A Quantitative Proteomic View on the Function of *Qfhb1*

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Utilizing 2D-DIGE and MALDI-MS/MS technology, we studied proteomic changes at 24 hai in the spikelets of a pair of near-isogenic lines (NIL) contracting *Qfhb1*.

spots. An additional evaluation confirmed that the DAP spots were specific to the spikelet from fhb1-NIL (50 spots), and fhb1+ NIL (seven spots). The proteomic data also suggest that the absence of *Qfhb1* makes the fhb1- NIL vulnerable to *Fusarium* attack by constitutively impairing several mechanisms including sucrose homeostasis by enhancing starch synthesis from sucrose. In the absence of *Qfhb1*, *Fusarium* inoculations severely damaged photosynthetic machinery; altered the metabolism of carbohydrates, nitrogen and phenylpropanoids; disrupted the balance of proton gradients across relevant membranes; disturbed the homeostasis of many important signaling molecules induced the mobility of cellular repair; and reduced translational activities. These changes in the fhb1- NIL led to strong defense responses centered on the hypersensitive response (HSR), resulting in infected cells suicide and the consequent initiation of FHB development. Therefore, the results of this study suggest that *Qfhb1* largely functions to either alleviate HSR or to manipulate the host cells to not respond to *Fusarium* infection.

Keywords: FHB, fhb1, FHB pathogeneses, FHB resistance, fusarium, hypersensitive response, proteomics, scab, wheat

• The Near-isogenic lines for *Qfhb1* used in this project were created and kindly shared by Dr. James Anderson's group at University of Minnesota





Detection of Major Proteomic Changes with 2D-DIGE at the 24th hour after FHB Inoculation



Experimental Design

Detection of Major Proteomic Changes During FHB with 2D-DIGE at 24 Hai

2C

2C + 4C

4C















Comparisons

- In-gel comparisons:
 - The mock-inoculated NIL260-1-1-4 vs. the mock-inoculated NIL260-1-1-2 (4C/2C)
 - The FHB-inoculated NIL260-1-1-4 vs. the FHB-inoculated NIL260-1-1-2 (4F/2F)
- Between-gel comparisons:
 - The FHB-inoculated vs. the mock-inoculated NIL260-1-1-2 (2F/2C)
 - The FHB-inoculated vs. the mock-inoculated NIL260-1-1-4 (4F/4C)
- Threshold for significance:
 - 1.5 fold difference
 - $p \le 0.05$

Representative 2D-DIGE Gel Image with the 80 Selected Protein Spots Marked



Example of RT-PCR Validation



The fold changes in expression of the genes of interest were either similar or insignificant between the two molecular levels



Protein spot number

Examples of Differentially Accumulated Proteins Identified by 2D-DIGE and MALDI-MS/MS Analyses and Their Top Ten Functional Annotations

