Transfer of new blackleg resistances into oilseed rape

Hendrik Winter[1], Antje Diestel, Susanne Gärtig, Nicole Krone, Karina Sterenberg and Maria Dolores Sacristán

Institute of Biology - Applied Genetics, Freie Universität Berlin, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany, [1]hewinter@zedat.fu-berlin.de

ABSTRACT

Blackleg caused by *Leptosphaeria maculans* (*Phoma lingam*) is the most significant disease affecting oilseed rape (*Brassica napus*) worldwide. Wild crucifers are important alternatives to current resistance sources. Genomic *in situ* hybridisation (GISH) is a powerful tool for the detection of alien chromatin in interspecific hybrids, although possible limitations in *Brassica* and related genera have to be considered. In this study GISH was applied to backcross progenies from interspecific hybrids between *B. napus* (2n=38) and *Sinapis arvensis* (2n=18), *Coincya monensis* (2n=24) and *B. juncea* (2n=36), respectively, exhibiting resistance to blackleg. Monosomic and double monosomic additions of *C. monensis* and *S. arvensis* chromosomes, respectively, could be identified in the *B. napus* background. Furthermore, putative recombination lines (2n=38) without GISH signals were obtained in all three groups. Plants showed high cotyledon and/or adult plant resistance to two aggressive (Tox^+, PG 4) isolates, W4 from Germany and M1 from Australia, the latter overcomes the *Brassica* B genome resistance. In the lines derived from *S. arvensis* or *C. monensis* adult plant resistance is inherited more readily than cotyledon resistance. Two genes conferring adult plant resistance in the *B. napus-C. monensis* lines were identified, while cotyledon resistance in *C. monensis* is probably due to two other genes. In the case of isolate M1 the monogenic inheritance of adult plant resistance in the *B. napus-B. juncea* lines is modified by an additional epistatic gene. Mixoploidies tend to decrease in subsequent backcross generations. Meiotic and RAPD-PCR analyses are also reported for these lines.

Key words: *Brassica* – *Sinapis arvensis* – *Coincya monensis* – *Leptosphaeria maculans* – GISH

INTRODUCTION

Blackleg caused by *Leptosphaeria maculans* (anamorph *Phoma lingam*) is the most significant disease affecting oilseed rape (*Brassica napus*, 2n=38) worldwide. As an alternative to current resistance sources which are mainly derived from the French winter cultivar "Jet Neuf" and *Brassica* species with the B genome, e.g. *B. juncea* (2n=36), the importance of wild crucifers, such as *Sinapis arvensis* (2n=18) and *Coincya monensis* (syn. *Rhynchosinapis cheiranthos*, 2n=24), is expected to increase in future. These species express high resistance to various aggressive (Tox^+) isolates of the fungus, including the Australian isolate M1 which has been found to overcome the B genome resistance, until now the major source for interspecific transfer of blackleg resistance (Purwantara *et al.* 1998, Winter *et al*. 1999). Effective bioassays are essential for accurate monitoring resistance behaviour in different developmental stages. Genomic *in situ* hybridisation (GISH) is a powerful tool for the detection of alien chromatin in interspecific hybrids, although limitations in *Brassica* and related genera have to be considered.

In this study, backcross offspring from intergeneric crosses *B. napus* x *S. arvensis* and *B. napus* x *C. monensis* exhibiting blackleg resistance were subjected to GISH. These plants showed high cotyledon and/or adult plant resistance to two Tox^+ isolates of *L. maculans*, W4 from Germany and M1 from Australia. GISH was also applied to *B. napus-B. juncea* lines (2n=38) with adult plant resistance to both isolates (which was unexpected in the case of M1).

MATERIALS AND METHODS

***B. napus-S. arvensis* lines**: BC_3, BC_3S_1, BC_3S_2, BC_3S_3, BC_4, BC_4S_1, and BC_4S_2 from crosses *B. napus* cv. "Madora" (winter oilseed rape) x *S. arvensis*, backcrossed with winter oilseed rape cv. "Ceres". ***B. napus-C. monensis* lines**: BC_2, BC_3, BC_3 S_1, BC_2 S_1*, BC_2S_2*, BC_2S_3*, BC_2S_4*, and BC_2S_5* derived from a hybrid *B. napus* cv. "Loras" (spring oilseed rape) x *C. monensis.* The

asterisk (*) indicates the open pollination (selfing is likely) of a BC_2 plant from which all relevant genotypes are derived from. ***B. napus-B. juncea* lines**: Dihaploid (DH) lines from crosses *B. napus* cv. "Liropa" x *B. juncea* were made and adult plant resistant ($_R$) as well as susceptible ($_S$) plants of the following generations were analysed: BC_2S_1-DH-S_2, BC_3S_1-DH-S_3, BC_3S_1-DH-S_3; made from them: reciprocal DH-F_1 (DH_R x DH_S), F_2, BC_1, and reciprocal *B. juncea* x DH-F_1.

Two aggressive (Tox+, PG 4) *L. maculans* isolates were used for resistance tests, W4 from Germany and M1 from Australia. Plants were grown in a greenhouse and were vernalised, if needed. Crosses, embryo rescue and classical mitotic analyses were described by Plümper (1995). Cotyledon (R_C) and adult plant resistance (R_A) or susceptibility (S_C/S_A) were mainly evaluated in tests with double inoculation (combined cotyledon and adult plant inoculation) and are reported in full by Snowdon *et al*. (2000). For Genomic *in situ* hybridisation (GISH) DNA was extracted from leaves according to Rogers and Bendich (1985, CTAB method). GISH was performed as described by Snowdon *et al*. 2000, with slight modifications. In some cases, 2-5 mm long styles from young flower buds were used instead of root tips for GISH.

