

A Quantitative Proteomic View on the Function of *Qfhb1*

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A Quantitative Proteomics View on the Function of *Qfhb1*, a Major QTL for Fusarium Head Blight Resistance in Wheat

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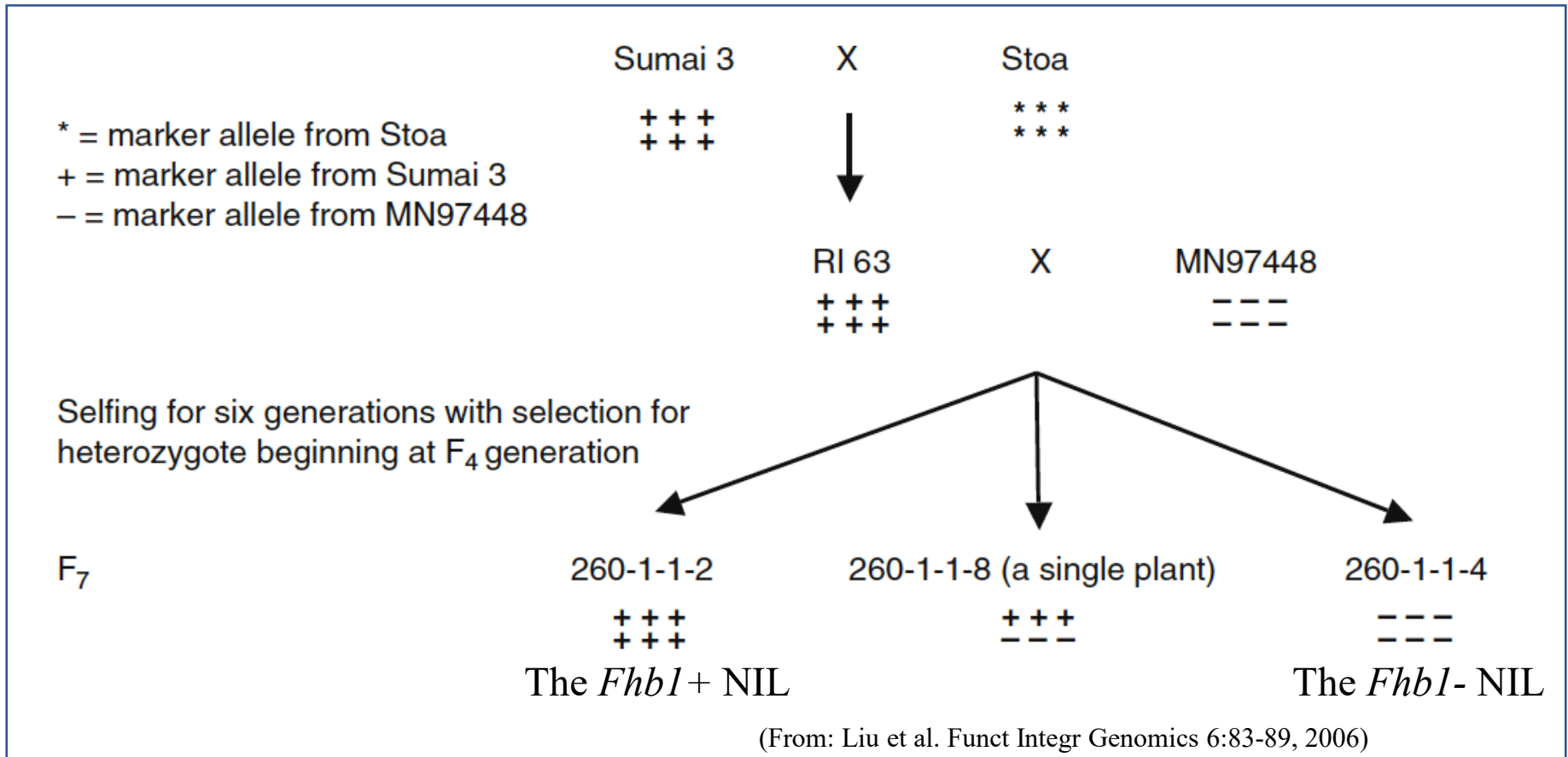
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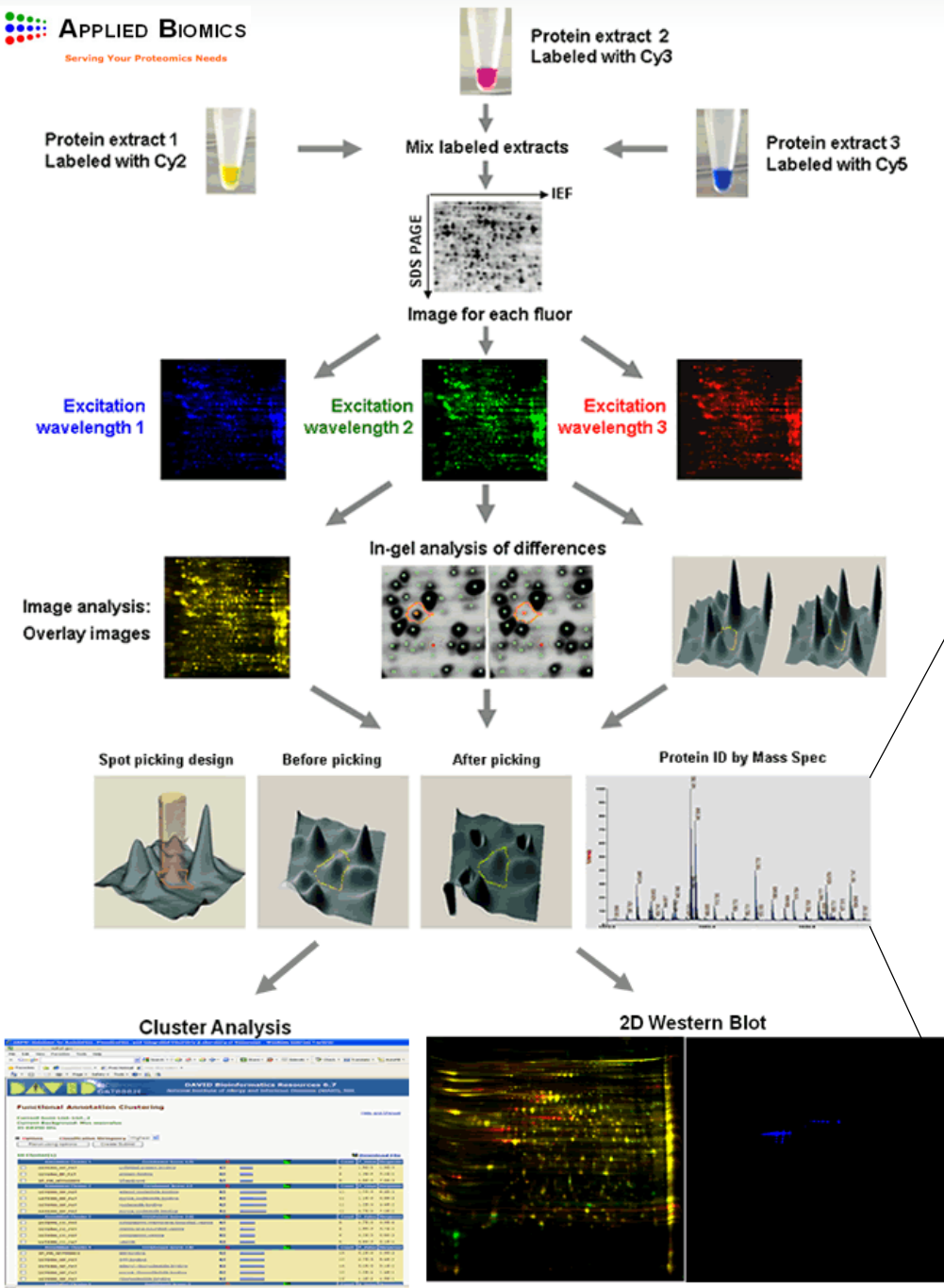
Utilizing 2D-DIGE and MALDI-MS/MS technology, we studied proteomic changes at 24 hai in the spikelets of a pair of near-isogenic lines (NIL) contracting *Qfhb1*.

