CHARACTERIZATION OF THE INFLUENCE OF BEET SOIL-BORNE MOSAIC VIRUS (BSBMV) ON THE AGGRESSIVENESS OF BEET NECROTIC YELLOW VEIN VIRUS (BNYVV) IN SUGARBEET

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Beet necrotic yellow vein virus (BNYVV) strains with different levels of aggressiveness have spread to all sugarbeet growing areas worldwide. In the US and Europe, resistance-breaking properties of some strains causing high yield losses have been observed (Liu et al. 2005, Pferdmenges et al. 2008). Both BNYVV and *Beet soil-borne mosaic virus* (BSBMV) belong to the genus *Benyvirus* and are vectored by the plasmodiophoromycete *Polymyxa betae* and occur in mixed infections in the Red River Valley (RRV) (Bornemann and Bolton, *unpublished observations*). As the *Rz1* resistance gene has been used in most of the commercial varieties since its identification, it had to be anticipated that resistance-breaking variants would be selected. Specific mutations allow the virus to infect resistant sugarbeet genotypes (Koenig et al. 2009), but it is not known what factors influence the generation of resistance-breaking strains of the virus. Research was done in the past to identify factors that might influence the occurrence of resistance-breaking strains of BNYVV. This includes the influence of the temperature (Bornemann & Thiel 2011), the vector *Polymyxa betae* (Bornemann & Varrelmann 2011), and the sugarbeet genotype (Bornemann & Varrelmann 2013). This project focusses now on the influence of other soil-borne pathogens with BSBMV in particular. Previous studies showed an antagonistic effect ("cross protection") on BNYVV (Mahmood & Rush 1999), but this has not been confirmed in BNYVV strains commonly found in the RRV. Likewise, it is not known if mixed infections of BSBMV and BNYVV promote mutations in P25. An exchange of genome components between the two viruses may be involved in antagonism or "cross protection". An exchange of genes between the two viruses can result in new virus strains that may result in changes of virus aggressiveness or resistance- breaking BNYVV strains. Recently, Ratti et al. (2009) used virus clones to show that BNYVV can utilize genes from BSBMV, but it is not known to what extent this occurs in nature. If this does occur in viral strains found in the RRV and southern Minnesota, Rhizomania-resistance may break down.

The aim of this study was to show the influence of BSBMV on the aggressiveness of BNYVV. Furthermore, supportive information to predict future resistance durability of cultivars with the *Rz2* resistance gene was obtained. It was also analyzed whether BSBMV affects resistance durability against Rhizomania as well as the occurrence of different mutations in mixed infections with BNYVV and BSBMV using different sugarbeet genotypes. The following questions were addressed by performing different greenhouse experiments: (i) can resistance durability of Rhizomania-resistant cultivars be compromised in mixed infections with BNYVV? (*Polymyxa betae* loading), (ii) does the sugarbeet genotype have an influence on the amino acid composition of the P25 pathogenicity factor P25? (BNYVV/BSBMV competition), (iii) is the target for the *Rz2* resistance the same as for the *Rz1* resistance? (BNYVV competition), and (iv) do virus mutants accumulate in *Rz1*-resistant plants? (Characterization of resistance-breaking strains).

Materials and Methods

BSBMV-BNYVV competition experiments: Five BNYVV strains from Europe and the US with different levels of aggressiveness and one BSBMV strain from Colorado were used in combination. Two different sugarbeet genotypes (susceptible and *Rz1*) were grown in infested soil for five weeks. Roots were harvested and virus titer was determined by means of ELISA.

***P. betae* loading experiment:** The same experimental design was used as described in Bornemann & Varrelmann, 2011. Young sugarbeet seedlings were mechanically inoculated in a single or double infection with BSBMV and/or two local BNYVV strains with a different level of aggressiveness.

BNYVV competition experiments with *Rz2* resistant cultivars: Infected roots of five different BNYVV strains from the US and Europe with different levels of aggressiveness were mixed in different combinations with sterile soil. Three different sugarbeet genotypes (susceptible, *Rz2*, *Rz1+Rz2*) were planted and cultivated

under standardized greenhouse conditions for five weeks. Roots were harvested and virus titer was determined. RNA was extracted from positive samples and further analyzed by means of RT-PCR and sequencing.

Characterization of resistance-breaking BNYVV strains: In order to characterize virus strains with resistance-breaking and non-resistance breaking properties, a greenhouse experiment with different sugarbeet genotypes and two virus strains from different origins was performed. Plants were grown for five weeks and virus titer was determined by ELISA. Five rotations with back-to-back planting were planned to increase the number of virus particles in the soil.

Results and Discussion

The results of the BSBMV-BNYVV competition experiments need to be verified. Typical Rhizomania symptoms were observed during harvest.

After mechanical inoculation of young sugarbeet seedlings in order to load a virus-free vector population, symptoms of BNYVV single infected plants were observed after ten days of inoculation. Symptoms of BSBMV infected plants were observed after three weeks. Although many plants showed symptoms, the virus titer in the roots was low. The experiment has been repeated and is currently ongoing.

Susceptible treatments of BNYVV competition experiments showed almost 100% infection rate, whereas Rz2 and Rz1+Rz2 treatments showed low infection rates when using infected roots as a source of inoculum. This experiment has been repeated by using field soil to inoculate the plants.

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