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Supplementary Material

Physiological, proteomic and transcriptional responses of wheat to combination of drought or waterlogging with late spring low temperature

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Table S1. Explanations of selected JIP-test parameters used in the present study

Subscript "o" indicates that the parameters refer to illumination onset, when all RCs are assumed to be open

Fluorescence parameters	
F_t	Fluorescence at time t after onset of actinic illumination
F_O	Minimal fluorescence, when all PSII RCs (reaction centers) open
$F_V=F_t-F_O$	Variable fluorescence at time t
F_M	Maximal recorded fluorescence intensity
$W_{OJ}=(F_t-F_O)/(F_J-F_O)$	Ratio of variable fluorescence F_t-F_O to the amplitude F_J-F_O
$W_{OI}=(F_t-F_O)/(F_I-F_O)$	Ratio of variable fluorescence F_t-F_O to the amplitude F_I-F_O
$W_{IP}=(F_t-F_I)/(F_P-F_I)$	Ratio of variable fluorescence F_t-F_I to the amplitude F_P-F_I
TR_O/RC	Trapped energy flux (leading to Q_A reduction) per RC
ET_O/RC	Electron transport flux (further than Q_A) per RC
RE_O/RC	PSI acceptor per RC
$\varphi_{PO}=1-F_O/F_M$	Maximum quantum yield for primary photochemistry
$\varphi_{EO}=(1-F_O/F_M)(1-V_J)$	Quantum yield for electron transport (ET)
$\varphi_{RO}=\varphi_{PO}\ (1-V_I)$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$\psi_{EO}=PSI_O$	Probability that an electron moves further than Q_A
$TR_O/CS_O=\varphi_{PO}$ (ABS/CS)	Trapped energy flux per CS
$ET_O/CS_O=\varphi_{PO}\psi_{EO}$ (ABS/CS)	Electron transport flux per CS
RE_O/CS_O	PSI acceptor per CS
ABS/CS	Absorption flux per CS

Table S2. Identification of differentially expressed proteins in wheat leaves exposed to interaction of low temperature and water stresses through MALDI-TOF/TOF

Spot ID are named according to Fig. S5. GI refers to accession no.. NCBI refers to database accession no.. Mr/pI refers to molecular weight and isoelectric point of identified protein. Score refers to Mascot protein score. SC refers to Sequence Coverage. NMP refers to Number of Matched Peptides.

Spot ID	Protein name	GI accession no.	Mr/pI (theor.)	Score	SC (%)	NMP	Taxonomy
1	dehydroascorbate reductase	28192421	23457/5.88	780	21	13	*Triticum aestivum*
2	ribulose-1,5-bisphosphate carboxylase activase	37783283	22493/4.98	536	20	7	*Triticum aestivum*
3	ferredoxin-NADP(H) oxidoreductase	20302473	40491/6.92	510	21	9	*Triticum aestivum*
4	ATP synthase beta subunit	110915710	53017/5.17	287	13	7	*Vulpia microstachys*
5	S-adenosylmethionine synthase 1	223635282	43247/5.61	983	24	10	*Triticum monococcum*
6	glyceraldehyde-3-phosphate dehydrogenase subunit B	87331054	34541/6.01	252	12	9	*Spirogyra sp.*
7	mitochondrial aldehyde dehydrogenase ALDH2	15128580	59644/6.25	340	12	6	*Hordeum vulgare subsp. vulgare*
8	catalase-1	2493543	57000/6.52	600	28	13	*Triticum aestivum*
9	ascorbate peroxidase	15808779	27964/5.10	508	21	9	*Hordeum vulgare subsp. Vulgare*
10	ATP synthase subunit alpha, mitochondrial	114419	55515/5.70	771	22	10	*Triticum aestivum*
11	phosphoglucomutase	18076790	62978/5.66	914	16	10	*Triticum aestivum*
12	RuBisCO large subunit-binding protein subunit alpha, chloroplastic precursor	134102	57656/4.83	351	11	7	*Triticum aestivum*
13	chloroplast glutathione reductase	148250114	50868/6.17	163	14	6	*Dasypyrum villosum*
14	glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic-like	357148595	49774/6.18	608	18	7	*Brachypodium distachyon*
15	enolase 2-like	357113118	48372/5.50	624	20	7	*Brachypodium distachyon*