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.

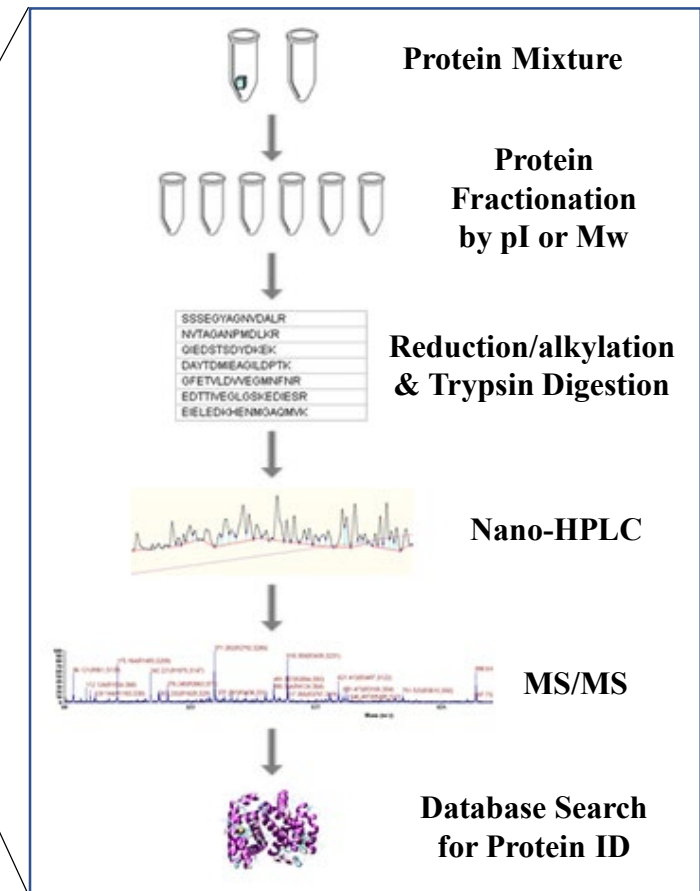
Keywords: FHB, *fhb1*, FHB pathogenesis, FHB resistance, fusarium, hypersensitive response, proteomics, scab, wheat

- **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**

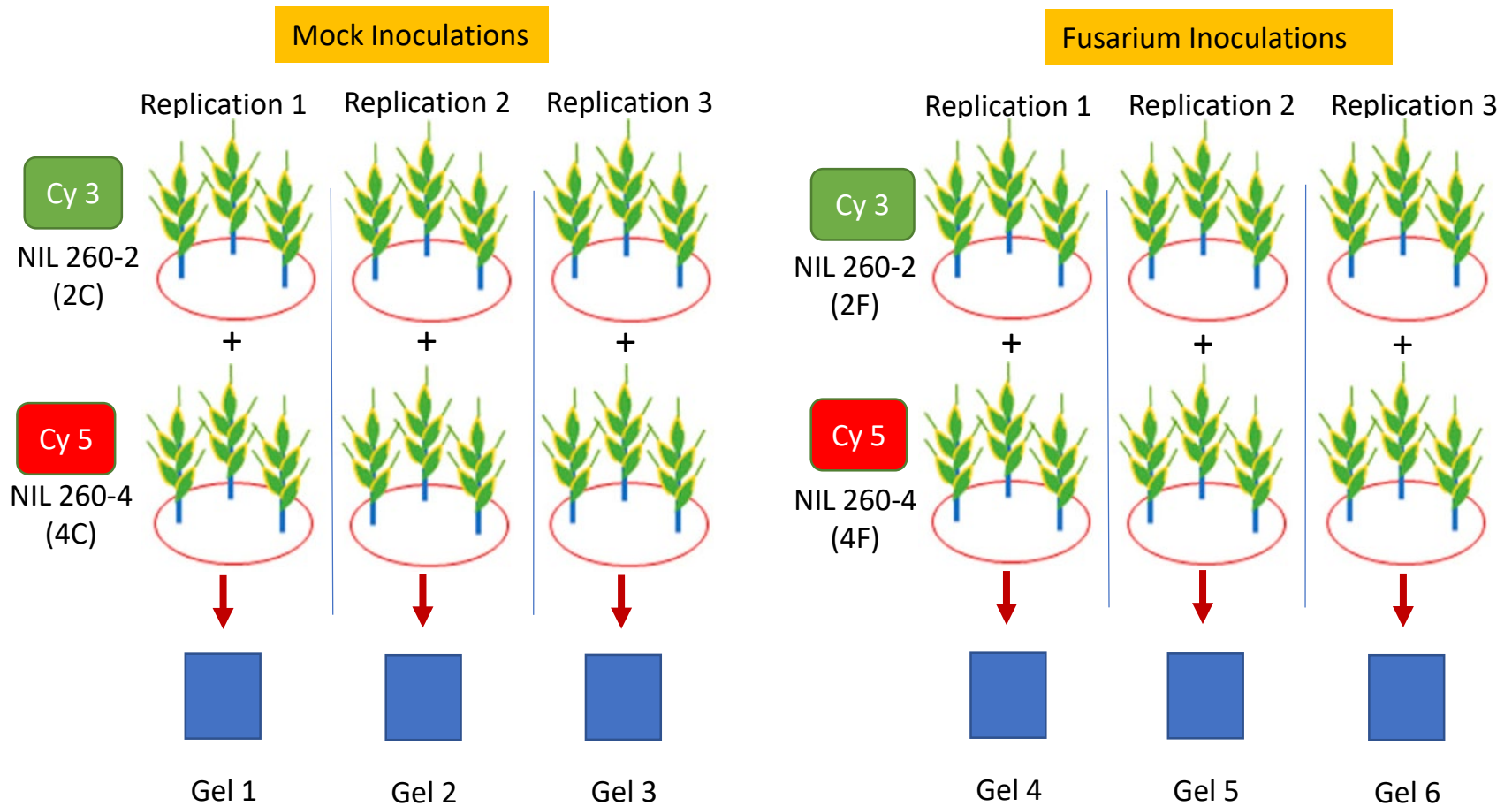




Procedure for Two-Dimensional Difference in Gel Electrophoresis (2D-DIGE) for Protein Expression Profiling



