Development of resynthesized oilseed rape (*Brassica napus)* with improved resistance against *Verticillium longisporum*

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Abstract

Oilseed rape is today the most important oil crop in Europe. The importance of rapeseed oil as a renewable raw material has led to an increase in cultivation area in Germany up to 1.4 million ha in Germany in 2006, and production of up to 2 million ha is projected for the coming decades. Because of the relatively short crop rotation and the increased area under rapeseed cultivation, diseases can be a significant problem. *Verticillium* wilt of oilseed rape is caused by the host-adapted pathogen *Verticillium longisporum* and can cause yield loss of up to 50%. *Verticillium longisporum* poses a threat to Sweden, Denmark, Great Britain and in the north of Germany. Since no certified fungicides for *V. longisporum* are available this disease is expected to be one of the major problems for rapeseed production in Northern Europe in the coming decades. The aim of this study was to identify new resistance sources among *Brassica rapa* and *Brassica oleracea* accessions and introduce these into the *B. napus* gene pool via resynthesized rapeseed.

Key words: *Brassica napus*, *Brassica oleracea*, *Brassica rapa*, *Verticillium longisporum*, resistance, resynthesized rapeseed

Introduction

Verticillium is a genus of the Deuteromycotina characterized by conidiophores. The fungus survives as microsclerotia or as mycelium or conidia in the vascular system of perennial plants. Germination of microsclerotia is benefited by moist soils and a temperature range of 21-27 °C. Disease severity is influenced by inoculum density, which increases from year to year when susceptible oilseed rape varieties are continually cropped. Close-range transport of the pathogen occurs primarily by soil cultivation and movement of soil by wind or water, whereas the movement of infected plant material or seed can distribute *Verticillium* over long distances. The fungus occurs in highest concentration in the top 30 cm of the soil profile, even though it can be recovered from depths as low as 41 cm (Berlanger, I. and M.L. Powelson 2000). Chemical protection of plants against *Verticillium* wilt by fungicides is ineffective due to the survival of microsclerotia in soils, the wide host range, and the genetic and pathogenic diversity of *Verticillium* populations. Management of *V. longisporum* is difficult due to the fact that the main part of the fungus' lifecycle proceeds within the host. By development of microsclerotia in dead plant tissues the soil is sustainably infested (Figure 1).

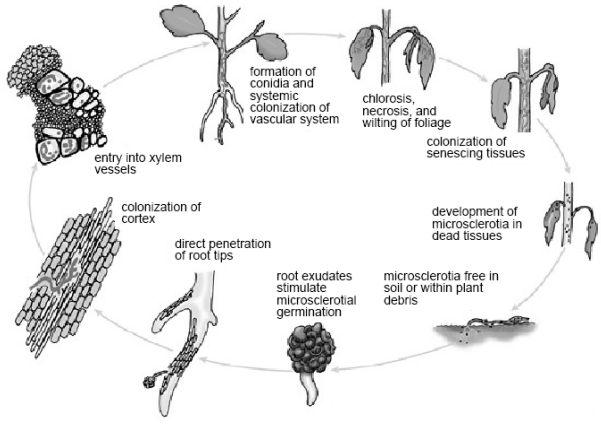

Figure 1: Disease cycle of Verticillium wilt caused by *Verticillium longisporum.* (Image from www.apsnet.org)

Breeding for resistance against *V. longisporum* is compounded by a very low degree of resistance in both winter and spring rapeseed varieties. Therefore, resynthesized rapeseed generated from interspecific hybridization between resistant forms of *Brassica rapa* and *Brassica oleracea* represents a potentially important resource to establish an enduring resistance against *V. longisporum* in oilseed rape. In this project resistance sources were detected in a resistance screening of turnip rape and cabbage genebank accessions and used to develop resynthesized oilseed rape with combined resistances from both A and C genome donors.

Materials and Methods

Resistance tests were performed using the *V. longisporum* isolates VL 43 and VL 40, which originate from *Brassica napus* grown in the North of Germany (Zeise and von Tiedemann, 2001, 2002a, 2002b). Seeds were surface-sterilized for 15 min. and were washed in sterilized tap water before being sown in double-autoclaved silica sand. Seven days later the roots of the seedlings were carefully washed out of the sand. Inoculation was performed by cutting 2 cm off the root apex and submerging the shortened roots in a mixed conidial suspension of both *V. longisporum* isolates for 30 min. Subsequently, 24 inoculated plants and 24 control plants of each accession were transferred into a sand-peat-compost (1:1:2) mixture in pots containing two plants each. Each plant was scored weekly for disease symptoms over a time period of four weeks using a nine-step assessment key slightly modified from Zeise (1992). Area under the disease progress curve (AUDPC) values were calculated from the disease severity values according to the formula of Campbell and Madden (1990). To compensate for a fluctuating infection levels between the trials a correction of the AUDPC value ($AUDPC_{corr}$) for each accession was made based on the reaction of the internal controls.

