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GENETIC DIVERSITY OF THE BNYVV VIRUS BY WHOLE GENOME SEQUENCING – SOME NEW INSIGHTS

Détermination de la diversité génétique du virus BNYVV par séquençage complet du génome – conclusions nouvelles / Bestimmung der genetischen Diversität des BNYVV-Virus durch Gesamtgenomsequenzierung – neue Erkenntnisse

ABSTRACT

The knowledge of the genetic diversity of the ss. RNA virus BNYVV (Rhizomania virus), one of the major plant pathogens in sugar beet, is a prerequisite for understanding the molecular and phenotypical interactions between different viral isolates and the genetic resistance sources in sugar beet. It is now accepted that the nucleotide variation at the RNA3 ORF P25 at nucleotide position 199 to 210 (aa position 67-70) plays a crucial role on the virulence level of the isolate in relation to natural resistance sources. To identify the exact nature of this variation RT-PCR of a short fragment of the P25 ORF is generally performed followed by single read sequencing. The sequence of the four amino acids (tetrad) can be used to classify field isolates in pathogenicity groups starting from infected root extracts.

In this study a different protocol was used to reveal the nucleotide variation of all RNA's present in the virus including the specific P25 region. Amplified cDNA was obtained using the the Ovation RNA seq kit (Nugen) starting from a number of virus infected beet samples and a purified viral isolate containing different tetrads. cDNA was then sequenced using 454 sequencing (Roche) and the in-house sugar beet genome sequence was used to identify the viral reads and sugar beet reads. The reads of viral origin were then mapped onto the genbank reference sequences available for BNYVV. First results indicated clearly that RNA 2, 4 and 5 if present showed the highest range of polymorphisms at nucleotide level. The result on RNA5 is in contradiction with our findings on RNA5 using the classical RT-PCR single read sequencing approach. At the level of the RNA3 coding region a number of heterozygous base callings were identified which suggests the presence of multiple isolates in the same sample. The use of this method to describe the genetic diversity in an infected sample is further discussed.