m	Donk	Top 10 Appendix	Seguence	Protein	Protein	Rep	Prot	ein	Tota	d Ion
m	канк	1 op 10 Annotations	Sequence	MW	PI	Coint	Score	C.I.%	Score	C.I.%
6	MASS.	APSRRAAASLLASLRAVTPATRCPNPAVPSTRAFDRRGFASWASTPSPASGVAWMTSRVKELKDAARAQMDAAKVAPERLKSAARESVRR	RLASALGPRAAESI	MSGTLSL	NPATHA	RNLTLR	ARAFRF	RHRAPL	AVAG	AGATC
	ATYG	AAVGAAERLRGRGDDDAORLDATSKLATLALSAGCVAAAATYLRARSRVDPERVHAAVMRLERHPGLCEVLGAPLVSSEHRAVVITGG	VWTSRTYTPRFRD	AKVHMAH	RVAGTI	RKLGLV	TVEAKK	RKAWG	GGVRA	SWHH
	FIGGL	PrHEYALVAVDVAADNGDEHKVYLAGGSEKYAKQGEVIGMHMKEALVSVASEAYEAEQKGEEAEEDEKLKKEKAEAKAESAPKPLDKGG Predicted protein [Micromones en: DCC200]	GMWPAERVIDYV	AQKRHEA 44054 3	10 40	LGVAD.	ARK 66	71 529	56	00 166
	2	Intern biolen protein [interning] Brachungdium distachung] pradictad	gi 255085775	170208.8	5.47		57	/1.556	50	99.100
	2	Intromoting protein against [Stachypotinin distaction], predicted	gi 2078/12007	33755 1	8 10	11	54	0		
	4	Typoucture in potent ACAP 1 DICAT	gi 297841295 gi 290489566	80834.2	9.00	16	54	0		
	5	Innamed protein protein process taril	gi 308799307	33417.7	6.09	10	51	0		
	6	Similarity protein product [Ostecococus dati]	gi 356543678	45164	8.81	11	49.00	0		
	7	Predicted protein IChlamydomonas reinhardtiil	gi 159468908	22756.3	6.22	9	47.00	0		
	8	Prediced protein [Ostaropocous lucimations CCF9901]	gi 145341341	1483051	5.65	24	47.00	0		
	9	Predicted protein [Ostromonas sn BCC200]	gi 255089182	60494	7.75	12	46.00	0		
	10	Innamed protein protein traduct [Vitis vinfera]	gi 296090673	11253.8	11 94	7	46.00	0		
	MVRH	OF MAIN TO THE PROBANCE AND A CHART OF THE AND A CHART OF A DESCRIPTION OF THE ADDRESS OF A DESCRIPTION OF A DESCRIPA DESCRIPTION OF A DESCRIPTION OF A DESCRIPTION OF A DESCRIP	PNRTLAPPLPPPSG	SKPPPTAT	MGFDV	GVVPFN	PDGWGP	PETPAA	PPSLG	GATAT
	ASIPF	AFFSRSDKLGRIADWTRNPGGPGMHPAASRDSVFDFTSADDSLAAAAEDSSFIL/DAKPPPRHPRFGPKWRFNQRPQLPQRDEEVEAKRR	EAEKERARRERHY	QNHRSHH	HQGFRG	NQSSSA	KPSVDI	OPDWTN	IREQIP	FANFT
	KLSFT	$\label{eq:constraint} VNDQPEDLLVCGAVEFYDRAYDRVNPKAARRLERFKSRNFFKVTTTDDPVIRRLAEEDKATVFATDAILAALMCTPRSILSWDIVVQRVGNVQRVQRVQRVQRVQRVQRVQRVQRVQRVQRVQRVQRVQRV$	KLFFDKRDGSQLD	LLTVNET	AQEQLPH	ENKDDI	SAHAL	AVEATY	INQNFS	SQQVL
	VHEG	${\tt EKVTF} DEPNPFASEGEDAASNGYRYRRWKLDEE ISIIARCEVHAASADASGGRQFFTLNALNEFDPKITGVDWRQKLETQRGAVLATELKNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN$	ANKLARWTCQAL	LSGADMN	IKLGYV	SRVHPR	DHYNHS	ILTVMC	YKPRD	FAAQI
	NLNT	ANMWGIVKSIVDICMKFEEGKYVLVKDPAKPQVRIYSVPNDAFENDYVEEPLPEEEQVRLPTDDVDATAEEMDAAAEAEANKAATGGVGE	KNAEATVA	(5500.0	5.50		201	100		100
	1	Predicted protein [Hordeum vulgare subsp. vulgare]	gi 326533418	67502.8	5.52	21	296	100	161	100
	2	Eukaryotic translation initiation factor 3 subunit D-like [Brachypodium distachyon], predicted	gi 357128487	78267.8	5.67	11	148	100	109	100
7	3	Uncharacterized protein LOC100383775 [Zea mays]	gi 293336540	36090.9	4.80	4	119	100	109	100
	4	Hypothetical protein SELMODRAF [_425252 [Selaginella moellendorffii]	gi 302809182	30026.3	9.81	11	107	99.998	48	93.769
