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Arabidopsis thaliana-derived resistance against *Leptosphaeria maculans* in a *Brassica napus* genomic background

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Abstract Stem canker (blackleg) caused by *Leptosphaeria maculans* is a widespread disease of *Brassica napus*. In contrast, most *Arabidopsis thaliana* accessions are highly resistant. Hence, novel material derived from symmetric and asymmetric somatic hybrids between *B. napus* and *A. thaliana* was utilised in a screen for *L. maculans* resistance. Initially, both cotyledon and adult-leaf resistance traits were transferred from *A. thaliana* to *B. napus*. In later generations the two traits segregated and cotyledon resistance was lost. The adult-leaf resistance was investigated with respect to genome localisation and protein expression. Analyses of remaining *A. thaliana* DNA in resistant plants showed co-segregation between adult-leaf resistance and chromosome-3 molecular markers. Resistant offspring from asymmetric hybrid plants that contained fragments of chromosome 3 were studied in more detail. Two regions at positions 9.8–10.4 Mbp and 18–19.5 Mbp, where several defence-related genes are located, were identified. A proteomic approach was taken to further investigate genes involved in the defence interaction. Forty eight hours after inoculation with *L. maculans*, only a few proteins, such as glycolate oxidase, were identified as differentially expressed in the resistant line compared to *B. napus*, despite the presence of additional *A. thaliana* chromosomes. The plant materials described in the present study constitute a new genetic source of *L. maculans* resistance and are currently being incorporated into *B. napus* breeding programmes.

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Introduction

Wide crosses and interspecific hybridisation are used to investigate the genetic basis of complex traits (Bernatzky et al. 1995; Bradshaw et al. 1995; Bernacchi and Tanksley 1997; Eubanks 1997). For plant breeders, hybridisation also constitutes a bridge for the transfer of valuable traits from wild species into cultivated crops (Kuckuck et al. 1991). Interspecific, intergeneric or intertribal hybrids can be produced either by sexual crosses, often in combination with embryo rescue, or by employment of somatic-hybridisation techniques (Waara and Glimelius 1995; Glimelius 1999). Brassica crops constitute good examples in this respect. In the wild germplasm of Brassica allies, extensive genetic diversity occurs and genetic enrichment of Brassica crops from wild relatives is currently one of the major approaches in crop-improvement programmes (Prakash et al. 1999).

Even if agriculturally important traits can be identified in wild relatives, molecular markers or detailed knowledge of the genomes are normally lacking. A different situation exists, however, regarding hybrids where *Arabidopsis thaliana* is one of the parents. The availability of the *A. thaliana* genome-sequence (The Arabidopsis Genome Initiative 2000) provides unique opportunities for genetic and functional genomic analysis. Several interspecific hybrids of *A. thaliana* and closely related species have been produced (Nasrallah et al. 2000), but also a few with *A. thaliana* and the more-distantly related *Brassica napus* (Bauer-Weston et al. 1993; Forsberg et al. 1994, 1998a, b). *B. napus* and *A. thaliana* belong to different tribes, the Brassiceae and Sisymbrieae, respectively (Warwick and Black 1991; Price et al. 1994), but are both members of the family *Brassicaceae*. Despite the tremendous impact that *A. thaliana* research has provided on the understanding of fundamental plant biology, rather few agronomic characters have been revealed in

its genome. Several different disease resistance genes have, however, been cloned in *A. thaliana* (Jones 2000). The next step will now be to apply this knowledge in the rather-closely related Brassica crops, for example via syntenic relationships.

Stem canker or blackleg caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not. [anamorph: *Phoma lingam* (Tode ex Fr.)] is one of the most-damaging fungal diseases of Brassica crops worldwide (West et al. 2001). Resistance to *L. maculans* is mainly found in Brassica species containing the B-genome, i.e. *Brassica nigra*, *Brassica juncea* and *Brassica carinata* (Roy 1984; Sjödin and Glimelius 1988). Conventional breeding programmes are utilising this type of germplasm as a source of resistance, but breakdown of the resistance has already occurred in such breeding material (Salisbury et al. 1995; Chen et al. 1996). This highlights the need for new genetic sources, and also of more fundamental knowledge about DNA sequence, function and the regulation of resistance genes.

