenic method has the potential to be used to control important crop pests via RNA silencing of vital genes of the respective pests. It should be noted that dsRNA-mediated silencing of vital genes of pests has been exploited in transgenic plants with positive outcome against aphids, whiteflies and mites.

Keywords: *dsRNA, small interfering RNAs, siRNAs, Myzus persicae, Tetranychus urticae, Solanum lycopersicon, Trialeurodes vaporariorum*

References

- [1] Bass B (2000) Double-stranded RNA as a template for gene silencing. *Cell* 101:235-238.
- [2] Baulcombe D (2004) RNA silencing in plants. *Nature* 431:356-363.
- [3] Konakalla NC, Kaldis A, Berbati M, Masarapu H, Voloudakis AE (2016) Exogenous application of double-stranded RNA molecules from TMV p126 and CP genes confers resistance against TMV in tobacco. *Planta* 244:961-969.
- [4] Pitino M, Coleman AD, Maffei ME, Ridout CJ, Hogenhout SA (2011) Silencing of Aphid Genes by dsRNA Feeding from Plants. *PLoS ONE* 6:e25709.
- [5] Thakur N, Upadhyay SK, Verma PC, Chandrashekar K, Tull R, Singh PK (2014) Enhanced Whitefly Resistance in Transgenic Tobacco Plants Expressing Double Stranded RNA of *v-ATPase A* Gene. *PLoS ONE* 9:e87235.
- [6] Shen GM, Song CG, Ao YQY, Xiao YH, Zhang YJ, Pan Y, He Lin (2016) Transgenic cotton expressing *CYP392A4* double-stranded RNA decreases the reproductive ability of *Tetranychus cinnabarinus*. *Insect Science* 0:1-10.