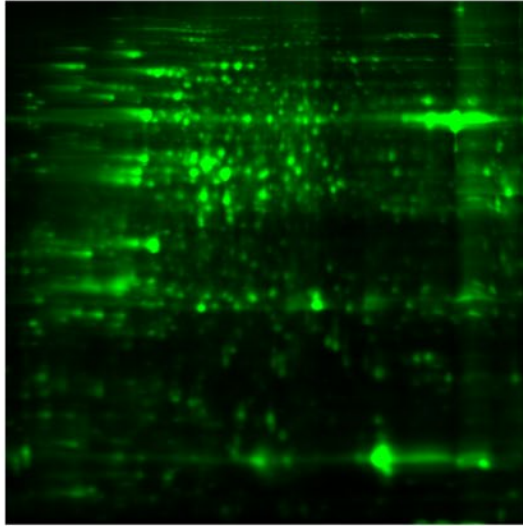
Detection of Major Proteomic Changes with 2D-DIGE at the 24th hour after FHB Inoculation



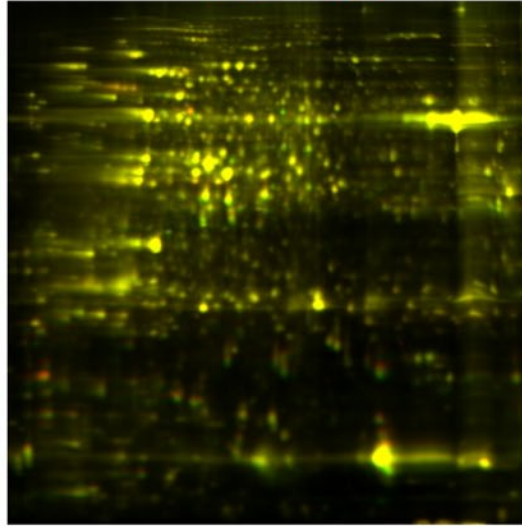
Experimental Design

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

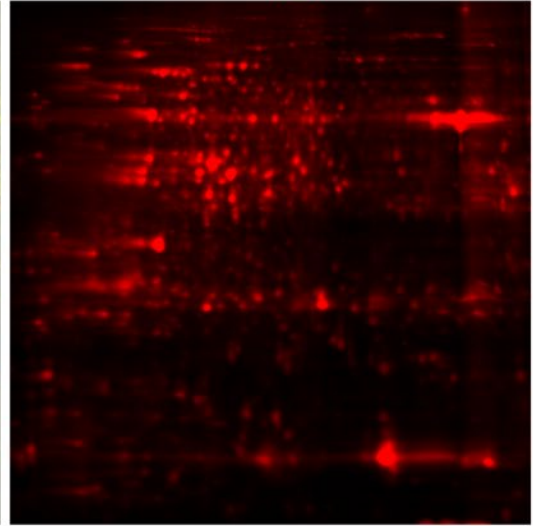
2C



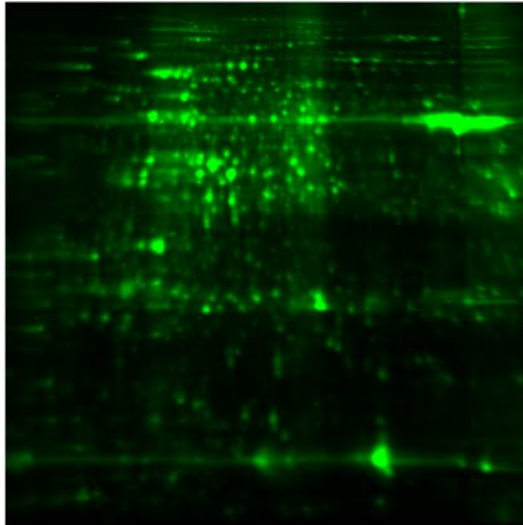
2C + 4C



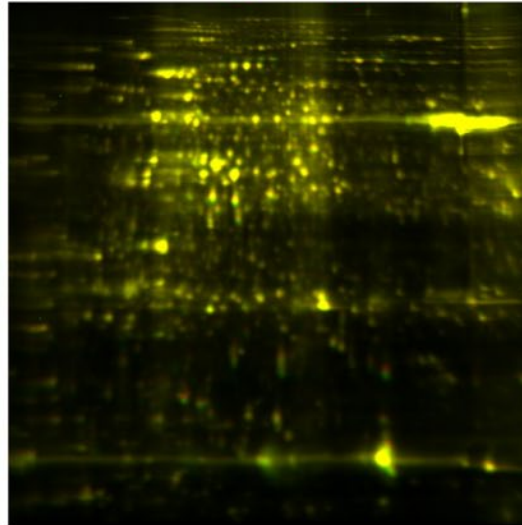