In this paper, we describe the analysis of offspring from hybrids between *B. napus* and *A. thaliana*. Properties of the progenies resulting from backcrosses and selfings are analysed with a focus on the expression of *L. maculans* resistance and the localisation of the corresponding genes in the *A. thaliana* genome.

Materials and methods

Plant material

Offspring from somatic hybrids between *B. napus*, 2n = 38, and *A. thaliana* Columbia (Col-0), 2n = 10 (Forsberg et al. 1994), have been used in the present study to generate resistant lines and to investigate linkage between *L. maculans* resistance and *A. thaliana* DNA. Somatic hybrids with all five *A. thaliana* chromosome pairs present together with the *B. napus* genome, were backcrossed once and selfed four-times during the study (Table 1). Asymmetric hybrids between *B. napus* and *A. thaliana*, Landsberg *erecta* (Ler-0), selected for integrated fragments of *A. thaliana* chromosome 3 and with approximately 38 chromosomes (Forsberg et al. 1998a, b; Bohman et al. 1999), were analysed in two backcrossed generations. The parents of the somatic hybrid plants, *B. napus* cv Hanna, *A. thaliana* Col-0 and Ler-0, were used as control groups in all experiments. The analyses of the hybrid-offspring material were partly restricted due to poor seed set and reduced seed germination. Plants were grown under controlled conditions in 16-h light, with an intensity of 200 µmol E/m²s at 21 °C, and in 8-h darkness at 16 °C, with a constant humidity of 65%.

Inoculation and phenotypic scoring of *L. maculans* resistance

L. maculans, isolate PHW 1245, pathogenicity group 2 (Koch et al. 1991), was cultured and promoted to produce pycnidiospores as described by Sjödin and Glimelius (1988). Two to three leaves per plant were wounded by a pipette tip and inoculated at day 19, after seed germination, with 10 µl of 10^7 spores/ml H_2O. To promote infection, plants were incubated in 100% humidity for 24 h. Disease symptoms were evaluated from day 14 post-inoculation using the disease severity scale described by Delwiche and Williams (1979).

Genomic DNA analysis

Analyses of genomic DNA content were performed on several generations to identify the remaining *A. thaliana* DNA and to correlate the information with *L. maculans* resistance. Isolation of DNA, electrophoresis, transfer and hybridisation were performed as previously described by Sharpe et al. (1995) with modifications (Forsberg et al. 1998a). The RFLP markers and their positions on the *A. thaliana* genome used in the present study were as described by Liu et al. (1996) and by Huala et al. (2001). The following RFLP markers were employed. Chromosome 1: mi372, mi443, mi324, mi62, mi133, mi72, mi441, mi230, mi425, mi157 and mi303. Chromosome 2: mi328, mi398, mi79a, mi54, mi193, mi320 and mi455. Chromosome 3: mi74, mi403, mi467, mi207, mi289, mi339, mi142, mi225, mi178, mi358, mi413, m249, mi456 and tai224. Chromosome 4: mi122, mi301, mi279, mi30, mi334, mi167 and mi330. Chromosome 5: mi121, mi90b, mi438, mi184, mi137, mi61 and mi69. PCR analyses were performed with DNA primers (TAG Copenhagen A/S) amplifying DNA on specific positions in the *A. thaliana* genome. The primers pairs are named after the bacterial artificial chromosome (BAC) clone that they were generated from, or from cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel 1993). The position of the BAC clones and CAPS markers are described by Huala et al. (2001). The PCR-based markers used on chromosome 3 were AT-DMC1, g4711, MDJ14, MGF10, F1B5, Gl1, F7N15, T29H11, T11D3, T21J18, F22E2, F9L2, F6I4, F2N6 and Bgl1. The markers were amplified under the following conditions: 8-min denaturation at 94 °C followed by 35 cycles of 94 °C for 30 s, 57–61 °C for 15 s, 72 °C for 2 min, with a final extension period at 72 °C for 10 min. The reaction mixture contained 1.5 units of Amplitaq Gold, 2.5 mM of MgCl, 1 × PCR Buffer (Applied Biosystems), 2.5 mM of dNTP (Roche Diagnostics) and 6 pmol of each DNA primer.

