N – N and N – RNA Interactions in TSWV

Gregor Bucher, Joachim Uhrig, Jana Hackbusch & Peter Schreier
Max-Planck-Institute for Breeding Research, Carl-von-Linné Weg 10, D-50829 Cologne, Germany
E-mail: Bucher@mpiz-koeln.mpg.de

Abstract: Interactions of TSWV nucleocapsid (N) protein with S RNA fragments were analyzed in Gel Electrophoretic Mobility Shift Assays (GEMSAs). The N protein was found to have a higher affinity for the 5‘ end of TSWV S RNA than for the 3‘ end, a result also observed in studies with nucleocapsid protein from Bunyamwera and influenza A. Mutant proteins with a reduced ability to multimerize shifted RNA at lower concentrations than wild-type N, which suggests a correlation between multimerization and RNA binding.

Because the multimeric state of the N protein may have a regulatory function in the TSWV life cycle, Blue Native Electrophoresis (BN-PAGE) was used to examine N protein complexes. As predicted by yeast two-hybrid interaction studies, mutants with impaired C-terminal binding sites were predominantly present as monomers and low molecular weight complexes in BN-PAGE gels, in contrast to wild-type N protein.