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ORF 5 OF GRAPEVINE VIRUS A AS GENE SILENCING SUPPRESSOR AND VIRULENCE FACTOR

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Post-transcriptional gene silencing (PTGS), is a sequence-specific RNA degradation process whereby the targeted RNA is cleaved, thus loosing its function. This phenomenon, recently discovered as a defence mechanism against virus infection in plants and invertebrates, seems to be a general RNA – targeting system whose natural functions include protecting hosts from invading viral RNAs. For successful infection to occur, plant viruses but also insect and, recently, a mammalian virus, were shown to counter this host defence system by means of proteins which operate as silencing suppressors. We have recently investigated the functions of the expression product of ORF5 (protein 10K) of *Grapevine virus A* (GVA), a member of the genus *Vitivirus* associated with "rugose wood" disease of grapevine, to verify its possible role as PTGS suppressor and virulence factor. To this aim, the *Agrobacterium* coinfiltration assay was used on wild or gfp-transgenic (16c) *N. benthamiana* plants. In both systems, GVA 10K protein was able to suppress local silencing of the gfp mRNA, although it showed a weaker activity than the HC-Pro, an extensively studied viral suppressor of PTGS, used for control. RNA blot analysis showed that increased gfp fluorescence, observed in ORF5 suppressed leaves, was associated with higher levels of gfp mRNA and lower levels of gfp-specific 21-25 siRNAs, by comparison with gfp-silenced leaves. A potential activity on systemic silencing was not observed in ORF5 agroinfiltrated 16c plants. A general feature of PTGS suppressors is to enhance disease symptom severity when expressed *via* a PVX genome; a synergistic effect attributed to an enhanced suppression of RNA silencing. Both GVA ORF5 and a non-translatable version of it (ORF5mut), were ectopically expressed in young non-transgenic *N. benthamiana* plants, using a PVX vector (pP2C2S) under a duplicated promoter of the PVX CP gene. About 6 to 8 days post inoculations (dpi), veinal chlorosis that soon evolved in strong necrosis and sometimes in plant death, appeared in systemically infected leaves of PVX-ORF5 inoculated plants. By contrast, plants inoculated with PVX and PVX-ORF5mut reacted with mild vein chlorosis and systemic mottling. RNA blot analysis showed that PVX-ORF5 RNAs accumulated similarly to PVX-ORF5mut and PVX in systemically infected tissues. A further demonstration of PTGS suppression ability of GVA ORF5 came from the double infection of 16c *N. benthamiana* plants with PVX gfp and PVX–ORF5. Systemic virus-induced gene silencing (VIGS) of gfp transgene, which is normally elicited by PVX gfp after 25 dpi, was strongly delayed in plants doubly infected by PVX gfp/PVX-ORF5.