Protein isolation, expression and sequencing

Leaves, wounded as described above, were inoculated on each side of the vein with 2 × 10 µl of 10^7 spores/ml H_2O and incubated in 100% humidity for 48 h. Discs with a diameter of 25 mm were removed, snap-frozen in liquid nitrogen and stored at –70 °C. Plant proteins were isolated according to Damerval et al. (1986) and Tsugita et al. (1994) with the following modifications. Eight leaf discs from three to four plants were ground in liquid nitrogen and suspended in 6 ml of 50 mM Tris-HCl buffer, pH 8.0, containing 0.35 M sucrose, 7 mM EDTA and 0.04% (v/v) of β-mercaptoethanol (ME). The suspension was filtrated through two layers of gauze fabric and two layers of miracloth, and centrifuged at 1,000 *g* for 10 min. The supernatant was precipitated with 34 ml of 10% (v/v) TCA in acetone with 0.07% (v/v) ME at –20 °C for 45 min. The precipitation was centrifuged at 34,000 *g* for 15 min and the pellet was washed twice with acetone containing 0.07% (v/v) of ME. The precipitation was finally lyophilised overnight. Five to ten milligrams of freeze-dried samples were agitated in 360 µl of 8 M urea, 2% CHAPS {3-[(3-Cholamidopropyl)-dimethylammonio]1-1propane-sulfonate (Sigma)}, 20 mM DTT, 0.001% (w/v) of bromophenol blue and 0.5% (v/v) of IPG buffer (pH range 3–10, Amersham Pharmacia Biotech) for 1 h at room temperature. Undissolved material was pelleted and all supernatant was applied to 18-cm Immobiline dry strips with an immobilised pH non-linear gradient, pH 3–10. The first dimension was performed on an IPGphor isoelectric focusing unit (Amersham Pharmacia Biotech) starting with a re-hydration step at 50 V for 12 h at 20 °C. The following steps were 500 V for 1 h, 1 kV for 1 h and 8 kV until the total of 38 kV was reached after about 19 h including the re-hydration step. Strips were equilibrated for 15 min in 10 ml of 50 mM Tris-HCl (pH 8.8), 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.001% (w/v) bromophenol blue and 65 mM DTT, followed by a 15-min alkylation step in 10 ml of the same solution but with 135 mM iodoacetamide instead of DTT. The second dimension was performed in 15% (w/v) polyacryla-

mide gels at a constant current of 24 mA until the dye front reached the bottom of the gel. Molecular-weight markers (Bio-Rad) were applied on a small piece of filter paper beside the strip before starting the second dimension. The gels were silver-stained according to Shevchenko et al. (1996).

Protein sequencing

Proteins were extracted, cleaved and analysed as described in Wilm et al. (1996). The analysis was performed on a Tandem Electrospray Mass Spectrometer (Q-Tof) equipped with a Nano-spray Interphase (Micromass).

Results

Selection of *B. napus* lines containing *L. maculans* resistance transferred from *A. thaliana*

