

# Supplementary Materials: The Regulatory Mechanism of Water Activities on Aflatoxins Biosynthesis and Conidia Development, and Transcription Factor AtfB is Involved in This Regulation

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**Table S1.** Comparisons of several global regulators in different  $a_w$  by transcriptome analysis.

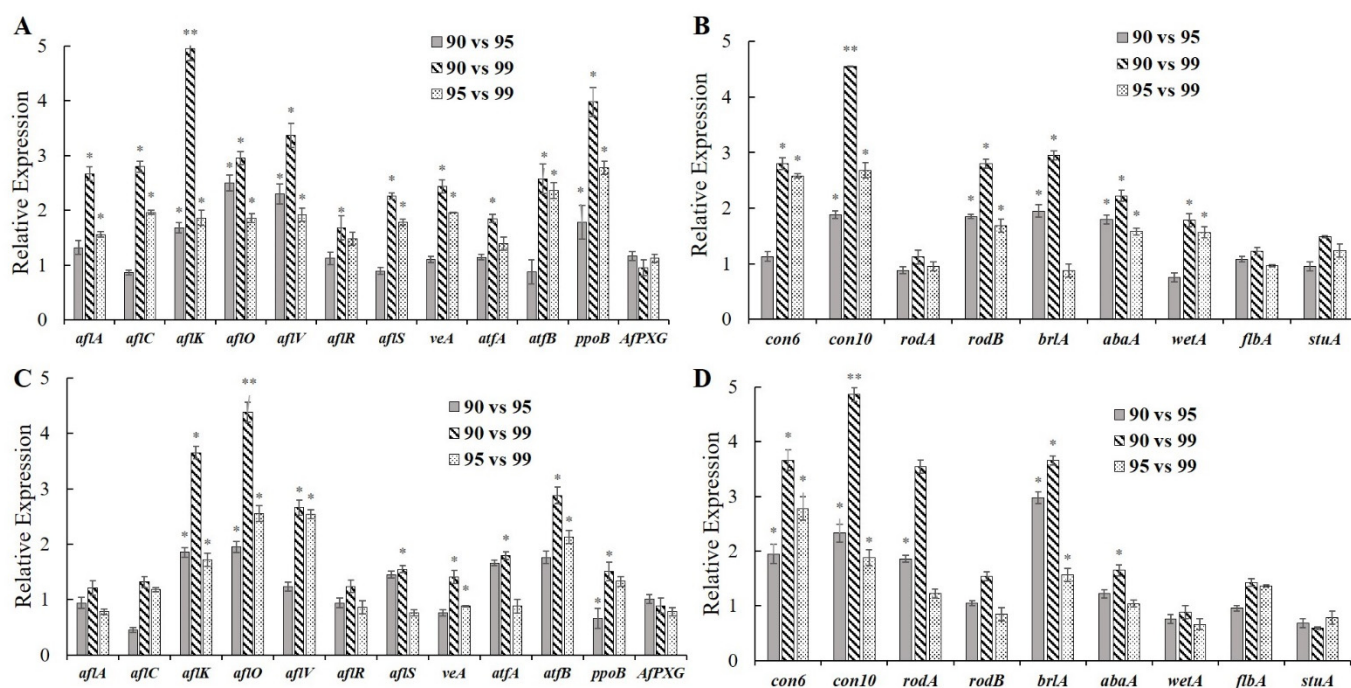
Gene ID (AFLA_)	Gene	Gene Description	log <sub>2</sub> (90/95)	log <sub>2</sub> (90/99)	log <sub>2</sub> (95/99)
066460	<i>veA</i>	developmental regulator AflYf / VeA	0.51	−0.95	−1.48
033290	<i>laeA</i>	regulator of secondary metabolism LaeA	−0.53	−0.12	0.39
084190	<i>velB</i>	conserved hypothetical protein	0.23	−0.47	−0.72
083380	<i>pbsB</i>	MAP kinase kinase (Pbs2) putative	0.42	0.16	−0.28
062500	<i>maf1</i>	mitogen-activated protein kinase MAF1	0.22	−0.19	−0.42
103480	<i>ste7</i>	MAP kinase kinase Ste7	−0.08	0.02	0.08
035530	<i>ste20</i>	serine/threonine kinase Ste20	−0.38	0.28	0.64
048880	<i>ste11</i>	MAP kinase kinase kinase Ste11	0.24	0.33	0.07
052570	<i>mpkA</i>	MAP kinase MpkA	0.14	0.58	0.42
051240	<i>map2k</i>	MAP kinase kinase (Mkk2) putative	−0.19	0.49	0.66
034170	<i>fus3</i>	MAP kinase FUS3/KSS1	−0.23	0.14	0.36
031560	<i>bck1</i>	MAP kinase kinase kinase (Bck1), putative	0.80	0.47	−0.34
068590	<i>sskB</i>	MAP kinase kinase kinase SskB putative	0.03	0.22	0.17
061090	<i>sakA1</i>	MAP kinase SakA	−1.38	0.87	2.23
099500	<i>sakA2</i>	MAP kinase SakA	−0.94	−1.51 *	−0.59
031340	<i>atfA</i>	bZIP transcription factor (AtfA), putative	−0.11	−0.35	−0.25
094010	<i>atfB</i>	bZIP transcription factor (Atf21) putative	1.06	3.69 *	2.60 *
129340	<i>ap-1</i>	conserved hypothetical protein	−0.27	−0.50	−0.24
110650	<i>msnA</i>	C2H2 transcription factor (Seb1) putative	−0.20	0.21	0.38
091490	<i>mtfA</i>	C2H2 finger domain protein putative	−0.72	−1.14	0.43
034540	<i>srrA</i>	stress response transcription factor SrrA/Skn7, putative	0.50	0.18	−0.34
062210	<i>sskA</i>	response regulator putative	0.15	0.44	0.28
026790	<i>ppoA</i>	fatty acid oxygenase PpoA, putative	−0.30	−2.59	2.29
120760	<i>ppoB</i>	fatty acid oxygenase, putative	6.59 *	12.54 *	5.94 *
030430	<i>ppoC</i>	conserved hypothetical protein	0.21	−2.19	−2.41
101920	<i>fluG</i>	extracellular developmental signal biosynthesis protein FluG	0.06	0.40	0.32
002850	<i>AfPXG</i>	calcium binding protein Caleosin, putative	−0.41	2.24 *	2.63*
025100	<i>gpdA</i>	glyceraldehyde 3-phosphate dehydrogenase GpdA	−0.43	0.29	0.71
046760	<i>gfdB</i>	glycerol 3-phosphate dehydrogenase (GfdB) putative	−0.75	0.81	1.54
060740	<i>gprA</i>	mating-type alpha-pheromone receptor PreB	0.94	0.84	−0.12
061620	<i>gprB</i>	a-pheromone receptor PreA	−0.51	0.66	1.15
074150	<i>gprC</i>	conserved hypothetical protein	−0.75	−0.94	−0.21
135680	<i>gprD</i>	G protein-coupled receptor GprD	−0.96	0.10	1.05
006880	<i>gprF</i>	PQ loop repeat protein	0.05	−0.13	−0.20
067770	<i>gprG</i>	PQ loop repeat protein	1.39	1.28	−0.13
006920	<i>gprH</i>	cAMP receptor-like protein, putative	2.03 *	0.36	−1.69
127870	<i>gprJ</i>	vacuolar membrane PQ loop repeat protein	0.55	−0.83	−1.40
009790	<i>gprK</i>	conserved hypothetical protein	0.98	0.22	−0.78