	5	Unknown [Glycine max]	gi 255634452	20542.2	4.54	3	73	95.277	62	99.72
	6	Usb5g056500 [Oryza sativa Japonica Group]	gi 297604927	65040.4	5.30	12	72	92.684		
	7	305 ribosomal protein \$1, chloroplastic;	gi 133872	44759.1	5.41	8	71	92.339		
	8	Uncharacterized protein yet45-like [Brachypodium distachyon], predicted	gi 357125252	71118.1	5.54	5	70	89.666	61	99.695
	9	Spermidne synthase 2-like [Glycine max], predicted	gi 356567616	36810.3	4.80	3	69	88.135	62	99.72
	10	F-box protein SKIP23 [Medicago truncatula]	gi 357492719	33514.1	8.44	3	68	85.403	60	99.562
	MASH	IVGYPRMGPKRELKFALESFWDGKSSAEDLEK VA IDLRASIWKQMAGAGIGKYIPSNIFA Y DQVLDITAMLGA VPDRYSW IGGEINLSI YI DUV VGDVSVI LI EV A VGVLPESEDI ELI SELI DVVEVIA ELI VA GA SUNOFREDIT VPDI EGUOLA A ES A VITEL ETI SCI DVV	SMARGNATVPAM	EMTKWEI	TNYHFI	VPELGP	DTKFSY	SSHKAI	NEYKE/	AKALG
	DDLA	Y VLVOY VSTELESKAAKO VERSFSFESEESSEEY TREVIAELKAAOASWIQP DET EV KDEESRQEAAFSAAT TEESTESOEN VEVET TAA. ASI ATT OST FAVIGKRKI VVERSFSFESTI MITAVDI VNETKI DDEIKSWI AFAAOKVVEVNAI KATA AGOKDEAVEA ANAAAT ASRDSSPRVT	NEEVOKAATALKO	SOHRRAT	TVSARL	DAOOK	KI NI PVI	PTTTIG	SEPOTA	VELRR
	VRRE	YKAKKISEEEYTNAIKEEISKVVKIOEELDIDVLVHGEPERNDMVEYFGEOLSGFTFTANGWVOSYGSRCVKPPIIYGDVSRPNPMTVFWSKN	AQSMTARPMKGM	ILTGPVTII	NWSFVI	RNDQPR	FETCYO	IALAIKK	EVEDL	EAGGI
	QVIQI	DEAALREGLPLRKSEHAFYLDWAVHSFRITNCGVQDTTQIHTHMCYSNFNDIIQSIINMDADVITIENSRSDEKLLSVFREGVVYGAGIGPGVVGAGIGPGVVGAGIGPGVVGAGIGPGVVGAGIGPGVVGAGIGPGVVVVGAGIGPGVVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVVGAGIGPGVVVVGAGIGPGVVVGAGIGPGVVVGAGIGPGVVVVGAGIGPGVVVVGAGIGPGVVVVGAGIGPGVVVVGAGIGPGVVVVGAGIGPGVVVVVVGVVVVVVVVVV	DIHSPRIPSKEEIA	DRVNKML	AVLDTN	ILWVN	DCGLK	TRKYAE	VKPAL'	TNMVE
	AAKQ	IRAELAKAQ								
	1	Methionine synthase 1 enzyme [Hordeum vulgare subsp. vulgare]	gi 68655495	84511.3	5.74	27	1,100	100	928	100
	2	Cobalamin-independent methionine synthase [Triticum monococcum]	gi 115589740	26146.4	6.10	11	622	100	546	100
8	3	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase-like [Brachypodium distachyon], predicted	gi 357155679	84641.5	5.97	21	573	100	468	100
	4	Os12g0623900 [Oryza sativa Japonica Group]	gi 115489652	84531.5	5.93	19	535	100	448	100
	5	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative, expressed [Oryza sativa Japonica Group]	gi 108862992	84582.4	5.93	18	529	100	451	100
	6	Predicted protein [Hordeum vulgare subsp. vulgare]	gi 326513870	84376.2	5.82	18	528	100	449	100
	7	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative, expressed [Oryza sativa Japonica Group]	gi 108862990	78929.9	7.19	17	522	100	448	100
	8	Os12g0624000 [Oryza sativa Japonica Group]	gi 115489654	84612.4	5.93	17	521	100	451	100
	9	5-methyltetrahydropteroyltriglutamatehomocysteine methyltransferase-like [Brachypodium distachyon], predicted	gi 357119807	84451.3	6.05	16	513	100	449	100
	10	LOC100286153 [Zea mays]	gi 226531522	84417.3	5.85	16	441	100	379	100