To generate *L. maculans*-resistant *B. napus* lines, ten seeds from six independent somatic hybrids between *B. napus* and *A. thaliana* that had been backcrossed to the parental oilseed-rape Hanna cultivar were sown (BC_1). The choice of material was based on the information that the original hybrid plants had all five *A. thaliana* chromosome pairs present together with the *B. napus* genome. Forty three plants germinated and were scored phenotypically for resistance to *L. maculans* (Table 1). Five plants showed cotyledon and adult resistance, eight plants showed only adult-leaf resistance and one plant expressed cotyledon resistance. A range of phenotypic variation was observed in the BC_1 population. Several plants had an intermediate rosette leaf structure, a decreased size of stamens and a dark-green leaf colour, more similar to *A. thaliana*. Most morphological characters were, however, similar to *B. napus*, such as plant height, leaf size, colour of the flower and size of the fertile seeds. Two plants expressing both adult-leaf and cotyledon resistance, and one plant with only cotyledon resistance, were selfed. From each line, 30 seeds were planted but only 34 seeds (38%) germinated. One plant showed both cotyledon and adult resistance, five plants maintained only cotyledon resistance and four plants expressed only adult-leaf resistance in the BC_1F_1, while the remaining plants were susceptible. Two plants that expressed cotyledon resistance, two plants that showed adult-leaf resistance and one plant with both types of resistance were selected for further self-pollination. None of the 150 plants screened in the BC_1F_2 showed cotyledon resistance but 12 plants maintained adult-leaf resistance. To generate a stable resistant line two additional selfings were performed. When plants in the BC_1F_4 generation were analysed for resistance they showed a significant increase in resistance ($P < 0.01$, q = 2.39, n = 62) compared to *B. napus*, using the scoring system developed by Delwiche and Williams (1979) (Fig. 1). The plants in the BC_1F_4 generation also differed from *B. napus* in morphological characters in that they had a more dark-green leaf colour, a reduced size of stamens (Fig. 2) and a much-poorer seed set.

Table 1 Inheritance of *L. maculans* resistance in offspring deriving from *B. napus* (+) *A. thaliana* symmetric hybrids

Material	No. of plants analysed	No. of resistant plants		
		Cot.R[a] + AdultR[b]	Cot.R[a]	AdultR[b]
BC_1	43	5	1	8
BC_1F_1	34	1	5	4
BC_1F_2	150	0	0	12
BC_1F_3	20	0	0	7
BC_1F_4	46	0	0	46

[a] Plants showing cotyledon resistance
[b] Plants showing adult-leaf resistance

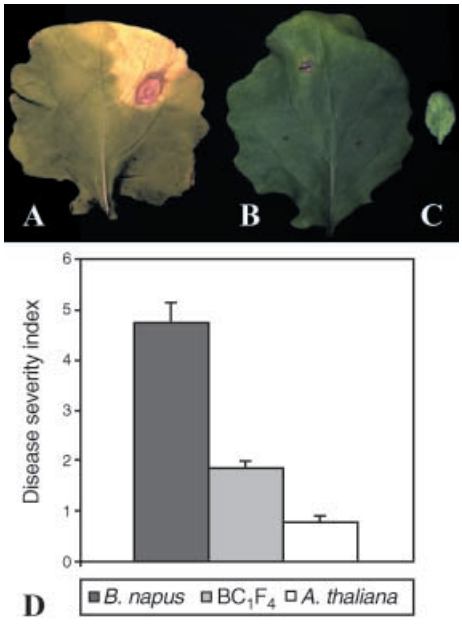

Fig. 1 A *L. maculans*-inoculated leaf of **A** *B. napus* cv Hanna, **B** a resistant plant from BC_1F_4, and **C** *A. thaliana* ecotype Columbia. **D** Mean disease severity index (Delwiche and Williams 1979) 20 days post-inoculation with *L. maculans* of *B. napus* cv Hanna (n = 16), *B. napus* BC_1F_4 (n = 46) and *A. thaliana* ecotype Columbia (n = 24). Error bars represent the standard error of the mean

Fig. 2 Morphological variations observed in the BC_1F_4 generation compared to *B. napus*. **A** *B. napus*. **B** and **C** Plants with retarded stamens and some changes in petal arrangements. **D** Normal development of stamens and anthers in *B. napus*. **E** Reduced stamen length, and **F** significantly reduced stamen and anther development

A biased co-segregation of *A. thaliana* chromosome 3 with *L. maculans* resistance

