

## Supplementary material

### Article title

**Root colonization by fungal entomopathogen systemically primes belowground plant defense against cabbage root fly**

### Authors

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**Table S1.** List of the fungal strains used in the study (see (Hettlage, 2018)).

Strain	Crop regime	Location
GC1I	Semi-natural permanent grassland for at least 7 years. Grassland harvested twice a year	51°34'10.7"N 10°03'54.1"E
Gd12		
Cb15III	Winter wheat, barley or oilseed rape crop rotation, conventional farming	51°33'58.3"N 10°04'10.1"E
Cb17b		
CC5		

**Table S2.** Primers used for qPCR amplification of genes of *Brassica napus* root tissues

ID	Gen/accession	Pathway	Gene description	Sequence 5' --> 3'	Reference
<b>ABA2</b>	LOC106300040	Absciscic acid biosynthesis	Xanthoxin dehydrogenase	GCATCGCTCGTCTGTTCCAC	Karssemeijer et al., 2021
	XM_013736089			CGGCGAAGTCAACAGCGTTA	
<b>ERF2</b>	At5g47220	Ethylene (ET) signaling	Ethylene Response Factor 2	ATGTACGGACAGAGCGAGGT	Yang et al., 2010
				AAGCTTCGAAACCAACAAGTAACTG	
<b>ACO</b>	EV102889	ET biosynthesis	ACC oxidase	TCCGTCTGGGCTATCACTCT	Maag 2014
				GTGAGTGGGTCGATGTTCTT	
<b>PR1</b>	XM_013877950	Salicylic acid (SA) signaling	Pathogenesis-related protein 1	AAAGCTACGCCGACCGACTACGAG	Alkooranee 2017
				CCAGAAAAGTCGGCGCTACTCCA	
<b>PAL</b>	LOC106342153	SA synthesis-Phenylpropanoid pathway	phenylalanine ammonia-lyase 1	TCGCTATGGCTTCTTACTGCTCTG	Karssemeijer et al., 2021
	XM_013781008			GAGGTCTTACGAGATGAGATGAGTCC	
<b>AOS</b>	LOC106327419	Jasmonic acid (JA) synthesis	Allene oxide synthase	ACCGCTTGCGACTAGGGATC	Karssemeijer et al., 2021
	XM_013765565			CAAAGTCCTTACCGGCGCAC	
<b>MYC2</b>	EV120351	JA signaling	Basic helix-loop-helix (bHLH) DNA-binding family protein	GCAAAGCCCAGACAGAGAAC	Maag et al., 2014
				AGCTCACGCAACACCTTCTT	
<b>TPI</b>	EV144353	JA signaling	Trypsin inhibitor B-like	GTGGTATCACCATGAACCTTG	Maag et al., 2014
				GTTGACCACCTTAACCGGAA	
<b>PDF1.2</b>	EV163328	JA signaling	Defensin-like protein 16	TCCATCACCTTCTCTTTGC	Maag et al., 2014
				TTTTGGCACGCATAGTCGTA	
<b>ACTIN</b>	AF111812	Housekeeping gene	Housekeeping gene	ATCGTCTCAGTGGTGGTTC	Maag et al., 2014
				TTGATCTTCATGCTGCTTGG	
<b>GTR1A2</b>	Bra018096	Glucosinolate (GSL) transport	Glucosinolate Transporter 1 A2	ATCACCTTCGGGGAACTGG	(Sontowski et al., 2019)
				TCGCTTGCTTCTGCTTGGTC	
<b>CYP79B2</b>	At4G39950	Indole GSLs biosynthesis	CYTOCHROME P450, FAMILY 79, SUBFAMILY B, POLYPEPTIDE 2	AAGAGGTTGTGCTGCTCCG	Tytgat et al., 2013 Also in Marthur
				TCCAAGTGAAACCTGAAGAAGTC	
<b>CYP83A1</b>	At4G13770	Aliphatic GSL biosynthesis	CYTOCHROME P450, FAMILY 83, SUBFAMILY A, POLYPEPTIDE 1	CTCCTTATCCCTCGTGCTTG	Mathur et al., 2013
				TGTCGTAACCAGCGATCTTG	
<b>BABG</b>	LOC106429220	Myrosinase biosynthesis	Beta-glucosidase 27-like	CCGAGCGAGCTATGGAGTTT	This study
	XM_022718558			CGGCTTGTCTGGATCCACTT	
<b>BnMyr4</b>	LOC106430598	Myrosinase biosynthesis	PREDICTED myrosinase 4-like	TCAACTGCGACAATCCCCTT	This study
	XM_013871387			ATCACAAGCAAGGTCTCCGG	
<b>Myr2.Bn1</b>	LOC106382545	Myrosinase biosynthesis	B.napus myrosinase, thioglucoside glucohydrolase	TTGAAGGAGGGAGAGGTCGT	This study
	NM_001316199			AGCATTGAGTTGCCCATCA	
<b>DTCMT.a</b>	LOC106392535	Phytoalexin brassinin biosynthesis	Dithiocarbamate S-Methyltransferase	TGTTCCACTGGACCTAACACG	This study
	XM_013833342			GGCCAAAGAAAGATCCGGGA	