Annotation Thresholds

- Protein score:
 - High confidence: protein score ≥ 72
 - Low confidence: protein score ≥ 31 but ≤ 71
 - No confidence: protein score ≤ 30
- Total ion score:
 - The higher, the better fits
- Confidence Interval \geq 95% for both protein scores and ion strength

Differentially Accumulated Proteins (DAP) Revealed by 2D-DIGE Followed by MALDI-MS/MS



Group	ID	Annotation	GenBank ID	kDa	pI	4C/2C	2F/2C	4F/4C	4F/2F
	80**	60S ribosomal protein L, ent-kaurene synthase like 1 or thylakoid-bound ascorbate peroxidase	gi 360042516	34.44	9.30	-6.87	3.53	19.54	-1.24
	24	Myo-inositol-1-phosphate synthase	gi 90289596	49.68	4.90	-2.51	1.20	3.43	1.14
	52	Vacualar invertase	gi 3219509	56.40	5.80	-1.87	1.11	1.62	-1.27
	12	Disardelaria ammonia luca	=12024262	76.90	6.10	1.67	1.40	2.60	1.22
	12	Characteristic antiformativase		13.09	6.00	-1.57	1.40	2.09	1.23
1	1	Giycosyitransterase/Pm3	gi 56409844	65.08	6.00	-1.71	-1.18	1.19	-1.21
	47	Abscisic stress ripening protein	gi 357163453	25.95	5.10	-1.86	-1.33	1.23	-1.14
	69	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic	gi 68566191	23.71	8.50	-2.19	-1.10	-1.05	-2.10
	67	Eukaryotic translation initiation factor 5A1	gi 74048999	17.35	5.70	-1.85	1.14	-1.06	-2.24
	79	Ribulose bisphosphate carboxylase small chain clone 512	gi 132107	13.05	5.80	-1.71	1.05	1.02	-1.76
	17	Protein phosphatase 2A structural subunit	gi 328775741	65.46	5.00	1.63	-1.07	-1.47	1.18
	62	Glutathione transferase F5	gi 23504745	23.42	5.80	1.57	1.08	-1.58	-1.08
2	16	Protein disulfide isomerase	gi 222446340	56.64	5.00	1.74	-1.10	-1.53	1.25
	20	Phosphoethanolamine methyltransferase	gil17887465	56.82	5.20	1.87	1.23	-1.78	-1.18
	64	Salt tolerant protein	gi 63021412	17.05	4.70	2.88	1.09	-2.84	-1.08
	77	Thylakoid lumenal 15 kDa protein 1, chloroplastic, predicted	gi 225449424	23.42	6.50	1 14	-1.28	-1.86	-1.27
	72	Thioradoxin M tuna abloranlactic: productor	ail11125474	10.12	8 70	1.16	1.24	1.92	1.27
	70	Manualue ATDess submit E	-104527548	14.22	6.70	1.04	1.40	1.74	1.20
	70		gij94557548	14.37	5.50	1.04	-1.40	-1.74	-1.20
	39	Light-harvesting complex 1 enforophyll arb binding protein 5	gi 79520445	23.75	5.00	1.05	-1.22	-1.07	-1.31
	68	Eukaryotic translation initiation factor 5A1	gi 74048999	17.35	5.70	1.29	-1.05	-1.66	-1.22
	66	Thioredoxin-dependent peroxidase	gi 256708473	17.39	5.20	1.10	-1.07	-1.62	-1.38
3	74	Ribulose bisphosphate carboxylase small chain clone 512 (Fragment)	RBS3_WHEAT	13.05	5.80	1.13	-1.30	-1.59	-1.08
	34	Glutamine synthetase isoform GSr1	gi 40317416	38.71	5.40	1.45	-1.06	-1.59	-1.04
	61	Oxygen-evolving enhancer protein 2, chloroplastic	gi 52548238	23.31	5.60	-1.05	-1.15	-1.55	-1.42
	56	Adenylate kinase, chloroplastic-like	gi 357139457	31.43	7.10	1.15	-1.12	-1.54	-1.19
	51	Chlorophyll a-b binding protein of LHCII type III, predicted	gi 357122389	28.56	5.00	1.01	-1.39	-1.51	-1.07
	57	Chlorophyll a-b binding protein	gi 225690794	26.96	5.40	1.12	1.03	-1.79	-1.64
	71	Peroxiredoxin-2E-2, chloroplastic-like, predicted	gi 357139104	23.21	6.70	1.20	1.15	-1.59	-1.52
	22	Alpha tubulin-2A	gil90289596	49.68	4.90	1.10	1.32	1.92	1.60
	4.	COP9 signalocome subunit	gi[159463648	50.44	6.00	-1.05	1.12	1.52	1.20
	4	Cory signalosonic subunit	-169655405	04.51	6.00	-1.05	1.12	1.52	1.29
	8	Methodine synthase 1	gi 08055495	64.51	5.70	-1.10	1.25	1.54	1.11
	10	Adenosyihomocysteinase	SAHH_WHEAT	53.40	5.70	-1.10	1.29	1.52	1.07
	45	Malate dehydrogenase 1, mitochondrial-like, predicted	gi 357132456	35.37	8.50	-1.23	1.04	1.54	1.20
	5	Predicted barley protein	gi 326503600	99.43	5.20	-1.04	1.34	1.56	1.13
	28	Phosphoglucomutase	gi 18076790	62.75	5.70	-1.03	1.36	1.60	1.13