RFLP analysis was performed on resistant and susceptible plants in each generation described. After one backcross (BC_1) all five *A. thaliana* chromosome pairs were present either as parts or as whole chromosomes. When the plants were selfed (BC_1F_1), analysis with molecular markers showed that chromosome 1 was present in both susceptible and resistant plants; in the latter case together with chromosomes 2 and 3. Part of chromosome 2 was identified only in one adult-leaf resistant plant. The entire chromosome 3 was present in two of three analysed adult-leaf resistant plants, and in the third only on the north side. Chromosomes 4 and 5 could no longer be found in any plant analysed. In the next selfed generation (BC_1F_2), only offspring derived from plants containing the whole of chromosomes 1 and 3 exhibited adult-leaf resistance. All resistant plants in the BC_1F_2 maintained chromosome 3, whereas in the susceptible plants four out of six plants had lost all markers on chromosome 3. Two resistant and two susceptible plants analysed also had chromosome 1 present. No other chromosomes could be detected. After two more selfings (BC_1F_4), concomitant genomic analysis revealed that chromosome 3 was maintained in all 46 plants and among the plants 18 (40%) still carried chromosome 1. RFLP markers located on both sides of the centromeres on chromosomes 2, 4 and 5 did not show any presence of these chromosomes (Fig. 3).

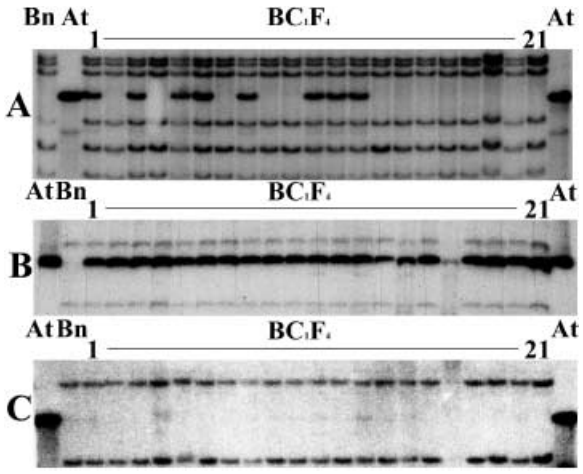

Fig. 3 RFLP analysis of parental lines (Bn = *B. napus*, At = *A. thaliana*) and of 21 plants from BC_1F_4. **A** Segregation of marker F24B9 positioned on chromosome 1. **B** Presence of mi456 positioned on chromosome 3. **C** Absence of mi30 positioned on chromosome 4

Two regions of chromosome 3 are linked to adult-leaf resistance

The co-segregation of chromosome 3 with adult-leaf resistance to *L. maculans* prompted us to investigate if smaller chromosomal regions that were governing the resistance could be identified. For this purpose, a second set of plant material, derived from asymmetric hybrids between *B. napus* and *A. thaliana*, was used. The material was characterised for its reduced content of *A. thaliana* DNA. In an initial screen, seven plants with different parts of chromosome 3 present in the *B. napus*-background were chosen. Three of the plants also contained fragments of chromosomes 2, 4 and 5 (Table 2). The plants were backcrossed and the offspring (BC_2) tested for resistance. One line (BC_2-1) that produced resistant plants with markers detecting only chromosome 3 was chosen for further screening. An additional 60 plants of the chosen line were screened for adult-leaf resistance and ten were classified as highly resistant. Of the resistant plants, eight maintained *A. thaliana* DNA corresponding to two areas located on both sides of the centromere of chromosome 3 (Fig. 4). These two areas are located between approximately 9.8–10.4 Mbp and 18–19.5 Mbp on the Arabidopsis Genome Initiative (AGI) map. None of the ten-tested susceptible plants and two of the resistant plants maintained corresponding fragments according to the molecular markers used. One resistant line was selected and backcrossed (BC_3-1). When analysing the BC_3-1 plants the results were similar to the previous generation. Five highly resistant plants, in which three could be linked to the positions described above, were found. For the susceptible plants and the two remaining resistant plants, no *A. thaliana* DNA at all could be detected.