**Table S3.** Acquisition parameters for phytohormones analysis.

Compound	RT [min]	Polarity	Parent Ion [m/z]	Fragmentor V	Collision Energy V	Product Ion [m/z]
<i>Trans</i> -zeatin	1.93	+	220.1	100	15	136.1
					9	202.1
					22	148.1
<i>Trans</i> -zeatin-d5	1.92	+	225.1	105	16	137.1
					10	207.2
Absciscic acid (ABA)	4.68	-	263.1	85	4	153.1
					5	219.1
					12	204.1
Absciscic acid-d6	4.67	-	269.1	88	4	159.1
					8	225.1
Jasmonic acid (JA)	5.20	+	211.2	85	8	133.1
					8	151.1
					5	193
Jasmonic acid-d5	5.19	+	216.1	85	8	135.1
					9	153.2
					7	198.2
Salicylic acid (SA)	4.05	-	137	140	15	93.1
					35	65.1
Salicylic acid glucoside (SA-Glu)	2.41	-	137	140	15	93.1
					35	65.1

**Table S4.** Statistical summary of GLM analysis with binomial distribution of the total mortality on in vitro experiments. Mortality includes mycosed larvae, pupae, and adults that developed mycosis after emerging.

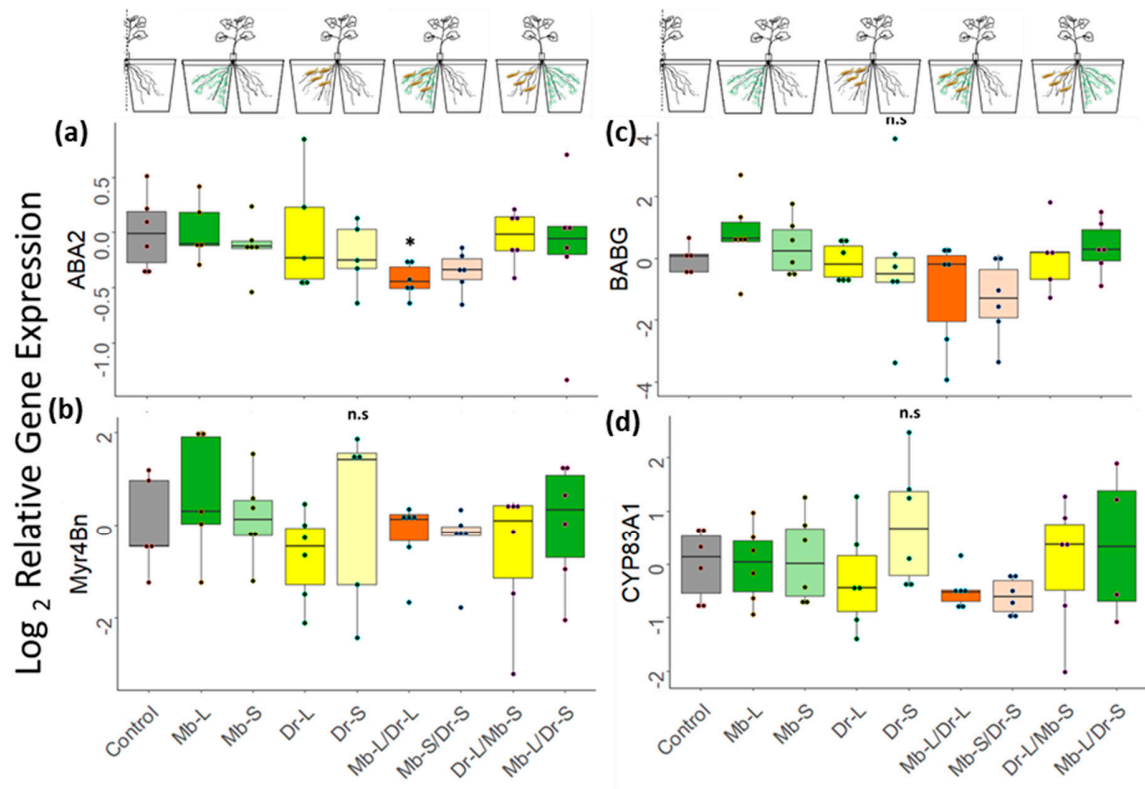
Isolate	L3 Larvae			
	Estimate	SEM	z value	Pr(> z )
Intercept	-1.6398	0.3706	-4.425	9.65E-06***
Cb15III	2.339	0.4349	5.378	7.52E-08***
Cb17B	3.0474	0.4525	6.735	1.64E-11***
CC5	3.1991	0.4588	6.972	3.11E-12***
Gc1I	4.1194	0.5248	7.85	4.15E-15***
Gd12	4.1194	0.5248	7.85	4.15E-15***
Sand	-0.7047	0.2525	-2.791	0.00525**

**Table S5.** Statistical summary of GLM analysis with binomial distribution of the total mortality in planta experiment. Mortality includes mycosed larvae, pupae, and adults that developed mycosis after emerging. RCD: root collar diameter, included as covariant.

Factor	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	2.9015	0.9581	3.028	0.00246**