4C



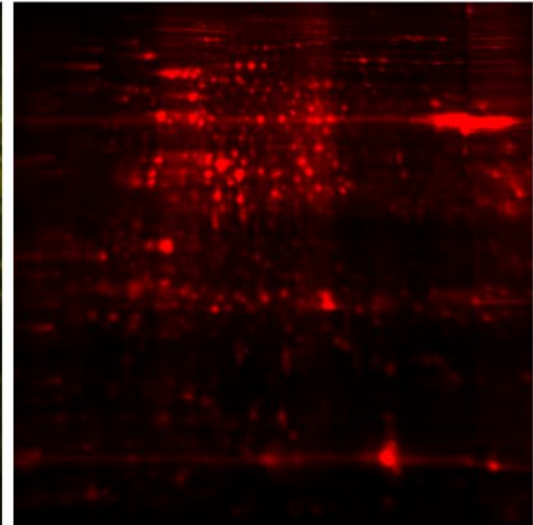
2F



2F + 4F



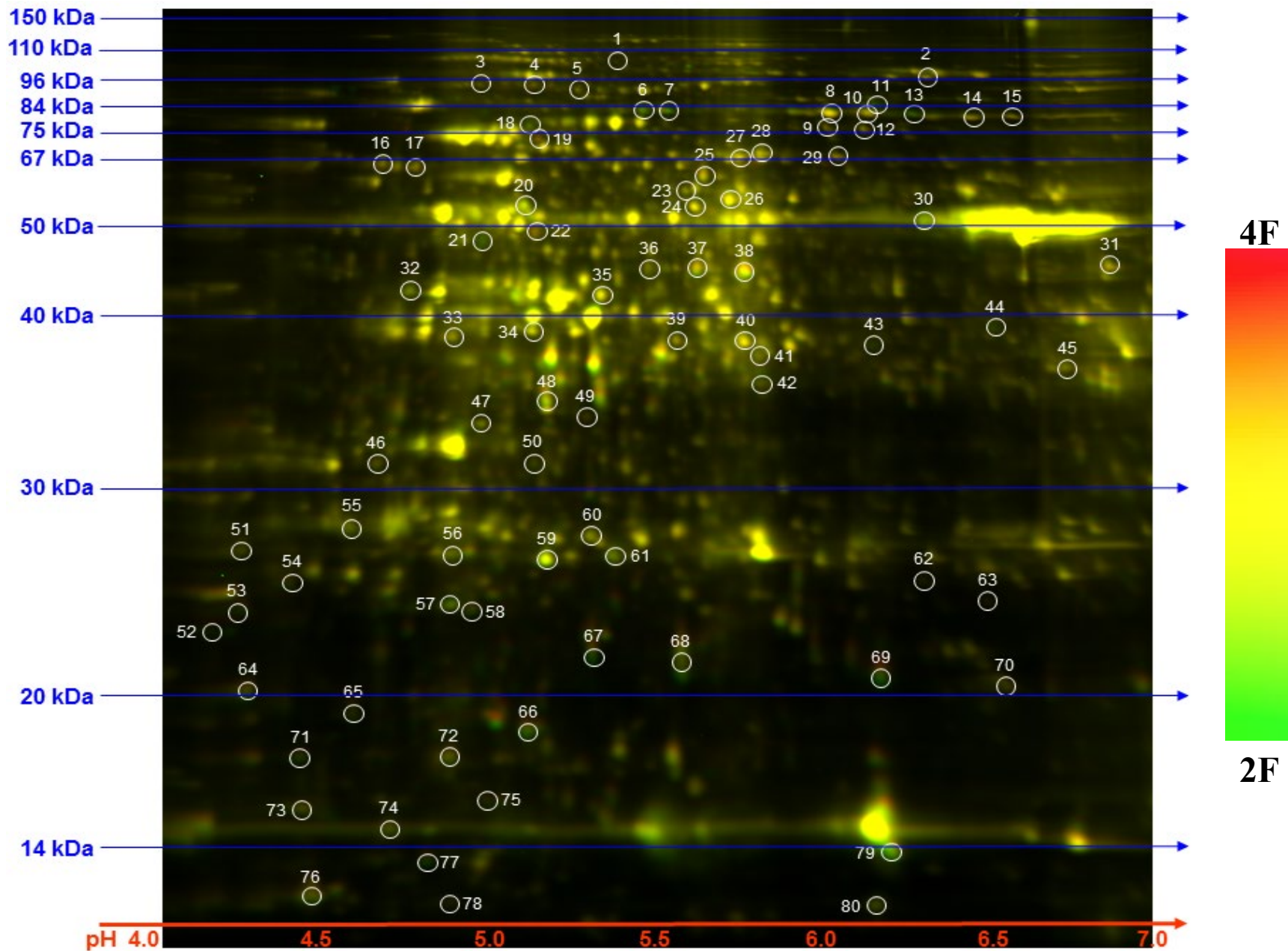
4F



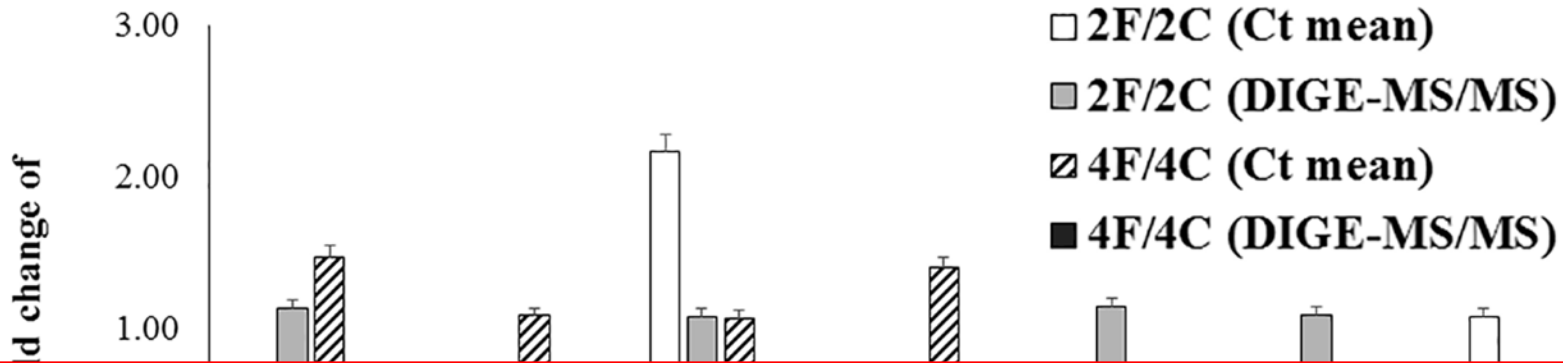
Comparisons

- 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
 - $p \leq 0.05$

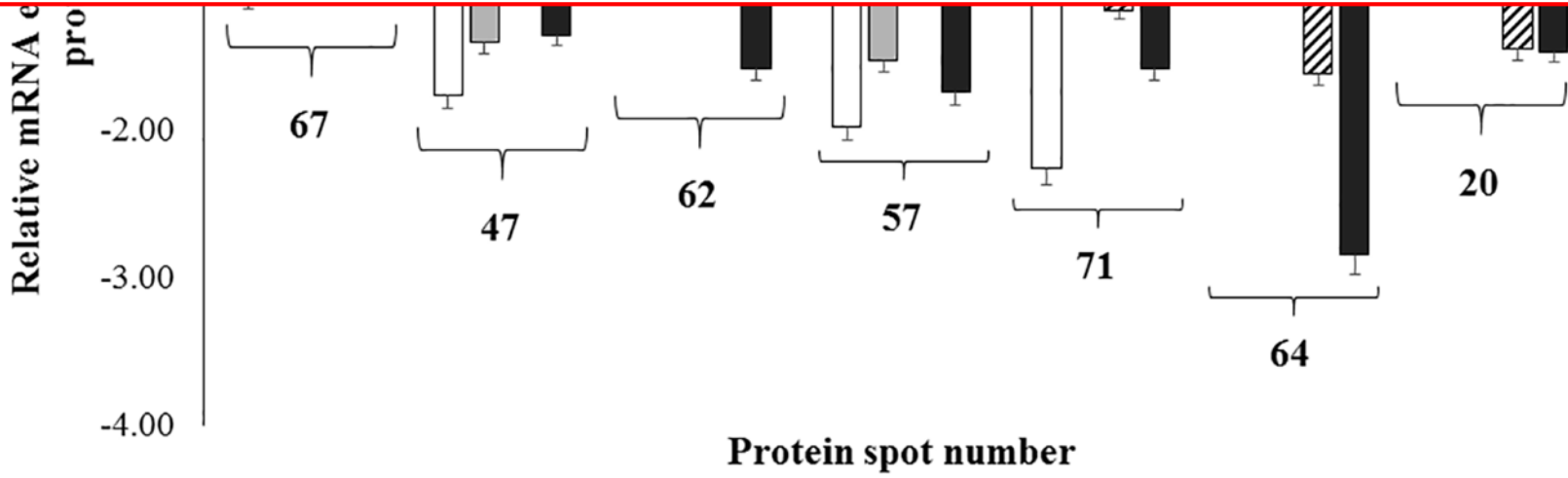
Representative 2D-DIGE Gel Image with the 80 Selected Protein Spots Marked