The *B. rapa* and *B. oleracea* genebank accessions were analysed concerning their response to *V. longisporum* infection. Resistant accessions were used to develop RS-rapeseed lines. For all crosses except one the *B. rapa* genotypes were emasculated and pollinated two days later with *B. oleracea* pollen. To enhance the efficiency of interspecific crossing the embryo rescue technique was used to regenerate *in vitro* haploid plants. Immature pods were harvested two weeks after pollination and surface sterilized with NaOCl. Ovules were dissected and cultivated in MS Media. Regenerated plants were transferred to soil and cuttings from the haploid hybrids were treated with colchicine in order to obtain amphidiploid *B. napus* plants that were self-pollinated for seed multiplication. From a total of ??? resynthesised *B. napus* genotypes that were able to be generated, 45 plants produced sufficient seed for phenotyping in the pathogenicity test to reveal the resistance phenotype.

Results

The tested plants of the *B. oleracea* accession showed predominantly good resistance. The resistance phenotypes of five *B. oleracea* lines that were already tested by Happstadius et al. (2003) were verified. In addition two new resistant *B. oleracea* genotypes were identified. The tested plants of both *B. oleracea* accessions showed consistently good resistance with a mean $AUDPC_{corr}$ value comparable to other cabbage accessions with resistance to *V. longisporum*. In contrary to *B. oleracea* the *B. rapa* lines showed mostly a susceptible phenotype, however two accessions showed a moderate resistance response comparable to that of some resistant *B. oleracea* genotypes. The 45 RS lines that produced enough seed for resistance testing showed a large variation in their response to *V. longisporum* infection (Figure 2). Statistical analysis of AUDPCcorr values of the RS lines confirmed that 37 RS lines (82%) had a significantly better resistance phenotype than the tolerant standard cultivar 'Express'. The seven most resistant RS lines were derived from crosses with the same pedigree, indicating that the two parental genotypes of these lines may contribute different resistance factors that interact to provide a highly effective resistance combination.

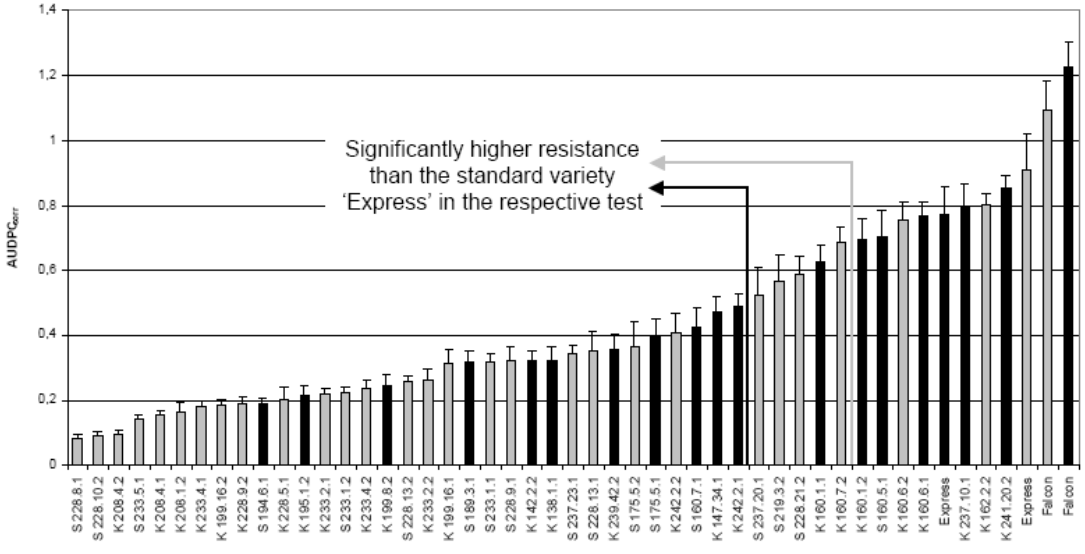

Figure 2: *Verticillium longisporum* resistance responses of 45 resynthesized rapeseed lines and the reference rapeseed cultivars 'Express' and 'Falcon' measured by area under the disease progress curve ($AUDPC_{corr}$). The black and grey bars represent two independent resistance experiments. Columns and whiskers represent mean values and standard errors from 20 infected plants of each accession.

Discussion and Conclusion

Although resynthesized rapeseed forms represent only pre-breeding material that generally exhibits unsuitable seed quality characters and poor yield, the genotypes developed in this study should provide a long-term source of genetically diverse *B. napus* for breeding of resistance against an increasingly important oilseed rape pathogen. The combination of C-genome resistance with resistance from A-genome donors can be expected to result in RS lines with a quantitative and

therefore potentially more durable polygenic resistance. The availability of a broad spectrum of genetic variation for resistance against this important pathogen will have considerable advantages for breeding towards integrated crop protection in coming decades. The potential benefits of resynthesis for rapeseed breeding demonstrated in the present study underline the importance of efforts dealing with germplasm conservation and well-directed use of *B. rapa* and *B. oleracea* genetic resources.

Furthermore, the developed material presents a promising basis for continuing research. After backcrossing with susceptible *B. napus* a mapping population will be generated for QTL analysis and marker development to support a marker-assisted backcrossing of the resistance into elite winter oilseed rape breeding material.

Acknowledgements

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