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Characterisation of soils from different geographic origin containing *Beet necrotic yellow vein virus* (BNYVV) which overcomes *Rz1* resistance in sugar beet.

ABSTRACT

BNYVV, the viral cause of Rhizomania, is transmitted by the soil-borne protist *Polymyxa betae* and spread world-wide in main sugar beet growing areas. Plant resistance represents the only effective measure to control the virus. A single dominant allele (*Rz1*), mainly used in commercial cultivars as the most important major rhizomania resistance gene, mediates partial resistance to avoid economical important root yield and sugar quality loss due to viral damage. The additional single resistance gene *Rz2* derived from *Beta vulgaris* subsp. *maritima* (WB42) represents a different resistance mechanism. Both commercially used resistance genes are located on the same linkage group (3) of the sugar beet genome. In the last decade, however, several soils containing different BNYVV isolates belonging to the A- and P-type have been described, inducing severe rhizomania symptoms in the field or in greenhouse experiments on partial resistant genotypes. Increased pathogenicity is attributed to high inoculum potential, genetic variability and additional fungal soil-borne pathogens, but experimental evidence is lacking. To understand variable pathogenicity, a cross-classified resistance test with BNYVV infested soil samples collected from different locations in Europe and USA was evaluated for virus resistance in greenhouse tests. Soil samples from Italy (Rovigo, referred to as A), Germany (Groß-Gerau; B), as well as soils from Spain (Diamiel; D), USA (Imperial Valley - IV and Minnesota - MN), France (Pithiviers; P), described to be more aggressive against BNYVV-resistance and an autoclaved sand control were chosen. Soil patterns were diluted in equal parts with autoclaved sand and mixed thoroughly in plastic bags. Soils were filled in 700ml PE-pots, for good drainage conditions each pot was filled with 100ml clay granulate and topped up with 650g soil sample. All samples were randomised in a complete block design with ten repetitions per genotype x soil origin. Various soil sample containers were parted at least 30cm to avoid splashing and contamination while irrigation. Seven days old seedlings, previously sown in sterile sand, were transplanted into fully water saturated soil. Seven days after planting, the beets were sprayed with fungicides (0,2 g/l Tachigaren 70) to stem damping-off due to *Aphanomyces* spp., *Phytium* spp. and *Rhizoctonia* spp.. Growing conditions in a climate chamber were maintained at +23°C and +20°C over day and night, respectively and 16h of light.

After 12 weeks of greenhouse cultivation, sugar beets were harvested individually. Beets were carefully removed from soil, the hair roots separated from the beet body with a knife and dried on paper towels. Leaves were cut of below the hypocotyledone and discarded. Fresh beet bodies were separately weight and scored for shape and discolouration. Quantification of BNYVV content in sugar beet hair-roots was carried out using BNYVV specific antibodies (Loewe and DSMZ) in DAS-ELISA, using dilution series from a *Chenopodium quinoa* BNYVV-virus purification with quantified total protein content. This series, used for developing a standard curve, was based on 4000, 2000, 1000, 500,..., 1,95ng virus protein / ml buffer, 0 ng ml^{-1} referred to the mean of tested health controls plus three times the standard error. The absorption (405nm) of the colour reaction was always measured after one hour incubation at 37°C.

To analyse the presence of other soil-borne pathogens, that might have influenced the sugar beet growth due to secondary infection, sugar beet pieces displaying root rot symptoms were used for isolation of outgrown fungal mycelium after surface sterilisation. Total DNA extracts were subjected to PCR amplification of ribosomal DNA containing the internal transcribed spacer 4 (ITS4), the 5.8S DNA and the nuclear small subunit (NS7) and subsequent sequencing.

Sequences were used for BLAST search to identify the fungal species.

Studies on BNYVV resistance towards different BNYVV-types in naturally infested soils were performed with a single-resistance variety (*Rz1rz1*, referred to as *Rz1* genotype) and as a control a susceptible sugar beet line (*rz1rz1*).

Root weight after 12 weeks showed that Pithiviers P-type as well as A-type D, IV and MN resulted high yield losses in the *Rz1* and the susceptible genotype due to virus infection. The mean values of those four types were determined between 1.6 to 5.1 g for the *Rz1* genotype and between 0.2 to 1.1 g for the susceptible genotype, whereas B gained 28.3 and 3.3 g, A 16.3 and 6.1 g and the sand control 17.7 and 18.4 g in the *Rz1* and the susceptible genotype, respectively. Thus, the yield losses in soils with severe symptoms were significantly higher in *Rz1* and susceptible sugar beet plants (ANOVA). Obviously, the *Rz1* genotype attained higher yield in the less aggressive A and B –type than in the sterile sand control, although fertilisation for all soils was conducted equally. The determination of infective BNYVV units transmitted by *P. betae* after 12 weeks displayed higher BNYVV concentration in hair roots from beets grown in Daimiel, Imperial Valley, Minnesota and Pithiviers soils. The BNYVV content of D, IV, MN and P arose from 39.3 to 123.0 ng ml^{-1} and 21.9 to 109,9 ng ml^{-1} in the *Rz1* and the susceptible genotype, respectively. Compared to significantly lower BNYVV-contents in A (1.9 ng ml^{-1}) and B (2.5 ng ml^{-1}) in *Rz1* plants. Susceptible sugar beets displayed similar infection levels for A (84.5 ng ml^{-1} and B (63.0 ng ml^{-1}) as already described for D, IV, MN and P. As expected, the sand control did not show any virus content in each of the genotypes tested.
Additional analysis of beets showed distinct infection with Pythium as well as *Fusarium* spec. in selected soils. Increasing pathogenicity of BNYVV P-type might be related to the presence of RNA5 and those of BNYVV A-type from Imperial Valley and Daimiel could be linked to high inoculum potential, the final proof of resistance breaking BNYVV strains however requires artificial infection methods in order to exclude all other factors which might interfere with viral pathogenicity in a synergistic manner.