Example of RT-PCR Validation



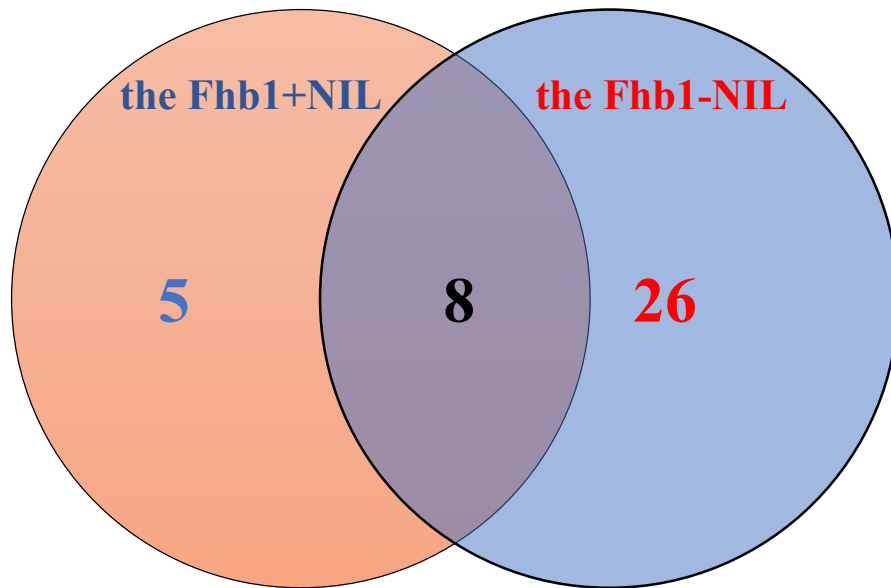
The fold changes in expression of the genes of interest were either similar or insignificant between the two molecular levels



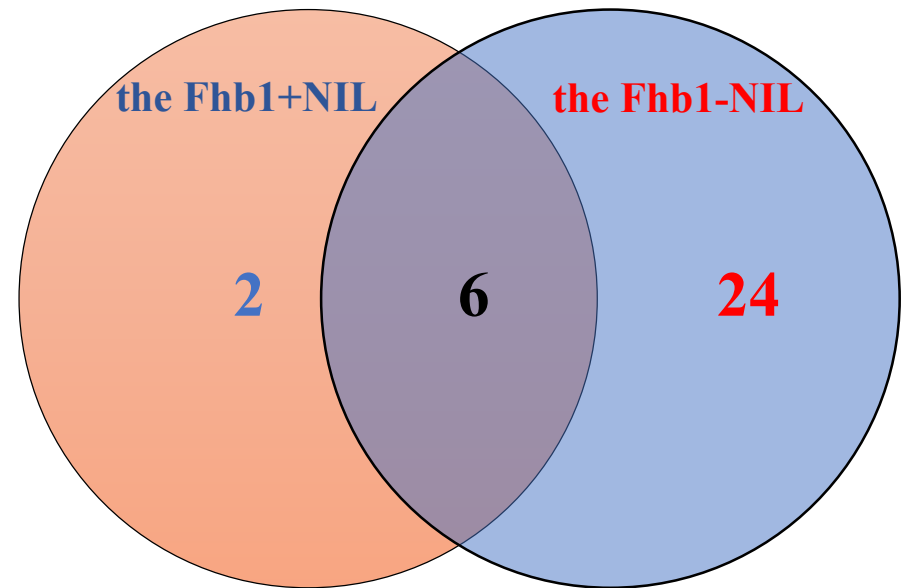
Annotation Thresholds

- 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

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



Up-regulated DAPs

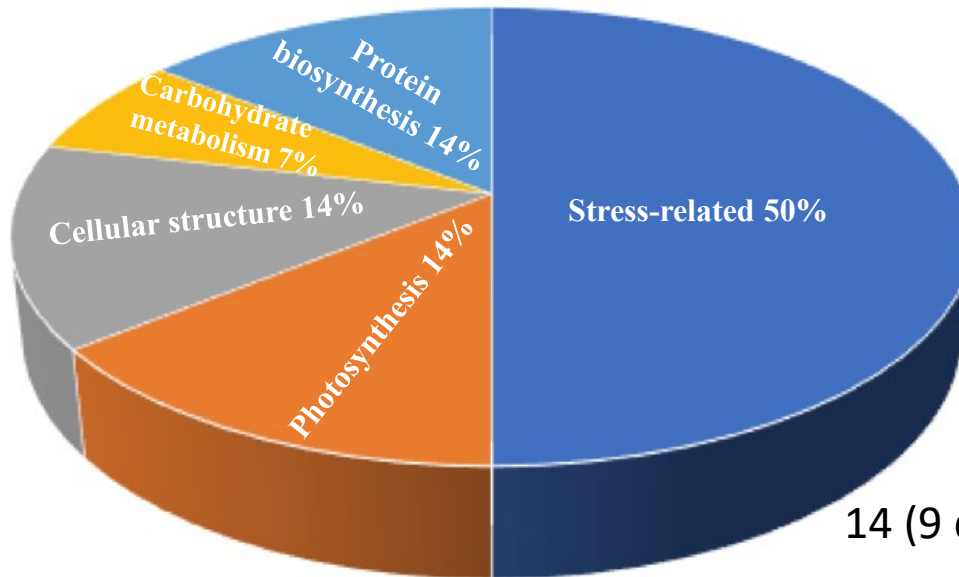


Down-regulated DAPs

- 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
1	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**	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 biphosphate carboxylase small chain clone 512	-1.71	1.05	1.02	-1.76
2	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
	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