075000	<i>gprM</i>	conserved hypothetical protein	−1.06	−1.35	−0.32
032130	<i>gprO</i>	hemolysin-III channel protein Izh2 putative	−0.23	−0.08	0.14
088190	<i>gprP</i>	IZH family channel protein (Izh3) putative	−0.42	0.03	0.43
023070	<i>gprR</i>	integral membrane protein	0.11	1.51 *	−1.39
006320	<i>gprS</i>	PQ loop repeat protein	−0.02	0.84	0.84
018340	<i>fadA</i>	G-protein complex alpha subunit GpaA/FadA	0.02	−0.18	−0.21
093240	<i>sfaD</i>	G-protein complex beta subunit SfaD	0.03	−0.19	−0.24
032870	<i>pkaR</i>	cAMP-dependent protein kinase regulatory subunit PkaR	0.13	1.54 *	1.40
135040	<i>pkaC</i>	cAMP-dependent protein kinase catalytic subunit PkaC1	0.07	1.76 *	1.67 *
018930	<i>capA</i>	adenylyl cyclase-associated protein (cap)	−0.16	−0.24	−0.09
071410	<i>somA</i>	cAMP-dependent protein kinase pathway protein (Som1)	0.37	0.25	−0.14
112560	<i>sok1</i>	cAMP-mediated signaling protein Sok1 putative	0.80	−0.10	−0.92
134680	<i>creA</i>	C2H2 transcription factor (CreA) putative	−0.24	0.45	0.68
049870	<i>areA</i>	GATA transcriptional activator AreA	0.67	1.74 *	1.06
030580	<i>pacC</i>	C2H2 transcription factor PacC putative	−0.50	−2.02 *	−1.53
012010	<i>farB</i>	C6 transcription factor (Ctf1B) putative	−1.27	−1.30	0.06