RESULTS AND DISCUSSION

GISH applied to the ***B. napus-S. arvensis* lines** (see Snowdon *et al*. 2000) revealed monosomic and double monosomic addition chromosomes. The BC_3S_1 progenies included fertile plants exhibiting high cotyledon and adult plant resistance associated with the presence of an acrocentric addition chromosome from *S. arvensis*. Individuals with only a metacentric addition showed the adult plant susceptible phenotype. Furthermore, other resistant genotypes were observed to have a normal *B. napus* karyotype with no visible GISH signals, indicating possible resistant introgression lines. Such putative introgressions could not be detected by GISH, probably because of its small size and/or localisation on the distal part of the chromosome arm. Introgressions seem to be likely because allosyndetic bivalent pairing has been reported for ASar, CSar and ACSar hybrids (e.g. Mizushima 1950, Kerlan *et al*. 1993).

All *S. arvensis* plants examined showed resistance to both isolates on the cotyledon and adult plant stages. *B. napus* cv. "Ceres", the backcross parent, revealed to be only moderate adult plant susceptible with some individuals scored resistant. This is due to the quantitative, partial and polygenic resistance from the "Jet Neuf" genes it carries. Therefore, severe resistance tests, like the test with double inoculation, are strongly recommanded. Resistance data from selfing progenies of double resistant plants show that cotyledon and adult plant resistance are conferred by different loci. Adult plant resistance is assumed to be oligogenically inherited (1-2 genes). Moreover, non-segregating, adult plant resistant lines were obtained.

The ***B. napus-C. monensis* lines** are derived from the highly resistant and widely sterile BC_2 genotype (mixoploid, for most cells 2n=50-54), which contains eight to twelve *C. monensis* chromosomes as revealed by GISH. Because it was not possible to obtain adult plant resistant BC_3 genotypes from this plant individuals originating from open pollinations of plant 16/1 were included in the tests and some of them showed cotyledon and/or adult plant resistance. For three of these putative BC_2S_1 (referred as BC_2S_1*) involved in GISH analysis four additional *C. monensis* chromosomes were found. Within the selfing offspring (BC_2S_2*) of one of these individuals a mixoploid plant with a main chromosome number of 2n=39 (monosomic addition) exhibiting adult plant resistance was selected. No GISH signals were obtained in resistant BC_2S_3* genotypes derived from it and in further selfing generations. Two genes (*Rlmca1*, *Rlmca2*) conferring adult plant resistance (R_A) were identified:

R_A:	**Rlmca1** _ _ _
	_ _ **Rlmca2 Rlmca2**

Among the individuals of the resistance donor, *C. monensis,* resistance and susceptibility at both developmental stages were observed. Crosses between *C. monensis* plants indicated two other genes (*Rlmcc1*, *Rlmcc2*) to be involved in cotyledon resistance (R_C):

R_C:	**Rlmcc1** _ **Rlmcc2** _
	Rlmcc1 Rlmcc1 _ _

For the *B. napus-S. arvensis* and the *B. napus-C. monensis* lines cytological analyses revealed a decrease in mixoploidies and an increase in regular meiotic behaviour in subsequent generations corresponding with a decrease of alien chromatin in the *B*. *napus* background.

In the interaction of the ***B. napus-B. juncea* lines** (putative recombination lines, 2n=38) with isolate W4 adult plant resistance is inherited as a monogenic trait. Surprisingly, when inoculated with the Australian isolate M1, certain backcross progenies showed adult plant resistance in spite of two susceptible parents, *B. napus* "Liropa" and *B. juncea*. Crosses and backcrosses with the DH lines and *B. napus* as well as with *B. juncea* gave evidence that a major resistance gene (*R*) and an epistatic gene (*E*) are involved in the expression of adult plant resistance (R_A):

R_A:	**R R e _**
	R r _ _

Epistasis in resistant *B. napus-B. juncea* lines was also pointed out by Pang and Halloran (1996). No GISH signals could be detected in either adult plant resistant or susceptible genotypes and, furthermore, in cotyledon resistant *B. napus-B. juncea* lines provided by A.M. Chèvre (INRA, Le Rheu, France). This should be due to the similar reasons as discussed above.

CONCLUSION

Wild crucifers are useful alternatives for blackleg resistance transfer into oilseed rape. GISH is of great value in the selection process from aneuploid plants of early backcross generations to euploid, putative recombinant lines. However, the method is limited by a small size of the introgression and/or a location on the distal parts of the *Brassica napus* chromosomes. Resistances from *Coincya monensis*, *Sinapis arvensis* and *B. juncea*, respectively, are mono- or oligogenically inherited. Cotyledon and adult plant resistance are conferred by different loci.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Rod Snowdon, Justus-Liebig-University Giessen/Germany, and Dr. Ulrike Bellin, Georg-August-University Göttingen/Germany, for help concerning GISH and/or meiotic examinations. We thank Dr. Bernhard Plümper for producing and analysing the original hybrids and early backcross generations with the wild crucifers, and Yvonne Bernhardt, Hannelore Lehmann, and Sabine Gillandt for technical assistance. We gratefully acknowledge the financial support from DFG, NPZ, the Professor Werner Schulze-Stiftung, and UFOP.

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