16	6-phosphogluconate dehydrogenase	412991069	56923/5.56	323	8	6	*Bathycoccus prasinos*
17	Cysteine synthase	585032	34207/5.48	192	10	5	*Triticum aestivum*
18	ribulose-1,5-bisphosphate carboxylase activase isoform 1	167096	47365/8.62	122	5	5	*Hordeum vulgare subsp*
19	glyceraldehyde-3-phosphate dehydrogenase subunit B	87331054	34541/6.01	240	9	6	*Spirogyra sp.*
20	fructose-1,6-bisphosphatase, cytosolic	300681469	37854/5.38	503	22	7	*Triticum aestivum*
21	heat shock protein 70	30025966	71208/5.17	487	17	7	*Nicotiana tabacum*
22	Phosphoglycerate kinase, chloroplastic	129915	49980/6.58	575	15	9	*Triticum aestivum*
23	NADP-dependent isocitrate dehydrogenase	15982950	46917/6.54	128	14	5	*Triticum aestivum*
24	chloroplast fructose-bisphosphate aldolase	223018643	42217/5.94	273	17	6	*Triticum aestivum*
25	aestivum stearoyl-ACP desaturase	319739540	44712/7.15	122	15	7	*Triticum aestivum*
26	fructose-1,6-diphosphate aldolase	21913296	31210/6.99	222	17	7	*Metasequoia glyptostroboides*
27	S-adenosylmethionine synthase 3	122220777	43138/5.51	506	16	9	*Hordeum vulgare*
28	ferredoxin--NADP reductase, leaf isozyme, chloroplastic-like	357110920	40807/6.72	409	14	9	*Brachypodium distachyon*
29	vacuolar proton-ATPase subunit A	90025017	68754/5.23	428	16	9	*Triticum aestivum*
30	ATP synthase subunit alpha, mitochondrial	114411	55595/6.51	507	19	9	*Phaseolus vulgaris*
31	NADPH producing dehydrogenase of the oxidative pentose phosphate pathway	162463282	53307/5.92	655	16	10	*Zea mays*
32	UDP-glucose pyrophosphorylase	6136111	51783/5.20	512	21	9	*Hordeum vulgare*