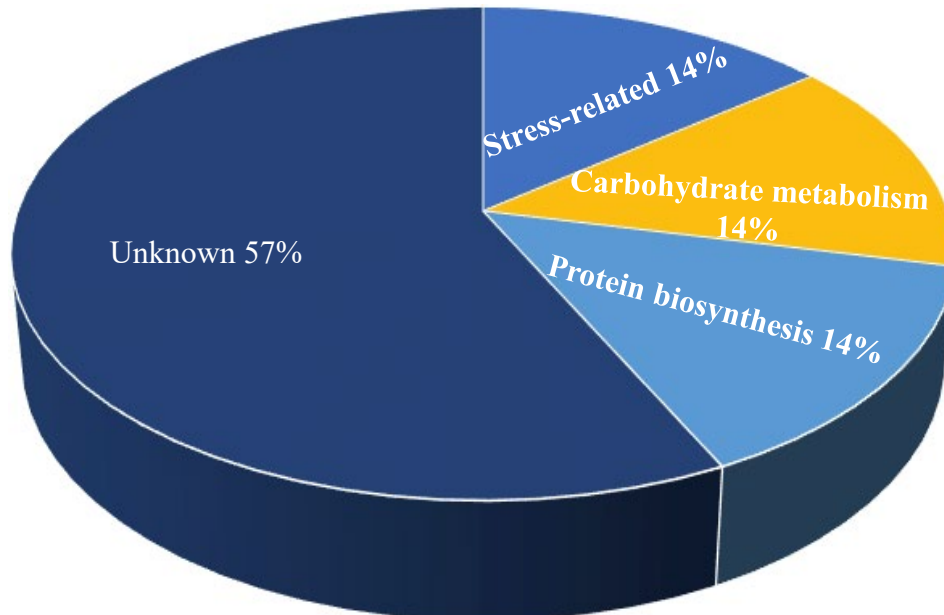
DAPs Between the Two NILs After Fusarium-infection

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 synthase Ila-3
#6	1.07	2.27	1.24	-1.72	Unknown

- *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



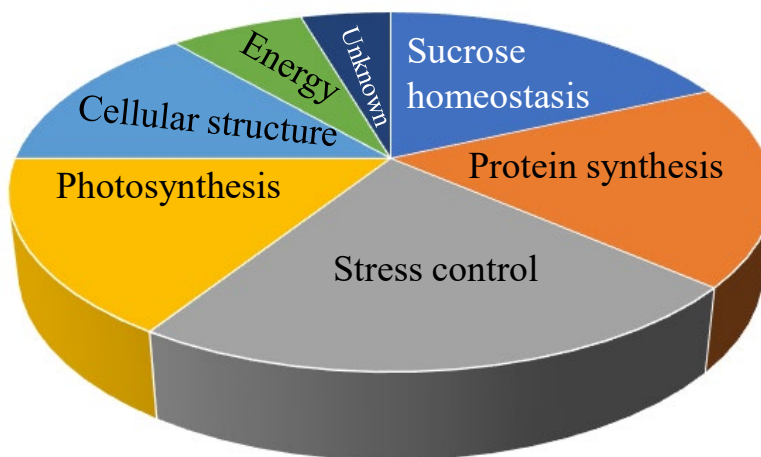
5 up and 2 down

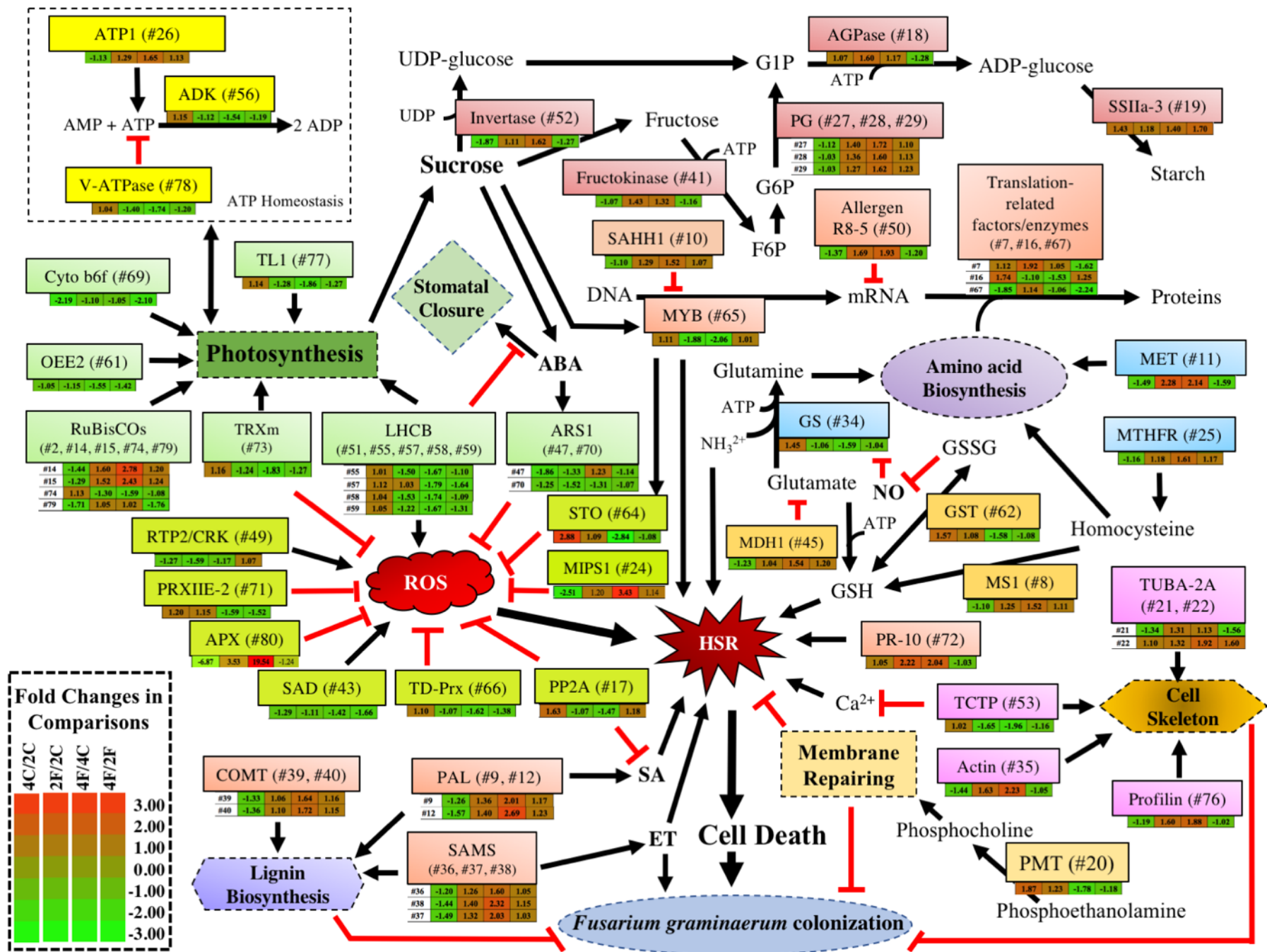
ID	Annotation	4C/2C	2F/2C	4F/4C	4F/2F	ID	Annotation	4C/2C	2F/2C	4F/4C	4F/2F
24	Myo-inositol-1-phosphate synthase	-2.51	1.20	3.43	1.14	71	Peroxisome protein 2E-2, chloroplast-like	1.20	1.15	-1.59	-1.52
12	Phenylalanine ammonia-lyase	-1.57	1.40	2.69	1.23	22	Alpha tubulin-2A	1.10	1.32	1.92	1.60
52	Vacuolar 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 biphosphate 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, chloroplast	-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, chloroplast; 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 biphosphate 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



