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Generation and Characterisation of Recombinant Antibody Fragments Against Non-Structural Proteins of Potato Leafroll Virus

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Abstract

Molecular biotechnology has provided powerful new measures for the control of crop disease. Crops can now be engineered to be resistant through creation of transgenic plants producing recombinant proteins, pathogen related proteins, or antisense RNAs that block pathogenesis. The principle of generating resistant plants by genetic engineering is to express a protein or nucleic acid that interferes with pathogenesis in a transgenic plant. However, this strategy bears a recombination or transcapsidation risk which can occur between the resistance-mediating transgene and an innocuous virus and so leading to increase virulence. For example viroid RNA can be encapsidated within *potato leafroll virus* (PLRV) particles. Therefore, these approaches can only cautiously be used, and alternative strategies should be employed that do not share this risk.

In contrast, antibody engineering is a novel approach to create pathogen-resistant plants, which is based on the expression of recombinant antibody fragments that inactivate pathogens and pathogen proteins. The effectiveness of antibody-based resistance is related to the antibody affinity and specificity to the target protein. Antibody-mediated resistant plants provide higher security levels and avoid the use of undesirable pesticides currently used in agriculture. PLRV, a member of the family *Luteoviridae*, is transmitted by aphids and confined to the phloem tissue of the host plant. In the course of infection, PLRV produces yellowing and leafrolling symptoms diminishing crop yield. Monoclonal antibodies (mAb) against different PLRV proteins were isolated and used for generating single-chain fragments of the variable domains (scFv). The variable heavy chain (VH) and variable light chain (VL) coding sequences were cloned by RT-PCR in a bacterial expression vector. After periplasmatic expression in *E. coli* the soluble scFv were isolated and purified via Ni-NTA affinity chromatography. The purified scFv were evaluated through Western blot and ELISA analysis demonstrating specific binding to selected recombinant viral target proteins.

The obtained scFvs were identified as promising candidates to establish resistance against PLRV upon engineering transgenic potato plants which is currently ongoing to study the relevance of the different PLRV proteins during the PLRV infection cycle.

Keywords: Potato leafroll virus (PLRV), antibody engineering, single-chain fragment of variable domain (scFv)

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