Table 2 Analysis of regions on chromosome 3 for linkage to *L. maculans* adult-leaf resistance in *B. napus* (+) *A. thaliana* backcrossed BC_1 asymmetric hybrid lines

Plant	RFLP markers present											AdultR[b]
	C1 3[a]	C1 23[a]	C2 15[a]	C2 17[a]	C3 6[a]	C3 10[a]	C3 19[a]	C4 7[a]	C4 15[a]	C5 6[a]	C5 24[a]	
1	–	–	–	–	–	+	+	–	–	–	–	+
2	–	–	–	–	+	–	–	–	–	–	–	–
3	–	–	+	–	–	–	+	–	–	–	–	+
4	–	–	+	+	–	+	+	–	–	+	–	+
5	–	–	–	–	+	+	–	–	–	–	–	–
6	–	–	–	–	–	–	+	–	+	–	–	–
7	–	–	–	–	+	–	–	–	–	–	–	–

[a] Approximate position (Mbp) of RFLP markers used on the AGI map
[b] Plants showing adult leaf resistance in the next (BC_2) generation. C = Chromosome. (+) = presence, (–) = absence

Induction of *A. thaliana* proteins after *L. maculans* inoculation

The BC_1F_4 generation was significantly more-resistant to *L. maculans* than *B. napus*. Molecular markers provided the information that *A. thaliana* chromosome 3 was stably maintained in the genome of generation BC_1F_4. To investigate whether the presence of additional *A. thali-*

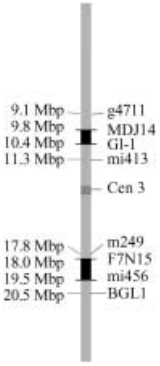

Fig. 4 Two areas on *A. thaliana* chromosome 3 linked to *L. maculans* resistance integrated into *B. napus*. The areas were located approximately between the BAC clone MDJ14 and the CAPS marker GL-1 and the BAC clone F7N15 and the RFLP marker mi456. The positions are given in Mbp according to the AGI sequence map

Table 3 Sequence data from proteins identified in BC_1F_4 after inoculation with *L. maculans*

Identified protein	Accession no.	Chromosome	Homology to *A. thaliana*
Glycolate oxidase	At3g14420	3	100%
A. thaliana photosystem I subunit-II precursor	At1g03130	1	100%
Chloroplast ribosomal L1-like protein	At3g63490	3	100%