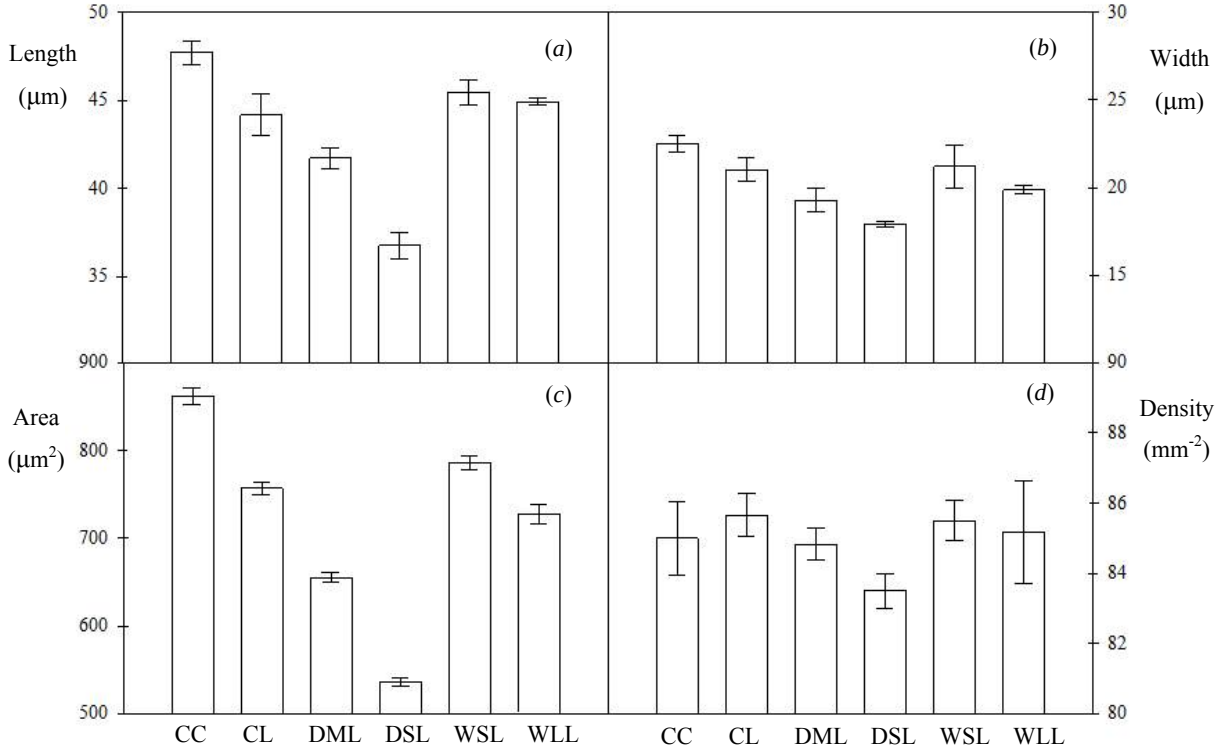

Fig. S1. Mean values of stomatal pore aperture length (a), width (b), area (c) and density (d) on both surfaces of leaves exposed to combination of freezing and water stress at the jointing stage in wheat.

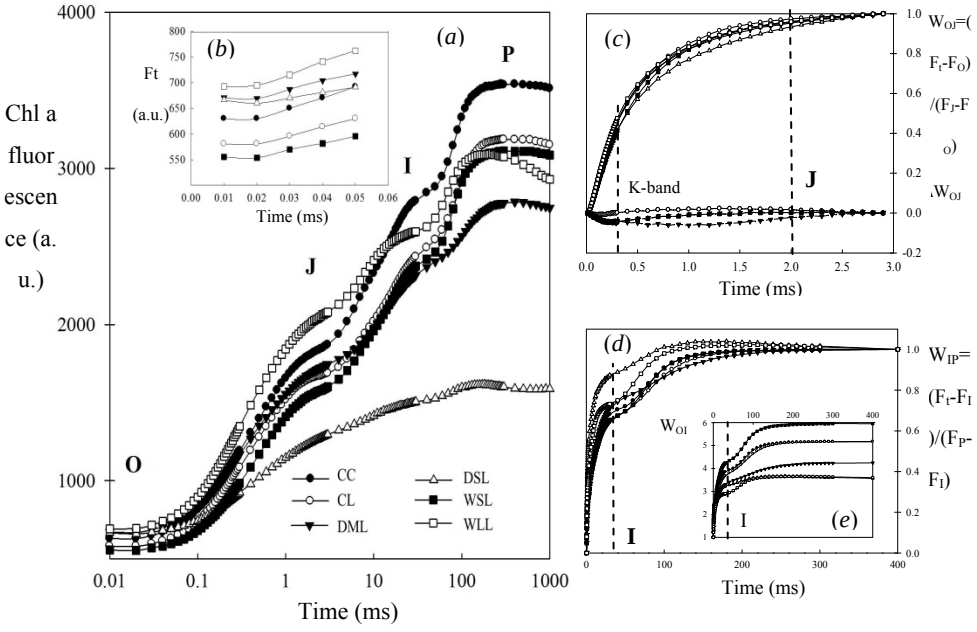

Fig. S2. Chlorophyll fluorescence transient of dark adapted leaves exposed to combination of low temperature and water stress at the jointing stage in wheat. (a) fluorescence intensity on logarithmic time scale; (b), fluorescence intensity from onset of actinic illumination to 0.05s; (c), average kinetics between step O and step J, WOJ=(Ft-FO)/(FJ-FO) and kinetic difference of WOJ, ΔWOJ=WOJ[stress treatment]- WOJ[cc], K-band and J step was plotted, respectively; (d), average kinetics between step I and step P,WOI=(Ft-FI)/(FP-FI); (e), average kinetics between step O and step I,WOI=(Ft-FO)/(FI-FO). Abbreviations of treatments are explained in Fig. 1.

