Frank Schwach (2002): „Aufklärung des Resistenzmechanismus gegen Tospoviren von *Nicotiana tabacum* L. Pflanzen, transformiert mit dem Nukleokapsidproteingen des *Tomato Spotted Wilt Virus* mit und ohne Leadersequenz aus dem *Plum Pox Virus*-Genom."
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Abstract

Five transgenic tobacco-lines obtained from Bayer AG (Germany) were analyzed for their mechanisms of resistance to *Tomato spotted wilt virus* (TSWV). One of these lines contained only the wildtype-N gene of TSWV-L3 (wildtype-N-construct) under the control of the CaMV 35S-promotor, the other four harbored a modification thereof containing a 5'-leader sequence (XL-N-construct) consisting of the 5'-nontranslated region of the *Plum pox potyvirus*, a linker-sequence and the 5'-nontranslated region of the TSWV S-RNA. The translation of a part of this leader-sequence into a peptide of 23 aa, fused N-terminally to the TSWV N-Protein and resulting in a recombinant protein with a MW of 31,4 kDa (XL-N protein), was shown in western blot analysis. Immunodetection of this protein under native conditions using a polypeptide antibody specific for the leader-peptide was performed in ELISA-tests, proving the leader to be localized on the surface of the recombinant XL-N protein.

By purifying nucleocore and virions from TSWV-infected transgenic plants expressing the XL-N protein and analyzing the samples in western blots it was shown that the recombinant XL-N protein integrated into these structures like the wildtype-N-protein.

Temporal and spatial distribution of the recombinant protein was also monitored, revealing a strong dependence on plant and leaf age. The concentration of recombinant protein in leaf-material also varied considerably between the different plant-lines harboring the XL-N-construct.

The resistance of all plant lines was characterized by inoculating whole plants with three different tospoviruses: isolate TSWV-L3, from which the N-protein gene for the constructs was derived, isolate TSWV-DH37, known as highly aggressive from other experiments, and isolate SA05 belonging to the tospovirus-species *Groundnut ringspot virus* (GRSV). In contrast to the one line expressing only wildtype-N-protein, the XL-N-plants exhibited resistance not only to the homologous isolate TSWV-L3 but also to highly aggressive TSWV-isolate DH37 and the GRSV-isolate. Strong resistance to GRSV can not be due to a, highly sequence-specific, RNA-mediated mechanism and was never described before for TSWV-N-transgenic plants. The expression of the N-terminal elongated XL-N protein itself must therefor be responsible for the observed resistance-phenotypes.

Replication of TSWV in inoculated protoplasts and leaf-discs was analyzed by western blot assays using an antiserum against TSWV NSs-protein. Viral replication turned out to be slightly slower in XL-N-transgenic plants and significantly slower in the wildtype-N-transgenic plants in comparison to the non-transgenic controls. Using a 4x1 cm leaf-strip, inoculated only on one end, the viral spread

through the leaf was followed by western blot analyses of sections of these strips at different times post-inoculation. One of the XL-N-lines, the wildtype-N-line and the non-transgenic control were compared and a significantly slower spread was found in the XL-N-plant but not in the wildtype-N-plant. Viral spread was also examined in whole plants by inoculating one leaf and analyzing tissue prints of transversal sections of all leaves, longitudinal sections through the stem and the uncut root. In the XL-N-transgenic plants TSWV, as detected by an antibody against envelope protein, was most often restricted to the inoculated leaf and did not cause systemic infection. In those cases where the virus was detected outside the inoculated leaf it was almost completely restricted to the veins and could be detected in the roots as well but not in the mesophyll of non-inoculated leaves. In non-transgenic plants the infection always spread from the inoculated leaf to the phloem and ultimately to the mesophyll of all leaves. The wildtype-N-protein expressing plants also exhibited this kind of systemic spread or no spread at all, the infection was never restricted to the vascular tissue as in the XL-N-expressors.

From these data it is clear, that expression of the N-terminal modified TSWV N-protein (XL-N), in contrast to expression of unmodified N-protein, leads to inhibited transport of viral RNAs from cell to cell. Entry and exit into and from the phloem seem to be blocked very efficiently in these plants. Because of the fact that XL-N-protein was found to encapsidate TSWV-RNA a steric inhibition of the interaction between the NSm movement-protein of TSWV and the N-terminal modified N-protein seems likely to be the cause for the inhibited transport.

Although the mechanism of resistance of the XL-N-transgenic plants is clearly protein-mediated, plants expressing higher levels of the recombinant protein exhibited a weaker resistance than those with a low expression-level, especially when challenged with the highly aggressive isolate TSWV-DH37. One possible explanation for this phenomenon could be an inhibitory effect of the N-protein (wildtype-N and XL-N) on host defense-mechanisms such as virus-induced gene-silencing. High concentrations of the recombinant protein would then inhibit the host defense which could otherwise contribute to the overall resistance of the plant while low amounts of the recombinant protein would allow both effects, inhibition of movement by the XL-N-protein and host endogenous defense, to work simultaneously, thus conferring a very strong resistance. This hypothesis is supported by the observation, that the wildtype-N-transgenic plant line was not only susceptible to infection with GRSV but also developed symptoms faster and more severely than the non-transgenic-control. This effect and a possible silencing-suppressor activity of TSWV-proteins has not been described before.