	36	S-adenosylmethionine synthase	gi 223635315	43.15	5.60	-1.20	1.26	1.60	1.05
	25	5,10-methylene-tetrahydrofolate reductase	gi 115589742	64.83	5.90	-1.16	1.18	1.61	1.17
	29	Phosphoglucomutase	gi 18076790	62.75	5.70	-1.03	1.27	1.62	1.23
4	39	Caffeic acid 3-O-methyltransferase	gi 145321007	38.57	5.70	-1.33	1.06	1.64	1.16
	26	ATPase subunit 1	gi 81176509	55.27	5.70	-1.13	1.29	1.65	1.13
	23	Mitochondrial-processing peptidase subunit beta-like, predicted	gil357113428	58.38	5.60	-1.36	1.24	1.66	-1.01
	2.00	Change Manual and MAN and and an		66.09	6.00	1.01	1.40	1.69	1.14
	3.0	Diversional and the second sec	gij30409844	60.08	6.00	1.01	1.49	1.00	1.19
	27	Phosphoglucomutase	gi 18076790	62.75	5.70	-1.12	1.40	1.72	1.10
	40	Cattere acid 3-O-methyltransterase	gi 145321007	38.57	5.70	-1.36	1.10	1.72	1.15
	42	60S acidic ribosomal protein (Os08g0130500)	gi 115474653	34.36	5.40	-1.36	1.46	1.76	-1.13
	2	Ribulose bisphosphate carboxylase small chain PW9, chloroplastic	RBS2_WHEAT	19.44	8.50	-1.20	1.35	1.80	1.11
	9	Phenylalanine ammonia-lyase	gi 3024363	75.89	6.10	-1.26	1.36	2.01	1.17
	37	S-adenosylmethionine synthase	gi 151175359	46.83	5.50	-1.49	1.32	2.03	1.03
	38	S-adenosylmethionine synthase	gi 223635315	43.15	5.60	-1.44	1.40	2.32	1.15
	65	MYB-related protein	gi 359952782	48.05	8.90	1.11	-1.88	-2.06	1.01
	33	Predicted barley protein	gi 326512860	36.77	5.00	1.22	-1.76	-1.99	1.08
	53	Translationally-controlled tumor protein	gi 146285306	18.77	4.60	1.02	-1.65	-1.96	-1.16
5	54	Predicted barley protein	gi 326520557	16,18	4,80	-1.03	-1.55	-1.66	-1.11
	58	Chlorophyll a-b binding protein CP24 10A, predicted	gi 356525886	27.67	6.20	1.04	-1.53	-1.74	-1.09
	55	Chlorophyll a/b binding protein, predicted	gi 302566696	28.20	5.10	1.01	-1.50	-1.67	-1.10
	15	Pibulose 1.5 hierboerbate earbourdeee/argemente large subunit	gij302500050	\$2.92	6.20	1.01	1.52	2.42	1.24
	15	Nouiose-1,5-oispiiospilate earboxylase/oxygenase large subunit	-1200807845	14.10	4.00	-1.29	1.52	1.00	1.29
	76	Promin	gi 500807845	14.18	4.90	-1.19	1.60	1.88	-1.02
	14	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	gi 14017580	52.82	6.20	-1.44	1.60	2.78	1.20
6	35	Actin-97-like, predicted	gi 357135037	41.70	5.20	-1.44	1.63	2.23	-1.05
	50	Group 5/9 grass pollen allergen R8-5	gi 365769201	24.91	5.60	-1.37	1.69	1.93	-1.20
	72	Pathogenesis related protein 10	gi 196051131	17.05	5.20	1.05	2.22	2.04	-1.03
	- 11	5-methyltetrahydropteroyltriglutamate/homocysteine methyltransferase-like, predicted	gi 357155679	84.64	6.00	-1.49	2.28	2.14	-1.59
	13**	Mechanosensitive ion channel, pentatriconentide repeat- domain containing protein or serine/threonine protein kinase	gi 86439721	34 71	8 50	-1.21	2.51	1.48	-2.05
	6	Predicted Micromona protein	gi 255083775	44.95	####	1.07	2.27	1.24	-1.72
	7	Eukarwatic translation initiation factor 3 subunit D-like predicted	gi[357139497	78.07	5.70	1.12	1.02	1.05	10
7	/	nakaryone translation initiation factor 5 subunit D-fike, predicted	gij55/12848/	78.27	5.70	1.12	1.92	1.05	-1.62
	44	Uncharacterized maize protein LOC100273160	gi 226528643	39.94	6.10	-1.03	1.65	1.33	-1.27
	18	Glucose-1-phosphate adenylyltransferase large subunit, chloroplastic/amyloplastic (Fragment)	GLGL3_WHEAT	55.52	6.60	1.07	1.60	1.17	-1.28
	49**	Root phototropism 2, putative, expressed	gi 108862102	28.47	9.70	-1.27	-1.59	-1.17	1.07
8	70	Abscisic acid stress ripening 1 protein	gi 321155395	10.45	6,60	-1.25	-1.52	-1.31	-1.07
	10*	Starch synthase IIa-3	gj 8953573	86.74	6 30	1.43	1.19	1.40	1.70
9	21	Alaba tubulia.2A	ail00280506	40.69	4.00	-1.24	1.10	1.40	-1.56
-	42	Comment A //D Jacobieses	gij90269590	44.42	9.90	1.34	1.31	1.13	-1.30
	43	Sicaroyinaca ucsattirase	gij519739540	44.45	0.20	-1.29	-1.11	-1.92	-1.00