Transcriptome analyses were performed three biological replicates. Data was calculated with read counts. 90/95, 90/99 and 95/99 represented the comparisons of  $a_w$  0.90 vs 0.95,  $a_w$  0.90 vs 0.99 and  $a_w$  0.95 vs 0.99, respectively. Significances were marked as \* with  $p_{adj} < 0.05$  and  $\log_2 \text{ratio} \geq 1$  or  $\leq -1$ .

**Table S2.** Primers used for qPCR analyses.

Gene Name	Gene ID (AFLA_XXX)	Primers		Fragment Length (bp)
<i>aflA</i>	139380	F: aattgctcaacttctacc	R: ttgggttgccctcgccaaag	143
<i>aflC</i>	139410	F: ttggaaccgctctagtgtgcc	R: agcgactgcagcttaccgcc	140
<i>aflK</i>	139190	F: tgtccaggatggaactgcttc	R: ttgataccagtcataatagagg	152
<i>aflO</i>	139220	F: agcagtggaacccctacaag	R: tccgaagaatcgaccaagg	151
<i>aflV</i>	139180	F: taaggtccctggctccctgg	R: atcggtctatgtcaacctcc	151
<i>aflR</i>	139360	F: aggagaaacggcctgtgctcg	R: aagtcctgttccgacctgg	158
<i>aflS</i>	139340	F: tgaccatctccgaccgttc	R: acgccagcacctggaactcc	154
<i>veA</i>	066460	F: tgcggtgcaggtgcaaagtcc	R: ttccaaagtggcgctacagg	160
<i>atfA</i>	031340	F: ttcgctgtctaatataacc	R: agtcttcagttccaaagcg	148
<i>atfB</i>	094010	F: tctcacagcctgacctgatgg	R: aaggccctctccatgttggg	143
<i>ppoB</i>	120760	F: tgtggagagatctccgcgcc	R: aagctggatgatgcgttcc	143
<i>AfPXC</i>	002850	F: acggattcccgcgactcacatc	R: ataggacgtgctgctggagag	149
<i>con6</i>	044800	F: tggctacaaggccaccctc	R: tcggatcctgttctgctcacc	140
<i>con10</i>	083110	F: tcagcaaccgtccccacgagg	R: accgggttgaaagcttccgc	152
<i>rodA</i>	098380	F: tggcgtggccagcaagtgc	R: agcaaggagaccctctc	166
<i>rodB</i>	014260	F: tgtcgtgctctcttcgcc	R: agcagacggggtggtgcc	164
<i>brlA</i>	082850	F: agcttctcgccatggattcc	R: tagacggaaagcactccgc	153
<i>abaA</i>	029620	F: accagcagaccgttcgaatgg	R: agtaagattgcaccggcc	140
<i>wetA</i>	052030	F: agcagtcaccatcatctcc	R: atgaacggtgtcttgaatg	137
<i>flbA</i>	134030	F: atctctcgccattggagg	R: tccgataatggtggagtcag	146
<i>stuA</i>	046990	F: tcgacggccaatctcgg	R: accagccgctaagtcgcag	153
<i>actin</i>	055230	F: ctttctggtcgatgatgctc	R: tggagtgaaggacaccacg	138



**Figure S1.** Transcriptional expressions analyses of diverse genes by RT-qPCR. The RT-qPCR analysis of (A) AF biosynthesis related genes and (B) conidia developmental genes in different  $a_w$  conditions in *A. flavus* CA14. The RT-qPCR analysis of (C) AF biosynthesis related genes and (D) conidia developmental genes in different  $a_w$  conditions in *A. flavus* ACCC 32656. The different  $a_w$  comparisons were showed as diverse bars. Three independent biologic replicates were performed in each condition, and data were presented as means  $\pm$  SD.  $t$  tests were applied for significance analyses with \*  $p < 0.05$  and \*\* $p < 0.01$ .