
RNA silencing suppressor protein of CaCV Triggers Hypersensitive Resistance Response in Capsicum; Candidate Avr Determinant

S.M.K. Widana Gamage^a and R.G. Dietzgen^b

^a*Department of Botany, Faculty of Science, University of Ruhuna, Sri Lanka*

^b*Queensland Alliance for Agriculture and Food Innovation, Centre for Horticultural Science, University of Queensland, Brisbane, Australia*

Corresponding author: shirani@bot.ruh.ac.lk

Capsicum chlorosis orthotospovirus (CaCV) is an emerging pathogen of capsicum and chili crops in Australia and South-east Asia due to lack of resistant cultivars. In Australia, breeders are trying to incorporate CaCV resistance into commercial cultivars. However, a corresponding avirulence (*Avr*) determinant has not been identified. Therefore, the present study was aimed to identify a candidate CaCV protein functioning as an *Avr* determinant. The R-*Avr* interaction initiates a cascade of hypersensitive responses (HR) leading to the accumulation of reactive oxygen species (ROS). Therefore, detection of ROS is an indication of HR. All CaCV proteins except RNA polymerase were transiently expressed in CaCV resistant and susceptible cultivars. Briefly, CaCV open reading frames (ORFs) for the proteins of NSm, Gc and Gn glycoproteins, NSs, and N were cloned into plant expression vector (pSITE II) as fusion to flag peptide using Gateway® cloning system and transformed into plant using *Agrobacterium*. Three strains of *Agrobacterium* and various optical densities were tested to determine a suitable *Agrobacterium* strain and concentration that minimally influence leaves. Recombinants and empty pSITE II vector were transformed into *A. tumefaciens* strain EHA105 and infiltrated into 4-5th leaves of 5-6 weeks old resistance and susceptible capsicum. NSs expression led to severe necrosis in the infiltrated zone of resistance plants while all other treatments including empty vector showed chlorosis. Buffer-infiltrated leaves did not show any visible response. Therefore, chlorotic phenotype was considered as a plant's general response to agroinfiltration. Further, 3,3'-diaminobenzidine (DAB) histochemical assay provided evidence for accumulation of hydrogen peroxide only in the leaves of resistant capsicum expressing NSs. These data confirmed that CaCV NSs uniquely triggers a necrotic HR-like phenotype in CaCV resistant capsicum and NSs is the most likely viral *Avr* gene candidate. This is the first report of identifying NSs functioning as the *Avr* gene in capsicum.

Keywords: Avr gene, CaCV, Hypersensitive response, Resistance