Cb15III	-0.8301	0.3658	-2.269	0.02324*
Cb17B	-0.2827	0.3721	-0.76	0.44746
CC5	-0.4439	0.368	-1.206	0.22774
Gc1I	-0.853	0.3887	-2.195	0.02818*
Gd12	-1.0107	0.3561	-2.839	0.00453**
RCD	-0.1567	0.111	-1.412	0.15794

**Table S6.** Statistical summary of beta regression analysis with of percentage of damage of root collar in planta experiment.

Factor	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	1.1920	0.1789	6.662	2.71e-11 ***
TreatCb15III	-0.7290	0.2491	-2.926	0.003432 **
TreatCb17B	-0.8382	0.2379	-3.523	0.000427 ***
TreatCC5	-0.6718	0.2394	-2.807	0.005005 **
TreatGc1I	-0.8858	0.2623	-3.377	0.000732 ***
TreatGd12	-1.1614	0.2415	-4.808	1.52e-06 ***



**Figure S1.** Normalized expression of representative genes of (a) abscisic acid biosynthesis *ABA2*, (b) myrosinase synthesis *Myr4Bn*, (c) beta-glucosidase biosynthesis *BABG* and (d) aliphatic GSL synthesis *CYP83A1*. Plants grew in a split root setup in which each compartment had either *M. brunneum* inoculation (Mb) in the local (L) or adjacent (S) compartment, *D. radicum* egg infestation (Dr) in the local (L) or adjacent (S) compartment, both treatments in same compartment (Mb-L/Dr-L), or each in adjacent compartments of the same plant (Dr-L/Mb-S; Mb-L/Dr-S). Eggs were placed 4 weeks after Mb inoculation. Plants were harvested 7 days after egg infestation. Gene expression was normalized to the housekeeping gene *AUXIN*. The boxplot shows all data points from at least 4 independent biological replicates ( $n \leq 6$ ) in which the horizontal line represents the median, surrounded by the upper (25th) and lower (75th) percentiles.