Distribution of the 71 2D-DIGE-revealed differentially accumulated proteins (DAPs) (fold change $\geq 1.5, p \leq 0.05$)



Up-regulated DAPs

Down-regulated DAPs

- Most changes occur in the Fhb1- NIL
- Resistance may be due to less change in accumulation of these proteins

Constitutive DAPs

Group	ID	Annotation	4C/2C	2F/2C	4F/4C	4F/2F
	80**	60S ribosomal protein L, ent-kaurene synthase like 1 or thylakoid-bound ascorbate peroxidase	-6.87	3.53	19.54	-1.24
	24	Myo-inositol-1-phosphate synthase	-2.51	1.20	3.43	1.14
	52	Vacuolar invertase	-1.87	1.11	1.62	-1.27
	12	Phenylalanine ammonia-lyase	-1.57	1.40	2.69	1.23
1	1**	Glycosyltransferase/Pm3	-1.71	-1.18	1.19	-1.21
	47	Abscisic stress ripening protein	-1.86	-1.33	1.23	-1.14
	69	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic	-2.19	-1.10	-1.05	-2.10
	67	Eukaryotic translation initiation factor 5A1	-1.85	1.14	-1.06	-2.24
	79	Ribulose bisphosphate carboxylase small chain clone 512	-1.71	1.05	1.02	-1.76
	17	Protein phosphatase 2A structural subunit	1.63	-1.07	-1.47	1.18
	62	Glutathione transferase F5	1.57	1.08	-1.58	-1.08
2	16	Protein disulfide isomerase	1.74	-1.10	-1.53	1.25
	20	Phosphoethanolamine methyltransferase	1.87	1.23	-1.78	-1.18
	64	Salt tolerant protein (a B-box type Zn-finger protein)	2.88	1.09	-2.84	-1.08



14 (9 down & 5 up) in NIL260-1-1-4

Proteins	4C/2C	2F/2C	4F/4C	4F/2F	Biological functions		
#69	-2.19	-1.10	-1.05	-2.10	Photosynthesis		
#79	-1.71	1.05	1.02	-1.76	Photosynthesis		
#57	1.12	1.03	-1.79 -1.64		Photosynthesis		
#7	1.12	1.92	1.05	-1.62	Protein production		
#67	-1.85	1.14	-1.06	-2.24	Protein production		
#11	-1.49	2.28	2.14 -1.59		Protein production		
#22	1.10	1.32	1.92	1.60	TUBA-2A		
#21	-1.34	1.31	1.13	-1.56	TUBA-2A		
#71	1.20	1.15	-1.59	-1.52	Stress reduction		
#43	-1.29	-1.11	-1.42	-1.66	Stress reduction		
#13	-1.21	2.51	1.48	-2.05	Stress reduction		
#19	1.43	1.18	1.40	1.70	Starch synthease lla-3		
#6	1.07	2.27	1.24	-1.72	Unknown		

DAPs Between the Two NILs After Fusarium-infection

- *Qfhb1* is able to maintain photosynthesis, protein biosynthesis, sucrose homeostasis and stress control after *Fusarium* infection
- It will be interesting to know the function of protein #6

DAPs specific to the *Fhb1*+ NIL 260-1-1-2

Proteins	Functions	4C/2C	2F/2C	4F/4C	4F/2F	
49**	Root phototropism 2, putative, expressed	-1.27	-1.59	-1.17	1.07	ROS sensor
70	Abscisic acid stress ripening 1 protein	-1.25	-1.52	-1.31	-1.07	ROS scavenging
18	Glucose-1-phosphate adenylyltransferase large subunit, chloroplastic/amyloplastic (Fragment)	1.07	1.60	1.17	-1.28	Sucrose metabolism
44	Uncharacterized maize protein LOC100273160	-1.03	1.65	1.33	-1.27	
6	Predicted Micromona protein	1.07	2.27	1.24	-1.72	
13**	Mechanosensitive ion channel/pentatricopeptide repeat- domain containing protein/serine/threonine protein kinase	-1.21	2.51	1.48	-2.05	
7	Eukaryotic translation initiation factor 3 subunit D-like	1.12	1.92	1.05	-1.62	Protein synthesis