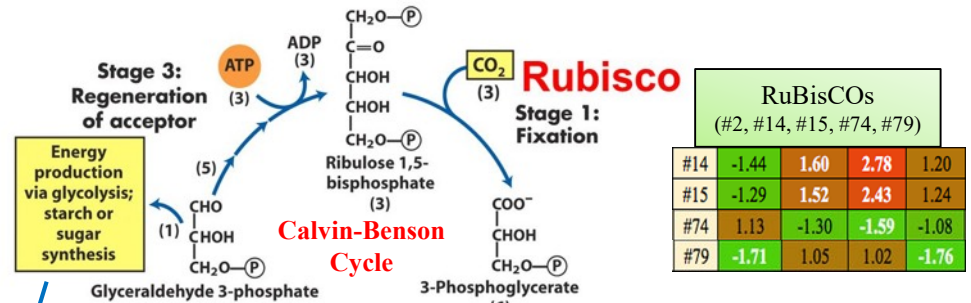


Impacts on Photosynthesis

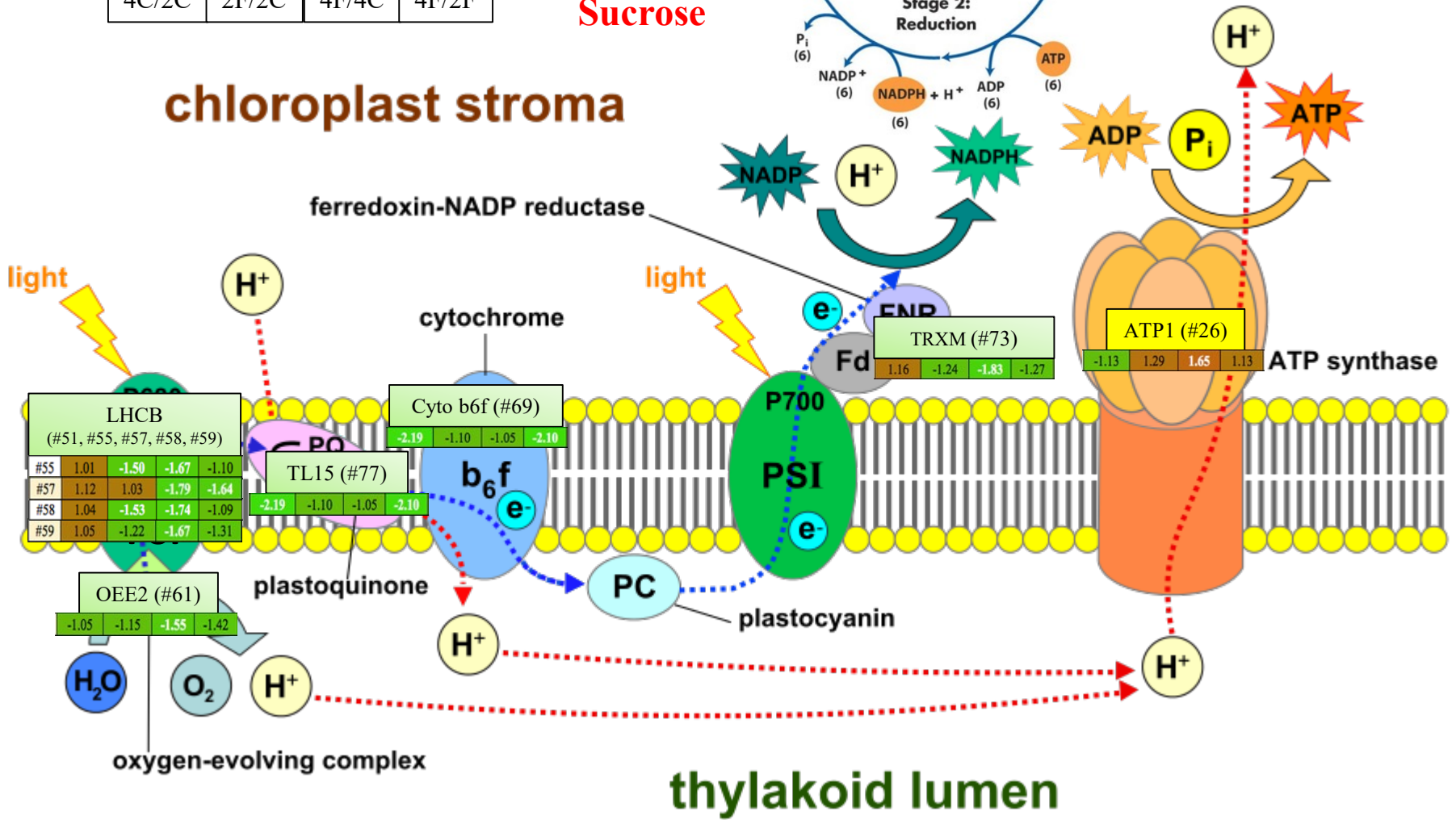
4C/2C	2F/2C	4F/4C	4F/2F
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chloroplast stroma

Sucrose



RuBisCOs (#2, #14, #15, #74, #79)				
#14	-1.44	1.60	2.78	1.20
#15	-1.29	1.52	2.43	1.24
#74	1.13	-1.30	-1.59	-1.08
#79	-1.71	1.05	1.02	-1.76



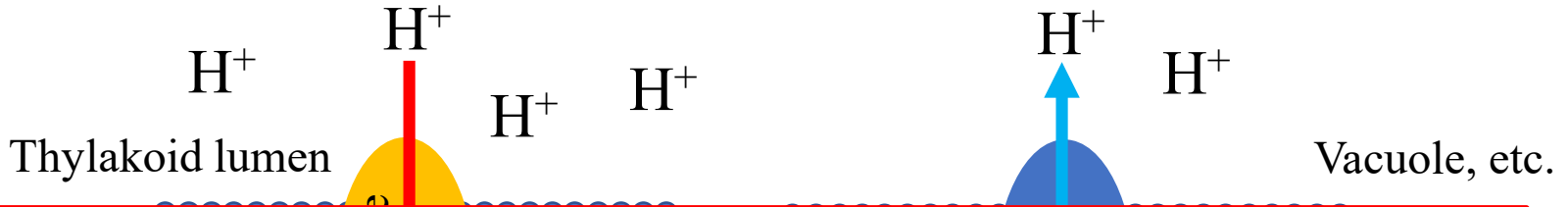
Impacts on Photosynthesis

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	(-1.71)	1.05	1.02	(-1.76)
#26	ATPase 1	-1.13	1.29	(1.65)	1.13

