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Tissue print immunoassay (TPIA) can be used to describe resistance of sugar beet against *Rhizoctonia solani*

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Introduction

The cultivation of varieties resistant to root and crown rot of sugar beet (pathogen: *Rhizoctonia solani*) has been a major achievement sustaining sugar beet production in infested areas. Mechanisms of quantitative sugar beet resistance against *R. solani* have not been studied comprehensively. Here we tested whether immunological detection of *R. solani* with specific antiserum can be applied to describe fungal spread within beet root tissues beyond visibly infected areas. Thereby a differentiation of resistance sources of sugar beet breeding lines may be achieved.

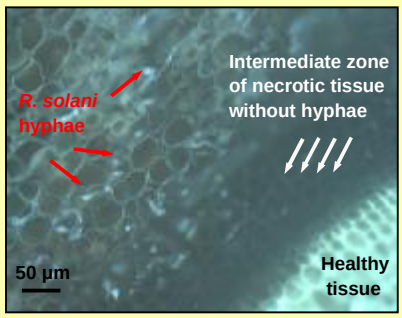

Fig. 1: Transverse section of *R. solani* infected sugar beet root (Calcofluor White)

Materials and methods

Three different *R. solani* isolates and four sugar beet breeding lines were used in greenhouse pathogenicity tests. Lines had been subject to field resistance screenings at the USDA-ARS, Fort Collins (USA) and expressed different resistance levels (L. Hanson, pers. comm.).

Eight week old plants were infested with liquid or barley inoculum of the different isolates and harvested 28 dpi. Disease severity was rated on root surface and on longitudinal cuttings used for tissue print immunoassay (TPIA) with polyclonal antibodies. PCR with specific ITS-primers was performed on necrotic and apparently healthy tissue segments. Fungal spread within infected tissue was shown by epifluorescence microscopy (Fig. 1). Differences in resistance of the sugar beet lines and interaction between sugar beet genotype and isolate were analysed.

Results

Detection of *R. solani* with TPIA was highly sensitive. The colour intensity of TPIA reflected disease severity and allowed a semi-quantitative analysis of fungus concentrations in tissues (Fig. 2). In cases of a strong TPIA signal in the centre of the tap root, no fungal mycelium nor DNA could be shown in microscope sections nor in specific PCR-analyses, respectively (Fig. 3). Disease severity after inoculation was sugar beet genotype specific with no significant interaction between genotype and isolate (Fig. 4). Disease severity was higher with barley inoculum in isolate IfZ 002 than with liquid inoculation.

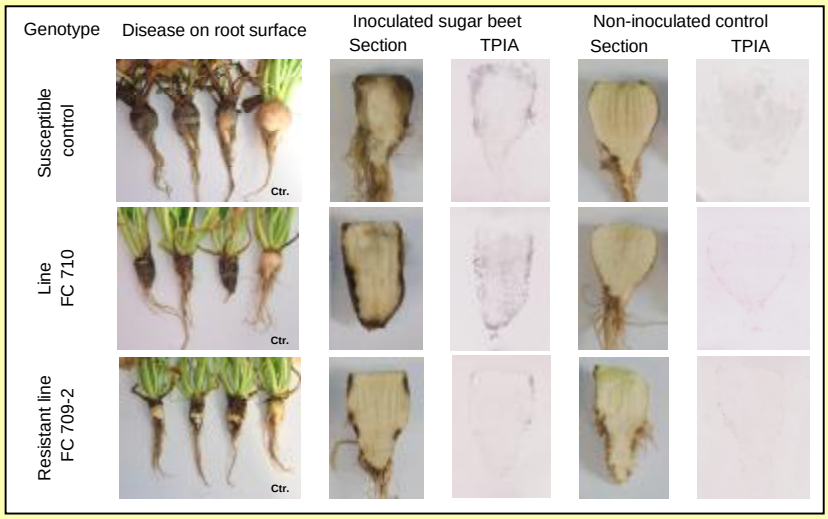

Fig. 2: Disease severity of sugar beet genotypes inoculated with *R. solani* isolates as determined on root surface, longitudinal sections and TPIA. Ctr: non-inoculated control.

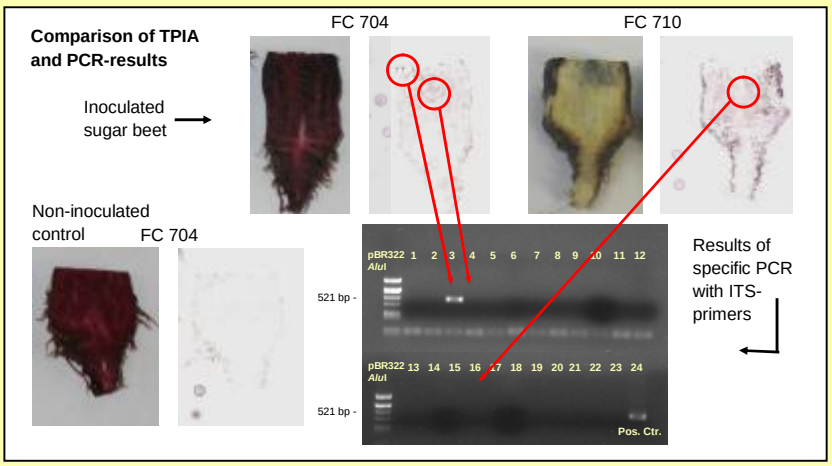

Fig. 3. Longitudinal sections of inoculated resp. control sugar beet, tissue print of same sections and results of specific PCR. Lane 24: positive control.

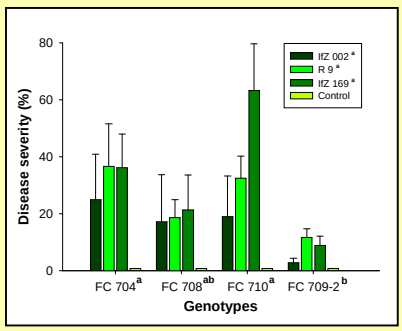

Fig. 4: Disease severity of sugar beet genotypes inoculated with *R. solani* isolates

Conclusions

The ranking of disease severity of genotypes was similar for all isolates, there was no interaction. Ranking in the greenhouse reflected rankings in field trials at the USDA, Fort Collins.

TPIA proved to be a valuable tool to describe infection progress in sugar beet tissue with *R. solani*. The intensity of the colour signal of the TPIA correlated well with disease severity.

The occurrence of a strong TPIA-signal was supported by microscopic and PCR evidence in surface tissue of beet roots, but not in central tissues. Subsequent studies are planned to verify a possible detection of soluble fungal proteins secreted into the root tissue by TPIA.

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