5 up and 2 down

ID	Annotation	4C/2C	2F/2C	4F/4C	4F/2F	ID	Annotation	4C/2C	2F/2C	4F/4C	4F/2F
24	Myo-inositol-1-phosphate synthase	-2.51	1.20	3.43	1.14	71	Peroxiredoxin-2E-2, chloroplastic-like	1.20	1.15	-1.59	-1.52
12	Phenylalanine ammonia-lyase	-1.57	1.40	2.69	1.23	22	Alpha tubulin-2A	1.10	1.32	1.92	1.60
52	Vacuolar invertase	-1.87	1.11	1.62	-1.27	3**	Glycosyltransferase/MYB-related protein	1.01	1.49	1.68	1.14
1**	Glycosyltransferase/Leucyl-tRNA synthetase/Pm3	-1.71	-1.18	1.19	-1.21	2	Ribulose bisphosphate carboxylase small chain PW9	-1.20	1.35	1.80	1.11
47	Abscisic stress ripening protein	-1.86	-1.33	1.23	-1.14	4*	COP9 signalosome subunit	-1.05	1.12	1.52	1.29
69	Cytochrome b6-f complex iron-sulfur subunit	-2.19	-1.10	-1.05	-2.10	5	Predicted protein [Hordeum vulgare subsp. vulgare]	-1.04	1.34	1.56	1.13
67	Eukaryotic translation initiation factor 5A1	-1.85	1.14	-1.06	-2.24	8	Methionine synthase 1 enzyme	-1.10	1.25	1.52	1.11

The absence of *Qfhb1* leads to significantly changes in photosynthetic, sucrose homeostasis, protein biosynthesis, and anti-ROS activities

61	Oxygen-evolving enhancer protein 2, chloroplastic	-1.05	-1.15	-1.55	-1.42	37	S-adenosylmethionine synthase	-1.49	1.32	2.03	1.03
66	Thioredoxin-dependent peroxidase	1.10	-1.07	-1.62	-1.38	39	Caffeic acid 3-O-methyltransferase	-1.33	1.06	1.64	1.16
68	Eukaryotic translation initiation factor 5A1	1.29	-1.05	-1.66	-1.22	40	Caffeic acid 3-O-methyltransferase	-1.36	1.10	1.72	1.15
73	Thioredoxin M-type, chloroplastic; Precursor	1.16	-1.24	-1.83	-1.27	42	60S acidic ribosomal protein P0 (Os08g0130500)	-1.36	1.46	1.76	-1.13
74	Ribulose bisphosphate carboxylase small chain	1.13	-1.30	-1.59	-1.08	45	Malate dehydrogenase 1, mitochondrial-like	-1.23	1.04	1.54	1.20
77	Thylakoid lumenal 15 kDa protein 1	1.14	-1.28	-1.86	-1.27	19*	Starch synthase	1.43	1.18	1.40	1.70
78	Vacuolar ATPase subunit F	1.04	-1.40	-1.74	-1.20	21	Alpha tubulin-2A	-1.34	1.31	1.13	-1.56
57	Chlorophyll a-b binding protein	1.12	1.03	-1.79	-1.64	43	Stearoyl-ACP desaturase	-1.29	-1.11	-1.42	-1.66

DAPs Specific to the *Fhb1*-NIL







Impacts on Photosynthesis

Proteins	Annotation	4C/2C	2F/2C	4F/4C	4F/2F
#51	LHCB	1.01	-1.39	-1.51	-1.07
#55	LHCB	1.01	-1.50	-1.67	-1.10
#57	LHCB	1.12	1.03	-1.79	-1.64
#58	LHCB	1.04	-1.53	-1.74	-1.09
#59	LHCB	1.05	-1.22	-1.67	-1.31
#61	OEE2	-1.05	-1.15	-1.55	-1.42
#77	TL15	1.14	-1.28	-1.86	-1.27
#73	TRXM	1.16	-1.24	-1.83	-1.27
#69	Cytob6f	-2.19	-1.10	-1.05	-2.10
#74	RuBisCO	1.13	-1.30	-1.59	-1.08
#2	RuBisCO	-1.20	1.35	1.80	1.11
#14	RuBisCO	-1.44	1.60	2.78	1.20
#15	RuBisCO	-1.29	1.52	2.43	1.24
#79	RuBisCO	-1.7)	1.05	1.02	-1.76
#26	ATPase 1	-1.13	1.29	1.65	1.13

 \bigcirc Significantly different (≥ 1.5 folds)

• In the absence of *Qfhb1*, *F. graminearum* infection severely damages photosynthetic machinery.

Impacts on ATP Homeostasis





4F/2F

2F/2C

4F/4C

4C/2C



The absence of *Qfhb1* makes the fhb1- NIL vulnerable ning to *Fusarium* attack by constitutively impairing sucrose homeostasis by enhancing starch synthesis from sucrose.



