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Patterns of Differential Gene Expression in *Brassica napus* cultivars infected with *Sclerotinia sclerotiorum*

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Introduction

Extensive genome duplication in *Brassica* species gives rise to gene families which in most cases are indistinguishable using *Arabidopsis thaliana* microarrays. Consequently, we used a recently developed oligonucleotide microarray representing 15,000 unique *B. napus* genes (Sharpe et al., 2006; Stasolla et al., 2008) to examine differential expression of genes associated with transcription, pathogen defence, hormone signalling and carbon metabolism in the partially resistant *B. napus* cultivar, Zhongyou 821, and the susceptible cv cultivar, Westar, in response to infection by the necrotrophic fungal pathogen *Sclerotinia sclerotiorum.* This research is part of several projects aimed at identifying alternative sources of resistance, development of molecular markers linked to resistance using segregating *B. napus* populations and association mapping of diverse *B. napus* germplasm.

Materials and Methods

Plant material, inoculation and tissue harvest

Brassica napus cv. Zhongyou 821 (ZY821), partially resistant to *S. sclerotiorum*, and a highly susceptible doubled haploid line of cv. Westar were inoculated with clone 321 of *S. sclerotiorum,* isolated from a Canadian canola field. The pathogen was grown on a glucose-rich media. To simulate natural infection, plants were inoculated at full flowering. Plugs (8 mm diam.) of mycelium from the growing margin of 3-4 day old cultures were attached to the main stem with Parafilm™ at three internodes. Control plants were inoculated with glucose agar plugs only. Stem tissues extending 10 mm beyond the inoculation site and 1 mm deep were excised at five time points 6, 12, 24, 48 and 72 hours post inoculation (hpi). There were six plants per time point and three replications of each cultivar for both the inoculated and mock-inoculated controls. The tissues harvested at each time point comprising 18 inoculation sites were pooled as one sample.

Microarray analysis

The experimental design included five time points, two cultivars, two treatments and three biological replicates for a total of 60 hybridization slides. Detailed description of slide preparation can be found in Zhao et al., 2009. Dye swap experiments consisted of two samples labelled with Cy3 or Cy5 and in reverse. Spot intensities from scanned slides were quantified using ArrayPro 4.0 (Bio-Rad Laboratories) and the data were imported into the BioArray Software Environment (BASE version 1.0). Gene Spring 7.3 (Agilent Technologies, Inc.) was used to select spots with intensity values $\geq$ 50 in at least half of the samples for further analysis. A one-way ANOVA was performed. A multiple test correction was applied and a False Discovery Rate (FDR) of $\leq$ 0.0001 was used. Genes differentially expressed more than 2.0 fold were identified.

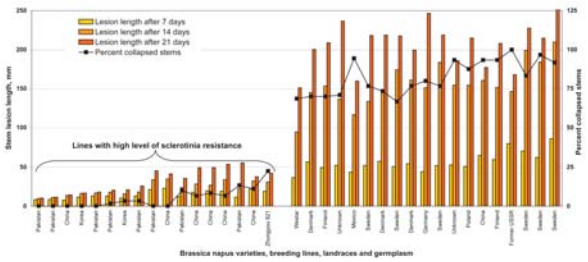

Figure 1. Assessment of *S. sclerotiorum* resistance on *B. napus* accessions.

Results and Discussion

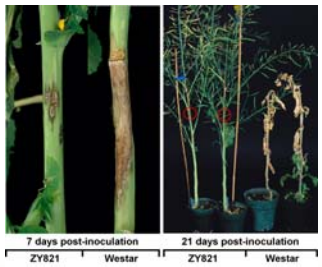

Figure 2. Phenotypes of *B. napus* ZY821 and Westar 21 days after inoculation of stems with *S. sclerotiorum*.

Screening of germplasm

So far 320 *B. napus* accessions have been screened for resistance to sclerotinia. ZY821 developed in China was one of the most resistant along with lines from Pakistan and Korea, while Westar was among the most susceptible along with lines from Sweden and Denmark (Fig. 1). Lesions on ZY821 were confined to the outer epidermis of the stem, but on Westar the pathogen penetrated into the pith and killed the plant within 21 days (Fig. 2).

Genes induced in response to *S. sclerotiorum* inoculation

Expression of 3659 and 4124 genes were significantly altered in ZY821 and Westar, respectively. Tables of differentially expressed genes are provided as supplements in Zhao et al., 2009. The data were also deposited in Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/index.cgi - Accession no. GSE13262). A total of 41 genes had increased transcript abundance only in ZY821 and are shown in Table 1. A subset of these have been cloned and expressed in susceptible *B. napus*.

Table 1. Classification of genes that were induced only in *B. napus* ZY821 stems after *S. sclerotiorum* infection.