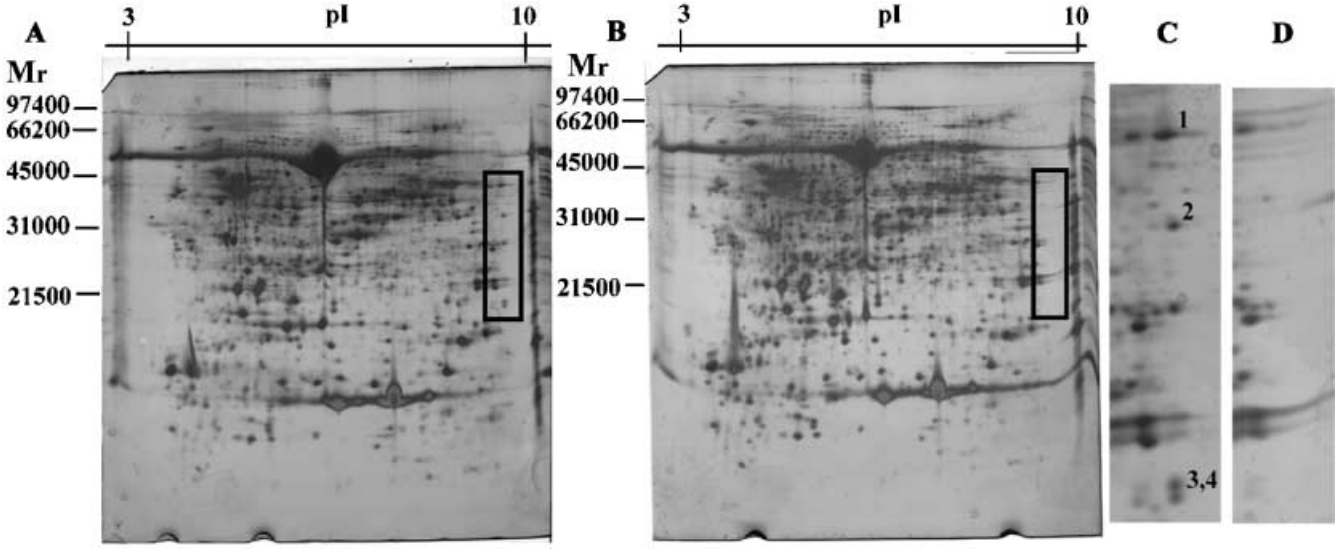

Fig. 5 2D protein gel-analysis of leaf-soluble proteins 48 h after *L. maculans* inoculation. **A** Resistant BC_1F_4 plants. **B** Susceptible *B. napus* cv Hanna. Magnification of two contrasting areas: **C** magnification of gel (**A**), spots (1–4) were chosen for sequence analysis; **D** magnification of gel (**B**)

ana DNA would generate a visible change in the proteome content, the expression of soluble proteins in BC_1F_4 was examined 48 h after inoculation with *L. maculans* on 2D gels. Despite the content of a rather large amount of extra *A. thaliana* DNA in the BC_1F_4, *B. napus* and the BC_1F_4 generation showed a very similar pattern of protein expression after inoculation with *L. maculans*. Among approximately 600 proteins visible on the 2D gels, six extra protein spots were identified in the BC_1F_4 sample that could not be observed in *B. napus* (Fig. 5). Another 14 proteins were present in *B. napus* but not in the BC_1F_4 plants. Four of the proteins expressed exclusively in the BC_1F_4 plants after inoculation were fractionated and analysed on a Q-tof MS. The resulting amino-acid sequences revealed that three of the proteins originated from *A. thaliana* and were located on chromosomes 3 and 1, respectively (Table 3). Further analysis at later time-points (50–72 h post-inoculation) identified another induced protein, polyubiquitin (*UBQ4*), not seen in *B. napus*.

Discussion

In previous screening work, where several-hundred *A. thaliana* ecotypes were analysed regarding their response to *L. maculans*, a majority were found to show resistance (Bohman et al. 1997). This observation made the hybrid material, produced earlier between a *L. maculans* susceptible oilseed rape cultivar and two resistant *A. thaliana* ecotypes, highly interesting. Indeed, it was clearly shown in the present study that these two ecotypes possessed genes coding for resistance to *L. maculans* which were functional in a *B. napus* genomic background. The resistance genes could be divided into those regulating cotyledon resistance and those coding for adult-leaf resistance. Previous genetic studies of *L. maculans* resistance in the *Brassica* B-genome have shown that cotyledon and adult-leaf resistance are two different, unlinked traits (Dixelius and Wahlberg 1999). Adult-leaf resistance was found to co-segregate with the presence of chromosome 3 whereas cotyledon resistance unfortunately was lost in the BC_1F_2 generation. To pinpoint genes of interest, all available material containing fragments of chromosome 3 integrated in the *B. napus* genome were investigated, and two potential regions of interest were found. According to database sequence information, the north region (9.8–10.4 Mbp) contained, among other genes, a P450 gene cluster. Another P450 gene in the