Impacts on Anti-Radical Oxygen Species Activities

Proteins	Annotation	4C/2C	2F/2C	4F/4C	4F/2F
#43	SAD	-1.29	-1.11	-1.42	-1.66
#71	Prox2	1.20	1.15	-1.59	-1.52
#57	LHCB	1.12	1.03	-1.79	-1.64
#51	LHCB	1.01	-1.39	-1.51	-1.07
#55	LHCB	1.01	-1.50	-1.67	-1.10
#58	LHCB	1.04	-1.53	-1.74	-1.09
#59	LHCB	1.05	-1.22	-1.67	-1.31
#66	TD-Prx	1.10	-1.07	-1.62	-1.38
#73	TRXM	1.16	-1.24	-1.83	-1.27
#53	ТСТР	1.02	-1.65	-1.96	-1.16

Significantly different (≥ 1.5 folds)

SAD: Stearoyl-ACP desaturase ; Prox2: Peroxiredoxin-2E-2 ; LHCB: Chlorophyll a-b binding protein; TD-Prx: Thioredoxin-dependent peroxidase; TRXM: Thioredoxin M-type; TCTP: Translationally-controlled tumor protein

There is a significant reduction in anti-ROS activities in the Fhb1- NIL but not much in the Fhb1+ NIL after the pathogen inoculation.

Proteins	4C/2C	2F/2C	4F/4C	4F/2F	Functions
#17	1.63	-1.07	-1.47	1.18	Inhibitors
#64	2.88	1.09	-2.84	-1.08	
#20	1.87	1.23	-1.78	-1.18	
#62	1.57	1.08	-1.58	-1.08	
#34	1.45	-1.06	-1.59	-1.04	
#51	1.01	-1.39	-1.51	-1.07	
#59	1.05	-1.22	-1.67	-1.31	
#66	1.10	-1.07	-1.62	-1.38	
#73	1.16	-1.24	-1.83	-1.27	
#53	1.02	-1.65	-1.96	-1.16	
#65	1.11	-1.88	-2.06	1.01	
#55	1.01	-1.50	-1.67	-1.10	
#58	1.04	-1.53	-1.74	-1.09	
#43	-1.29	-1.11	-1.42	-1.66	
#57	1.12	1.03	-1.79	-1.64	
#71	1.20	1.15	-1.59	-1.52	
#39	-1.33	1.06	1.64	1.16	
#40	-1.36	1.10	1.72	1.15	
#11	-1.49	2.28	2.14	-1.59	
#36	-1.20	1.26	1.60	1.05	Promoters
#37	-1.49	1.32	2.03	1.03	
#38	-1.44	1.40	2.32	1.15	
#25	-1.16	1.18	1.61	1.17	
#9	-1.26	1.36	2.01	1.17	
#72	-1.26	1.36	2.01	1.17	
#12	-1.57	1.40	2.69	1.23	

Impacts on Hypersensitive Reaction

• The absence of *Qfhb1* leads to significantly higher HSR

Summary

- The Fhb1- NIL responds to *Fusarium* infection by:
 - significantly reducing photosynthesis activities
 - significantly disturbing ATP homeostasis
 - significantly disturbing sucrose homeostasis
 - significantly reducing anti-stress activities leading to ROS build-up
- *Fusarium* infection induces strong HSR in the Fhb1-NIL leading to FHB
- The Fhb1+ NIL seems to launch much weaker response to *Fusarium* infection
- *Qfhb1* seems to help the wheat host to do less in response to *Fusarium* infection reducing HSR and thus FHB

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DAPs that Could be Encoded by Genes in *Qfhb1*

• Based on the published sequence of the *Qfhb1* marker interval by Schweiger et al. (2016), the following eight genes could be encoded by the genes in this interval:

Proteins	4C/2C	2F/2C	4F/4C	4F/2F	Annotation
#1	-1.71	-1.18	1.19	-1.21	Glycosyltransferase/Pm3
#3	1.01	1.49	1.68	1.14	Glycosyltransferase /MYB-related protein
#7	1.12	1.92	1.05	-1.62	Eukaryotic translation initiation factor 3 subunit D-like
#68	1.29	-1.05	-1.66	-1.22	Eukaryotic translation initiation factor 5A1
#20	1.87	1.23	-1.78	-1.18	Phosphoethanolamine methyltransferase
#26	-1.13	1.29	1.65	1.13	ATPase 1
#49	-1.27	-1.59	-1.17	1.07	Root phototropism 2
#53	1.02	-1.65	-1.96	-1.16	Translationally-controlled tumor protein/HRC

- We did not identify any DAP that is likely a PFT or a GDSL
- Protein #53 (TCTP) could be HRC because both TCTP and HRC have a calciumbinding domain
 - A significant down-regulation of its accumulation by *F. graminearum* infection was observed in both NILs though.
- Our proteomic data revealed a diverse number of pathways that contribute to FHB and thus probably more than one genes in this QTL are needed to work together
- Alternatively, *Qfhb1* gene must be a master regulator to all or at least most of the described pathways is required