

6 Abstract

Blackleg caused by *Leptosphaeria maculans* (*Phoma lingam*) is the most significant disease affecting oilseed rape (*Brassica napus*) worldwide. Wild crucifers and related species of the *Brassicaceae* family are known to be potential resistance sources. Therefore backcross offspring from intergeneric hybrids between *B. napus* x *C. monensis* (spring material) and *B. napus* x *S. arvensis* as well as *B. napus*-*B. juncea* dihaploid (DH) putative recombination lines (both groups: winter material) were examined according to their blackleg resistance behaviour. The progression from aneuploid (in some cases sterile) individuals from early backcross generations, with a high level of alien chromatin, to fertile euploid plants with *B. napus* karyotype ($2n=38$) and *B. napus* habitus, but still exhibiting resistance to blackleg, is presented for the first two groups. This progress is shown best for the *B. napus*-*C. monensis* lines.

The plant material was examined in different environments (greenhouse, growth chamber, field) with respect to its resistance response to two aggressive (Tox^+) isolates of the fungus, namely W4 from Germany and M1 from Australia. The latter was known to overcome the *Brassica* B genome resistance. Both isolates could be classified as PG (pathogenicity group) 4 isolates according to their compatible interaction in cotyledon tests with all three differential oilseed rape cultivars.

A test with double inoculation, performed in the greenhouse, is the method of choice for accurate evaluation of plant resistance levels, both at the seedling and adult plant stages. In this test an early inoculation of the cotyledon is followed by a later inoculation at the stem base. This test strategy was found to be superior to all other adult plant examinations (adult plant test on only cotyledon inoculated plants, stem base test, field test) because it allowed easier differentiation between resistant and susceptible individuals. Because environmental variation in symptom expression is reduced the test is especially appropriate for the study of resistance genetics.

Genomic *in situ* hybridisation (GISH) is a powerful tool for the detection of alien chromatin in interspecific hybrids and backcross offspring derived from them, although possible limitations in *Brassica* and related genera - due to small sizes of introgressions and/or their location on the distal parts of the chromosome arms - have to be considered. With GISH monosomic and double monosomic additions of *C. monensis* and *S. arvensis* chromosomes, respectively, could be identified in the *B. napus* background. The presence of an acrocentric addition chromosome from *S. arvensis* was always associated with adult plant resistance. Meiotic cells subjected to GISH gave evidence for an allosyndetic pairing of this chromosome with *B. napus* chromosomes. This is in agreement with a relatively high transmission rate observed for this chromosome. Furthermore, plants with a *B. napus* karyotype ($2n=38$) and no visible GISH signals, representing putative recombination lines, were obtained in both groups. In some cases plants showed small signals, however these signals were presumed to be hybridisation artefacts rather than introgressions. The same applies to selected *B. napus*-*B. juncea* backcross offspring. In the *B. napus*-*C. monensis* and *B. napus*-*S. arvensis* lines increased cytological stability in subsequent backcross generations could be demonstrated by a decrease in both, mixoploidies and irregular meioses. These decreases correspond with a reduction of alien chromatin.

In the lines derived from *S. arvensis* or *C. monensis* adult plant resistance is inherited more readily than cotyledon resistance. These traits are conferred by different loci, which is why

cotyledon resistance/susceptibility is not a suitable indicator for adult plant resistance/susceptibility.

Two genes were identified conferring adult plant resistance in the *B. napus*-*C. monensis* lines, while cotyledon resistance in *C. monensis* is due to two other genes. Finally, *B. napus*-*C. monensis* lines were selected that displayed adult plant resistance conferred by a single major gene.

In the group with resistances from *S. arvensis* adult plant resistance is probably also inherited oligogenically. Studies of resistance genetics in these lines are influenced by the backcross parent, *B. napus* “Ceres”, which has only a moderate adult plant susceptibility. This cultivar contains genes from *B. napus* “Jet Neuf”, which possesses moderate partial polygenic adult plant resistance. On the other hand, these lines could be of special practical value because they combine the mono- or oligogenic, vertical resistance from the wild species with the polygenic horizontal *B. napus* resistance following the modern breeding concept of pyramiding resistance genes. Cotyledon resistance, with an expected mono- or oligogenic inheritance, could not be studied in detail, however a temperature dependence was detected in some of the investigated lines. Higher temperatures led to the loss of cotyledon resistance to isolate W4, whereas in the case of the Australian isolate M1 the opposite was observed.

Comparative analysis of the virulence of isolates W4 and M1 did not reveal significant differences in adult plant resistance behaviour in the *B. napus*-*C. monensis* or in the *B. napus*-*S. arvensis* lines. In the *B. napus*-*B. juncea* lines - cotyledon susceptible to both isolates - adult plant resistance to isolate W4 was found to be inherited by a dominant gene. In the case of M1 adult plant resistance was surprisingly detected in several *B. napus*-*B. juncea* lines, even though the parents in the original cross, *B. napus* “Liropa” and *B. juncea*, were both susceptible. Results from offspring from crosses between resistant and susceptible DH plants (DH-F₁) as well as from interspecific crosses between DH-F₁ plants and *B. juncea* revealed that the monogenic inheritance of adult plant resistance to M1 is modified by an additional epistatic gene.

RAPD-PCR examinations using bulk segregant analysis were applied to selfing progenies from aneuploid (one *B. napus*-*C. monensis* line) and putative recombinant plants with 2n=38 (one *B. napus*-*C. monensis* and one *B. napus*-*S. arvensis* line) to find markers for adult plant resistance. The failure of these attempts was probably caused by the low reproducibility of the RAPD technique, the wide distribution of RAPD markers in the genomes, too few primers tested and genetic reasons (in some cases more than one resistance gene was present in the lines).

Adult plant resistances from *C. monensis* and *S. arvensis* in particular are considered to be valuable alternatives to current commercial and scientific sources of blackleg resistance for rapeseed breeding (*B. napus* “Jet Neuf”, *Brassica* B genome) as indicated by the interest of breeding companies in this material. Since, in wide parts of the world, e.g. in Europe, GM crops could not be grown commercially due to political reasons, the importance of interspecific trait transfer for plant breeding is assumed to increase in the next years.