Oligo ID[a]	Corresponding *A. thaliana* locus	Description	6[b]	12	24	48	72
Transcription							
BN11472	At3g57390	MADS-box protein	-	4.16[c]	6.28	12.8	4.95
BN26939	At2g47460	Myb transcription factor	-	2.21	2.17	5.28	5.71
BN27665	At1g28360	ERF domain protein 12	-	-	3.05	9.46	7.87
BN26396	At5g57520	Zinc finger	-	-	3.30	5.37	3.57
Stress							
BN25790	At3g16530	Lectin	5.18	15.8	50.5	109	108
BN22148	At3g57260	Endo beta-1,3-glucanase	2.27	3.52	6.11	9.91	7.56
BN18917	At2g38750	Annexin	-	3.65	2.66	25.1	33.5
BN27037	At4g22990	Multidrug resistance protein 2	-	2.20	2.57	8.02	9.01
BN22038	At4g34135	UDP-glucosyl transferase	-	-	3.95	8.95	3.40
BN16777	At4g15160	Protease inhibitor	-	-	2.92	3.02	18.4
BN20902	At4g27670	Heat shock protein	-	-	2.65	7.35	3.71
BN11639	At1g69530	Expansin	-	-	-	7.21	9.46
BN17923	At4g10265	Wound-responsive protein	-	-	-	6.03	7.79
BN18404	At5g27060	Leucine rich-repeat	-	-	-	5.58	5.00
BN15878	At5g65020	Annexin	-	-	-	4.26	7.39
BN13492	At2g37640	Expansin	-	-	-	3.90	7.79
BN12243	At5g06860	PGIP1	-	-	-	3.27	18.9
Metabolism							
BN22522	At4g27070	Tryptophan synthase	2.65	2.69	4.65	10.6	8.31
BN24404	At4g15417	Ribonuclease III	2.00	2.76	3.33	26.5	14.0
BN23990	At1g05680	UDP-glucosyl transferase	-	2.53	2.95	24.5	9.04
BN16989	At1g48920	Nucleolin	-	2.08	4.04	6.67	4.41
BN18865	At1g07720	Beta-ketoacyl-CoA synthase	-	-	3.09	28.8	15.7
BN15489	At1g17710	Phosphoric monoester hydrolase	-	-	3.01	73.0	54.4
BN23164	At4g18950	Ankyrin protein kinase	-	-	2.10	5.80	4.61
Others							
BN14638	At1g19770	Purine permease	2.13	2.10	4.59	10.9	10.1
BN20514	At1g76790	*O*-methyltransferase	2.00	7.87	12.5	25.3	31.1
BN13257	At1g54020	Lipolytic enzyme	-	3.15	14.2	-	-
BN14979	At2g30540	Glutaredoxin	-	2.16	4.74	17.4	7.81
BN13468	At4g22620	Auxin-induced protein	-	2.05	2.16	-	8.11
BN26740	At2g31990	Exostosin	-	2.04	2.17	10.4	7.10
BN23610	At5g43340	Inorganic phosphate transporter	-	-	4.66	27.5	14.8
BN20039	At1g15180	Membrane protein family	-	-	4.37	11.7	12.1
BN18437	At5g15440	Circadian clock coupling factor	-	-	3.75	13.4	11.5
BN24154	At5g01210	Transferase	-	-	3.63	5.48	7.55
BN17385	At5g10300	Hydrolase	-	-	2.14	3.91	7.93
BN26419	At5g17450	Copper homeostasis factor	-	-	-	11.1	9.69
BN19106	At5g01750	Expressed protein	-	-	-	9.41	6.90
BN20931	At5g60760	2-phosphoglycerate kinase	-	-	-	7.38	4.21
BN24358	At3g54040	Photoassimilate-responsive protein	-	-	-	5.07	-
BN17022	At3g13857	Expressed protein	-	-	-	2.94	10.5
BN13468	At4g22620	Auxin-induced protein	-	-	-	-	9.38

[a]Oligonucleotide designation from the *B. napus* gene-specific array. [b]Hours post inoculation. [c]Fold changes relative to mock-inoculated control.

Genes involved in hormone signalling

Both ZY821 and Westar exhibited an increased expression of genes associated with jasmonic acid (LOX, AOS, AOC, OPR) and ethylene (SAM, ACS, ACO) biosynthesis and a decrease in expression of a SA biosynthetic gene (ICS) (Fig. 3). This confirms that JA and ET broadly coordinate the response to necrotrophic pathogens, while salicylic acid (SA) is most often associated with the response to biotrophic pathogens.

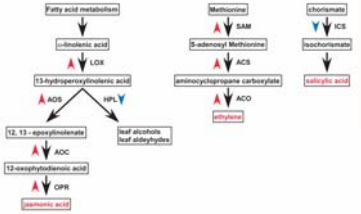

Figure 3. Increase (red arrows) and decrease (blue arrows) in the expression of genes in hormone biosynthetic pathways observed in *B. napus* tissue infected by *S. sclerotiorum*.

Expression of genes involved in carbon metabolism

Changes in expression of genes encoding enzymes involved in carbohydrate utilization and energy production (glycolysis, TCA and Calvin cycles) in both ZY821 and Westar suggested that photosynthesis was inhibited and carbon storage reserves (sucrose, starch and lipid) were shuttled through the glycolytic and photorespiration pathways (Fig. 4). The latter forms glyoxylate that enters the TCA cycle and hydrogen peroxide that may contribute to the defense response.

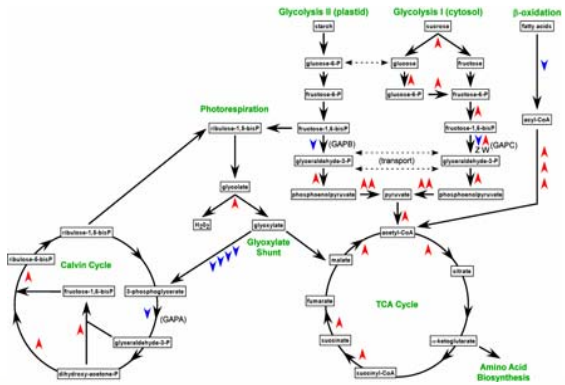

Figure 4. Expression of genes involved in carbon metabolism in *B. napus* ZY821 (Z) and Westar (W) after *S. sclerotiorum* inoculation. Increase (red arrows) and decrease (blue). Glyceraldehyde-3-phosphate dehydrogenase isoforms (GAP).

References

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Acknowledgements

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