Lehr- und Forschungszentrum für Landwirtschaft
Raumberg-Gumpenstein

62. Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs 2011, 19 – 20
ISBN: 978-3-902559-74-6

Mapping and marker development for breeding of oilseed rape with resistance to *Verticillium longisporum*

Christian Obermeier[1*], Muhammed Ali Hossain[1], Rod Snowdon[1], Jessica Knüfer[2], Andreas von Tiedemann[2] and Wolfgang Friedt[1]

Abstract

Resistance to *Verticillium longisporum* in oilseed rape has been found to show a quantitative inheritance which originates from the C genome of *Brassica napus*. Tightly linked, broadly applicable markers for marker-assisted breeding of *V. longisporum* resistance have been derived from QTL and validated in oilseed rape populations with diverse genetic backgrounds. Application of the QTL derived molecular markers within genetically diverse and similar DH populations did result in reproducible and pedigree dependent marker-disease-correlations for the markers derived from the C1-minor QTL and the C5-major QTL. The markers are valuable tools for a broader application in marker-assisted selection to combine different resistance loci from cultivated and wild *B. napus* material in breeding programmes. Based on metabolic profiling and co-localization of resistance QTL with QTL for hypocotyl phenylpropanoid compounds, genes from the phenylpropanoid pathway are suggested as candidates for *V. longisporum* resistance.

Keywords

Brassica napus, marker-assisted breeding, phenolics, quantitative trait loci, resistance, *Verticillium longisporum*

Summary

The vascular fungal pathogen *Verticillium longisporum* is causing one of the most important diseases of winter oilseed rape in northern Europe. Long-term control of *V. longisporum* can only be achieved by using cultivars showing effective quantitative resistance. The present study aimed to identify quantitative trait loci (QTL) for *V. longisporum* resistance in a segregating mapping population and develop markers useful in a broader range of breeding materials for marker-assisted breeding for *V. longisporum* resistance. In addition, the study aims to identify candidate genes for *V. longisporum* resistance. This objective was targeted by identifying phenylpropanoid metabolites in the hypocotyls of a mapping population which are correlated with resistance and produce metabolic QTL that co-localize with the major resistance QTL.

Resistance to *V. longisporum* was mapped in the doubled haploid (DH) population E×R53-DH, derived from a cross between the moderately resistant parent Express 617 and the resistant line R53, which was resynthesized from a kale, *B. oleracea* var. *acephala*, and a chinese cabbage, *B. rapa* var. *pekinensis*. Two QTL were identified in the E×R53-DH population. All two QTL originate from the C genome. One major QTL was identified on chromosome C5 and one minor QTL was identified on chromosome C1, respectively, explaining together about 30% of the phenotypic variation. The favorable allele within the QTL region on chromosome C1 is derived from the parent Express 617, whereas the parent R53 contributed the favorable allele within the QTL region on C5. A major QTL on C5 has been identified before in a mapping population with a very different genetic background. Markers flanking the QTL in this former study by RYGULLA et al. (2008) were also found to flank the QTL on C5 in E×R53-DH in our study. This chromosome region therefore appears to play an important role in the expression of *V. longisporum* resistance from very different C genome genetic backgrounds.

Thus, markers from the major QTL region together with markers from the minor QTL region identified in E×R53-DH were also applied to six new DH populations produced from crosses between commercial rapeseed breeding lines and other resynthesized *B. napus* lines with genetically diverse C genome donors. Correlations of resistance reactions with selected markers were determined to evaluate their putative usefulness for marker-assisted selection. Several markers were identified that allowed differentiation between the presence of different QTL combinations on chromosomes C1 and C5 in the new DH populations. These new QTL-derived markers are promising candidates for a broad application in breeding programs using marker-assisted selection for *V. longisporum* resistance.

Histochemical studies indicate that phenolic compounds in the hypocotyl of rapeseed plants may play an important role in the inhibition of systemic spread of *V. longisporum* (EYNCK et al. 2009). To identify specific phenolic compounds involved in *V. longisporum* resistance a high performance liquid chromatography (HPLC) analysis of soluble and cell wall-bound phenolics was performed for 100 lines of the DH mapping population using a mock- and

[1] Department of Plant Breeding, Justus Liebig University, Heinrich-Buff-Ring 26-32, D-35392 GIESSEN
[2] Department for Crop Sciences, Division of Plant Pathology and Crop Protection, Georg August University, Grisebachstr. 6, D-37077 GÖTTINGEN
[*] Ansprechpartner: Christian OBERMEIER, christian.obermeier@agrar.uni-giessen.de

V. longisporum inoculated set of plants. Total soluble phenolics concentration of the hypocotyl increased up to 16-fold in single DH lines upon *V. longisporum* inoculation in the hypocotyl 28 days after inoculation. Some phenylpropanoid acids were presumable identified by co-migration with external standards resulting in identical retention times. Significant variations in concentrations of a number of presumable identified phenolic compounds in the soluble phenolics fraction including sinapic, caffeic and ferulic acid were found. These soluble phenolics were also correlated weakly ($R^2 \approx 5\%$) with resistance measured as AUDPC (area under the disease progress curve), but did not produce significant metabolic QTL. In contrast, six yet unidentified HPLC peaks were found to show a weak to medium correlation (R^2 ranging from 7% up to 37%) with resistance and resulting in QTL positions co-localizing with the minor resistance QTL on C1 and the major resistance QTL on C5. One presumably identified compound, caffeic acid, did explain about 15% of the phenotypic variation in AUDPC in the mock-inoculated data set and produced a metabolic QTL co-localizing with the minor resistance QTL on C1. This suggest that caffeic acid is involved in a pre-existing fungal resistance mechanism. Caffeic acid is one of the most common secondary compounds in plants. It is a major precursor of lignin. Also simple phenylpropanoid acids including *p*-coumaric, ferulic, caffeic, sinapic and chlorogenic acids have been reported to exhibit antifungal properties. All these compounds are known to have a widespread distribution in plants and often accumulate after fungal infection (GRAYER and HARBORNE, 1994). Further dissection and identification of metabolic compounds in the soluble, cell wall-bound and lignin fraction which are correlated with resistance and the identification of metabolic QTL overlapping with resistance QTL in the mapping population might help in the near future to identify candidate genes involved in *V. longisporum* resistance.

Acknowledgements

This work was partially supported by the Association for Promotion of Private German Plant Breeding (GFP) and the Agency for Renewable Resources (FNR) of the Federal Ministry for Nutrition, Agriculture and Consumer Protection (BMELV). The DH populations used for verification of the markers were developed by German rapeseed breeding companies affiliated with the GFP. We thank Jutta Schaper, Anja Pöltl, Bashir Hosseini, and Liane Renno for excellent technical assistance, and Benjamin Wittkop and Florin Lipsa for help in RP-HPLC analysis.

References

EYNCK C, KOOPMANN B, KARLOVSKY P, VON TIEDEMANN A, 2009: Internal resistance in winter oilseed rape inhibits systemic spread of the vascular pathogen *Verticillium longisporum*. Phytopathology 99: 802-811.

GRAYER RJ, HARBORNE JB, 1994: A survey of antifungal compounds from higher plants, 1982-1993. Phytochemistry 37: 19-42.

RYGULLA W, SNOWDON RJ, FRIEDT W, HAPPSTADIUS I, CHEUNG WY, CHEN D, 2008: Identification of quantitative trait loci for resistance against *Verticillium longisporum* in oilseed rape (*Brassica napus*). Phytopathology 98: 215-221.