Transformation of *Cercospora beticola* with a Fluorescence Marker to investigate the Interaction with Sugar Beet

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Cercospora leaf spot (CLS) disease of sugar beet is caused by the fungal pathogen *Cercospora beticola*. The resistance against CLS is quantitative and based on 4-5 major genes. In the last years different QTLs were detected which are linked to Cercospora-resistance but are not further characterized regarding their impact on the formation of cellular resistance phenotypes (i.e. thickening of cell walls, plasmolysis and cell-collaps) and restriction of fungal growth in the plant tissue (Feindt, 1977; Fig. 1). In the present study *C. beticola* was transformed with a DsRed-gene from the reef coral *Discosoma sp*. encoding a red fluorescent protein using *Agrobacterium tumefaciens*-mediated transformation. No addition of substrates or cell activity is required for detection of DsRed and the observation of living infected tissue is possible. Therefore DsRed-transformed *C. beticola* isolates are a suitable tool for investigating the interaction with the host *Beta vulgaris* L. regarding the fungal growth and the formation of resistance mechanisms in the plant during the whole infection process.

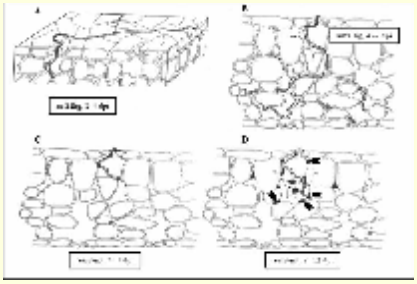

Fig. 1: Interaction of *C. beticola* and sugar beet in a resistant and a susceptible cultivar, analysed using electron microscopy (Feindt, 1977).

Material & Methods

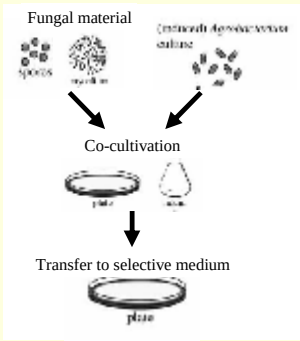

Fig. 2: Schematic overview of the *Agrobacterium tumefaciens*-mediated transformation (AMT) of fungi according to Michielse et al. (2005).

A mixture of conidia and mycelium of *C. beticola* strain Cb303b was transformed with the DsRed-plasmid pDR01 (Fig. 3) using ATM transformation. For transformation *A. tumefaciens* strain AGL-1 was used. The transformation plasmid contained the DsRed-gene as well as the *hph*-gene coding for hygromycin resistance. The co-cultivation of Cb303b and AGL-1 was conducted on induction medium containing acetosyringone as inducer for *vir* genes encoding the T-DNA transfer. Selection of transformants was conducted on V8-medium containing 70 ppm Hygromycin B. Mitotic stability was tested by subculturing the transformants on medium with and without the selection marker Hygromycin B. Pathogenicity of the transformants was revised by spraying a conidia/mycelium suspension on plants of a susceptible breeding line.

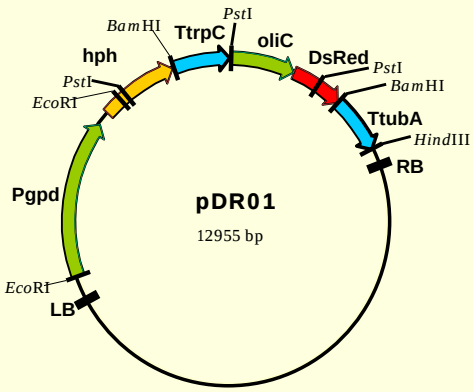

Fig. 3: Physical map of the DsRed-vector pDR01, an *Agrobacterium* binary vector constructed on the backbone of pPK2 (Covert et al., 2001) for transformation of *C. beticola*. The DsRed gene is placed under the control of the constitutive promoter *oliC* from *Aspergillus nidulans* and the *TtubA* terminator from *Botrytis cinerea*. The plasmid also contains the hygromycin gene (hph) conferring resistance to the antibiotic Hygromycin B under the control of the *A. nidulans* glyceraldehydes 3-phosphatase promoter *PgpdA* and followed by the *A. nidulans* transcriptional terminator *TtrpC*.

Results

A total of 34 transformants were isolated after transformation of *C. beticola* strain Cb303b using *A. tumefaciens* AGL-1. 23 out of 34 transformants showed mitotic stability of fluorescence and hygromycin resistance after repeated subculturing on media with and without selection pressure (Hygromycin B). Mycelium as well as conidia exhibited fluorescence after the transformation process (Fig. 4). All 23 fungal transformants maintained pathogenicity as shown after inoculation of a susceptible sugar beet breeding line with a mycelium/conidia suspension (Fig. 5).

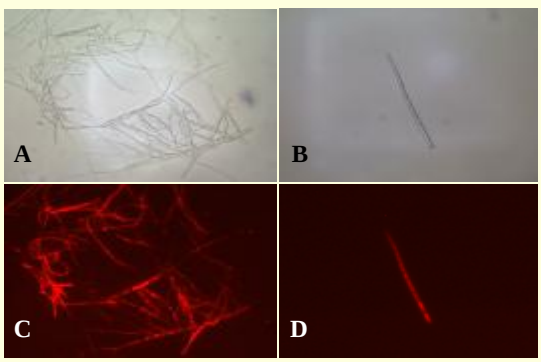

Fig. 4: Mycelium (A, C) and conidia (B, D) of a DsRed-transformed *C. beticola* isolate under bright field (A, B) and fluorescence (C, D).

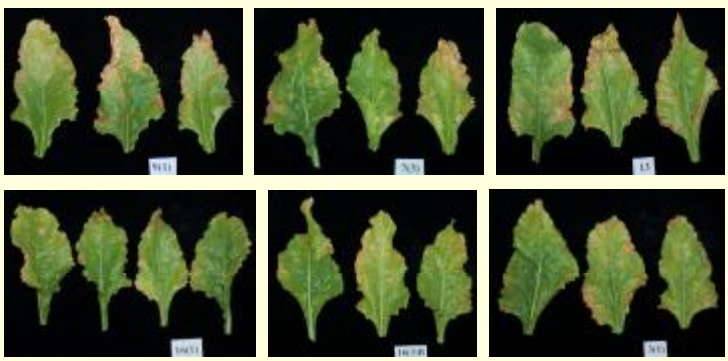

Fig. 5: CLS-symptoms on sugar beet leaves inoculated with a spore/mycelium suspension of the different DsRed-transformed *C. beticola* isolates.

Literature: Covert SF, Kapoor P, Lee M, Briley A, Nairn CJ (2001): *Agrobacterium tumefaciens*-mediated transformation of *Fusarium circinatum*. *Mycol. Res*. 105:259-264; Feindt F (1977): Untersuchungen zum Infektionsvorgang von *Cercospora beticola* Sacc. auf *Beta vulgaris* L. bei unterschiedlicher Anfälligkeit. Dissertation, Universität Göttingen; Michielse CB, Hooykas PJJ, van den Hondel CAMJJ, Ram AFJ (2005): *Agrobacterium*-mediated transformation as a tool for functional genomics in fungi. Curr. Genet. 48:1-17;

These results are part of a study supported within the BioChancePLUS programme of the BMBF