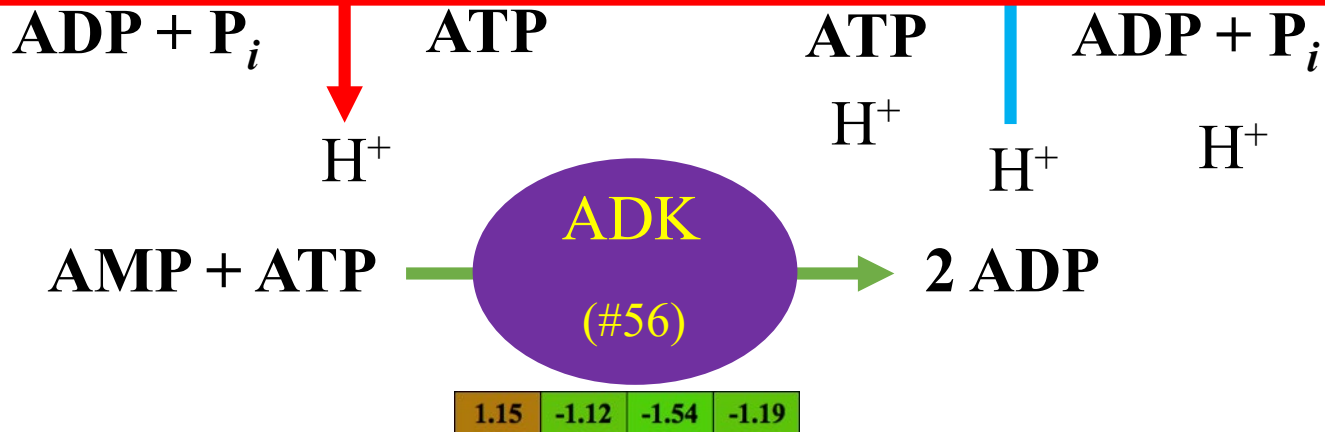
○ Significantly different (≥ 1.5 folds)

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

Impacts on ATP Homeostasis

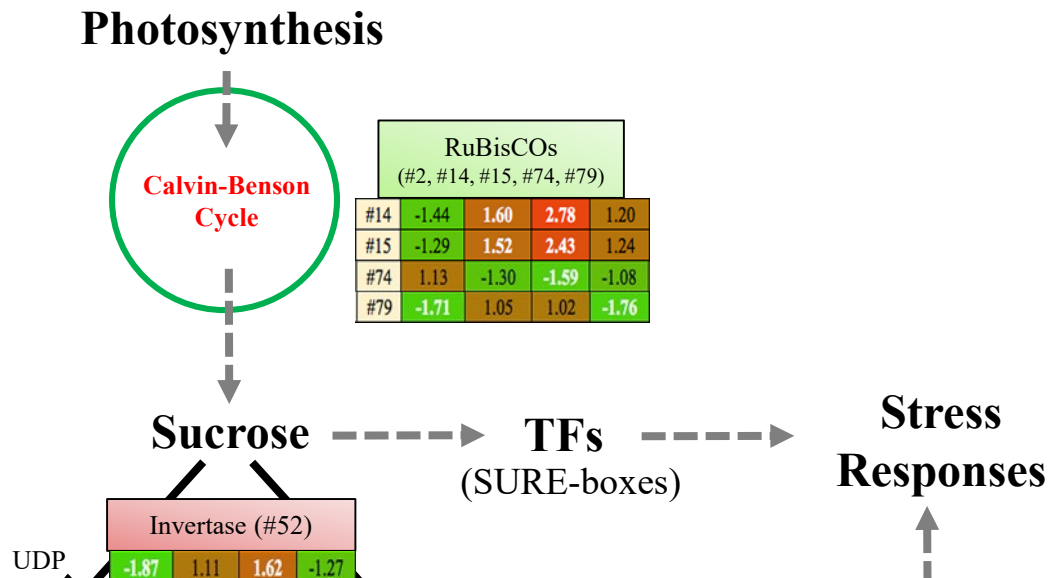


In the absence of *Qfhb1*, *F. graminearum* infection severely disrupts the balance of proton gradients across relevant membranes and significantly increases ATP in cytosol.

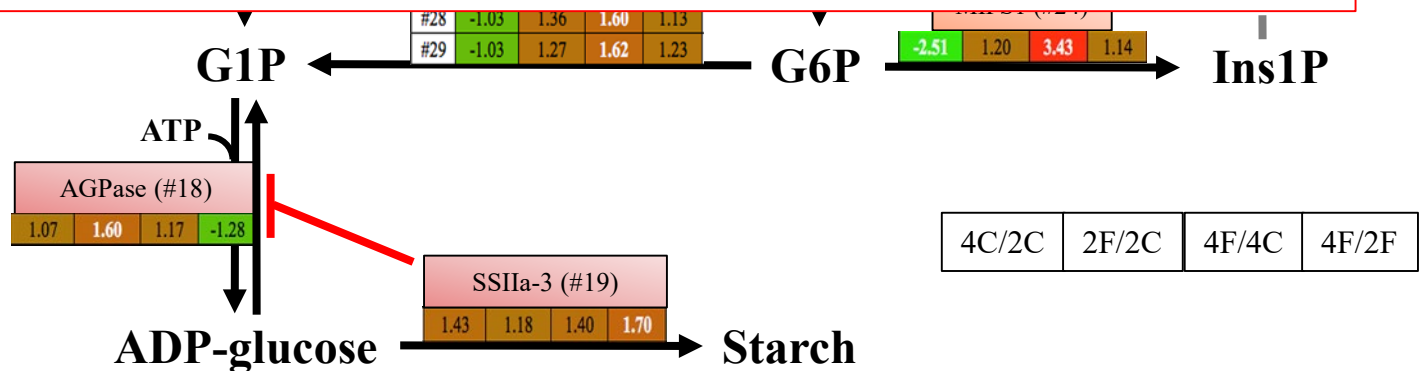


4C/2C	2F/2C	4F/4C	4F/2F
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Impacts on Sucrose Homeostasis



The absence of *Qfhb1* makes the *fhb1*-NIL vulnerable 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	TCTP	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.

Impacts on Hypersensitive Reaction

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	

- 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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SDSU Scholar and Research Initiative Fund to JR

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University of Minnesota: James Anderson

USDA-ARS-Dale Bumpers National Rice Research Center: Anna McClung

South Dakota State University: Shaukat Ali, Bimal Paudel, Tajbir Raihan

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 calcium-binding 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