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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Studies on RNA silencing-mediated resistance to tobamovirus in melon and tobacco plants (メロン及びタバコにおけるRNAサイレンシングが関与するタバモウイルス抵抗性に関する研究)

学位論文要旨：
Dissertation Summary

RNA silencing is sequence-specific mRNA degradation that is triggered by double-stranded RNA. The dsRNA is cleaved by an endonuclease, referred to as dicer, into small interfering RNA (siRNA). The siRNA is then incorporated into the RNA-induced silencing complex (RISC) and acts as a guide to recognize complementary RNA for its degradation. The expression of siRNA is a hallmark of the activated RNA silencing process. RNA silencing can also be induced by viruses and acts as a defense mechanism against viruses. The transgenic plants in which a virus-derived sequence or endogenous gene required for virus infection is silenced can be resistant to virus infections. One of the characteristics of RNA silencing in plants is transmission of RNA silencing by grafting. Here I present two types of virus resistance through RNA silencing; the one is virus resistance by virus derived sequence and the other is virus resistance by grafting procedure.

Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus and causes serious disease in the family *Cucurbitaceae*, which is a worldwide problem in cucurbitaceous crop production. For effective control of this virus using the transgenic approach, the direct repeat (DR) of the movement protein gene of CGMMV was used for transforming melon plants by *Agrobacterium tumefaciens*. PCR and Southern blot analyses of T_3 plants confirmed that they carried the transgene. Northern blot analysis with total RNA showed that transgene transcript RNA as well as siRNA was observed in all plants tested. After separate leaves or individual plants were inoculated with CGMMV, they were subjected to ELISA and RNA blot analysis using the coat protein gene probe of the virus. Compared to non-transgenic control, these plants were shown to have high virus resistance. Furthermore, siRNA from the transgene was detected. Thus, these results reveal that the transgenic lines were highly resistant to the virus through RNA silencing.

RNA silencing has been proven to be transmitted between scions and rootstocks through grafting using transgenic plants. *Arabidopsis thaliana* genes *TOM1* and *TOM3* are involved in tobamovirus multiplication. In the double null mutants of these genes, tobamovirus cannot multiply. These genes are conserved in other plants including tomato, tobacco and melon. It has been reported that RNA silencing of tobacco endogenous genes, *NtTOM1* and *NtTOM3* resulted in high resistance against several tobamoviruses. In the present study, I examined the graft transmission of RNA silencing for conferring virus resistance to non-transgenic scions of the same and different *Nicotiana* species grafted onto rootstocks in which both *NtTOM1* and *NtTOM3* were silenced. Non-transgenic *N. tabacum* (cvs. Samsun and Xanthi nc) and *N. benthamiana* were used as scions for grafting onto the rootstocks silenced with both genes. siRNA of *NtTOM1* and *NtTOM3* was detected in both the scions and the rootstocks eight weeks after grafting. The leaves were detached from the scions and inoculated with several tobamoviruses. The virus accumulation was tested by ELISA and northern blot analysis. The viruses were detected in grafted scions at extremely low levels, showing that virus resistance

was conferred. These results suggest that RNA silencing was induced in and virus resistance was conferred to the non-transgenic scions by grafting onto silenced rootstocks. The effect of low temperature on siRNA accumulation and virus resistance was not significantly different from that of normal temperature. In conclusion, the RNA silencing mediated virus resistance could be successfully obtained by using transgenic plants with DR of virus derived sequence or endogenous gene required for virus multiplication in combination with grafting. This approach for controlling virus diseases can be applied to other crops, which have seriously damaged by viruses.