same region is responsible for the production of camalexin (Zhou et al. 1999). A mutation in the P450 gene (*pad3-1*) gave enhanced susceptibility of *A. thaliana* to *Alternaria brassicicola* (Thomma et al. 1999) and susceptible symptoms in *A. thaliana* after *L. maculans* inoculation (Bohman et al., in preparation). No expression of camalexin could, however, be detected in the resistant offspring analysed in this study (data not shown). The absence of camalexin could be due to the lack of an appropriate substrate. One could speculate that novel phytoalexins might be formed by any of the P450 genes originating from *A. thaliana*. These putative novel phytoalexins could then, at least in part, be responsible for the transferred resistance. That *L. maculans* is unable to metabolise camalexin in vitro has been shown earlier (Pedras et al. 1998). The role of camalexin in this defence interaction is still far from clarified. The south region (18–19.5 Mbp) contains a range of genes linked to various defence responses, such as the ABC-type transport-like proteins, the *EDS-1*, proline-rich proteins, and some disease resistance-like proteins. The *A. thaliana* mutant *eds 1-1* is, however, not affected in its resistance to *L. maculans* (Bohman et al., in preparation). To dissect the individual role of these genes, recombination events with the *B. napus* genome are needed. Additionally, it can not be excluded that small genomic fragments from other parts of the *A. thaliana* genome have been integrated in the analysed material and that the molecular markers used have not detected these fragments. This could explain that a few plants were found that showed a resistant phenotype without any detected *A. thaliana* DNA.

Brassica species and *A. thaliana* have very similar genomes. When comparing *B. napus* to *A. thaliana*, it is estimated that 87% of the coding sequences are conserved between the two species (Cavell et al. 1998). Furthermore, comparative mapping between *A. thaliana* and *B. nigra* has revealed a common evolution of the two species (Lagercrantz 1998). However, several chromosomal duplication and rearrangement events have occurred, which can explain the phenotypic differences seen between them. Despite the high homology between *B. napus* and *A. thaliana*, recombination between the two genomes in the hybrid offspring was strongly restricted, suggesting that overall genomic structures interfere with the gene flow between the two species.

To identify differences in the plant material at the translational level, a comparative proteomic approach was taken. Very few proteins were found to differ between *B. napus* and plants containing extra *A. thaliana* chromosomes. A glycolate oxidase protein was, for example, found to be up-regulated 48 h after *L. maculans* inoculation. In contrast, microarray analysis of *A. thaliana* (Col) plants showed a down-regulation of this gene 72 h after inoculation by *A. brassicicola* (Schenk et al. 2000). This indicates a role of this gene in biotic stress, most-likely in downstream processes. Another significantly up-regulated protein was *UBQ4*, which is involved in protein degradation (Vierstra 1993). Since *UBQ4* is highly conserved among plant species (Burke et al. 1988) we were unable to determine from which parental species it originated.

Even though the intention was to study all resistant plants and their offspring, low-fertility and seed-germination frequencies in part of the material made this impossible. Hence, the population size was severely restricted. Such phenomena are often found in somatic-hybrid or alloplasmic lines where the nucleus of one species has been combined with the cytoplasm from another. Disturbed interactions between nuclear and mitochondrial genes lead to abnormal stamen development and suppressed pollen production (Kück and Wricke 1995). Such morphological disturbances were frequently observed in the offspring from the symmetric hybrids in the present study.

The biased selection towards resistance to *L. maculans* and fertility promoted the presence of chromosomes 1 and 3 in the material. If no selection was applied, these two chromosomes were eliminated, together with the other three *A. thaliana* chromosome pairs. Chromosome 3 was almost in all cases linked to the resistance trait, whereas plants containing only chromosome 1 in the *B. napus* genomic background were always susceptible to *L. maculans*. The presence of chromosome 1 may in this case be linked to fertility restoration, since resistant plants carrying both chromosomes 1 and 3 generally were more-fertile than plants with only chromosome 3. Further studies are needed to prove this hypothesis.

For the first time it has been shown that *A. thaliana* can provide a trait of great value for crop breeding. Novel oilseed rape plant-material was produced where resistance to *L. maculans* originating from *A. thaliana* was expressed. Despite the high homology between the two species, recombination was restricted and no tight linkage could be established to a particular *A. thaliana*-encoded resistance gene. However, new genetic sources of *L. maculans* resistance is needed to create durable cultivars since breakdown of Brassica B-genome-derived resistance has already been reported. Hence, this material will be further mutated to promote recombination before incorporation into *B. napus* breeding programmes.

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