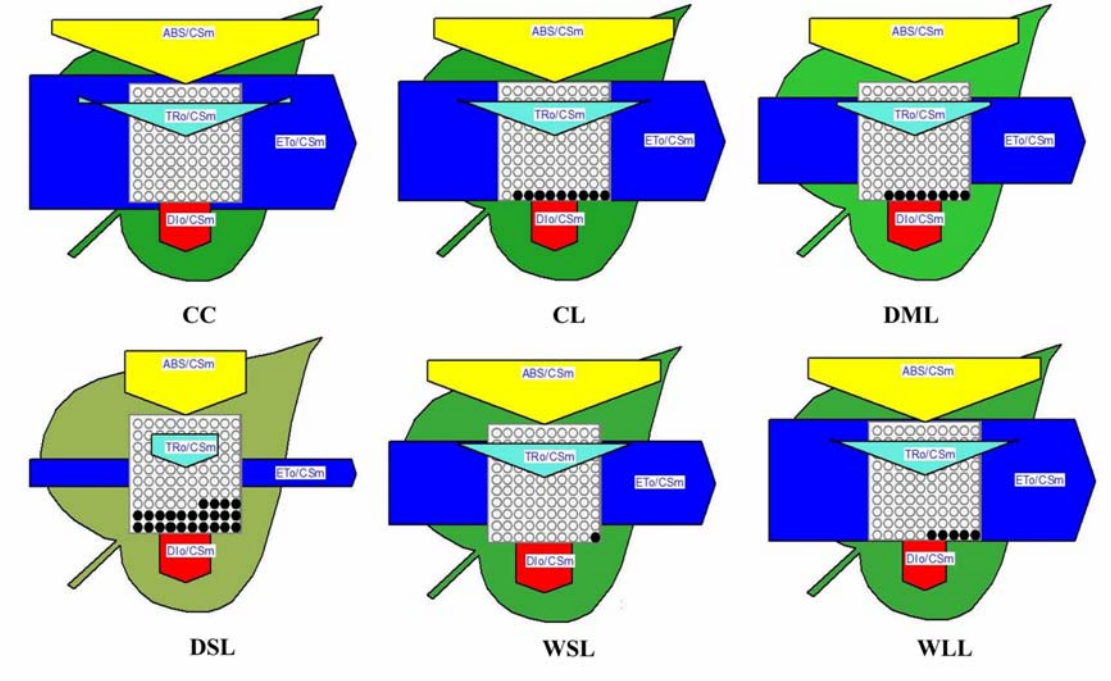

Fig. S3. Energy pipeline leaf model of phenomenological fluxes (per cross-section, CS) in top 1st leaves exposed to combination of low temperature and water stress at the jointing stage in wheat. The value of each parameter was shown as the relative changes in width of each arrow. Active RCs are shown as open circles, while inactive RCs are closed circles. Abbreviations of treatments are explained in Fig. 1.

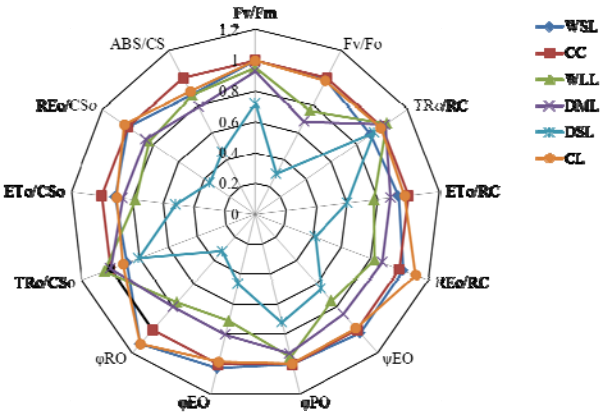

Fig. S4. Fluorescence transient chlorophyll a parameters deduced from analysis of the JIP-test of wheat leaves exposed to interaction of spring freeze and water stress at the jointing stage.

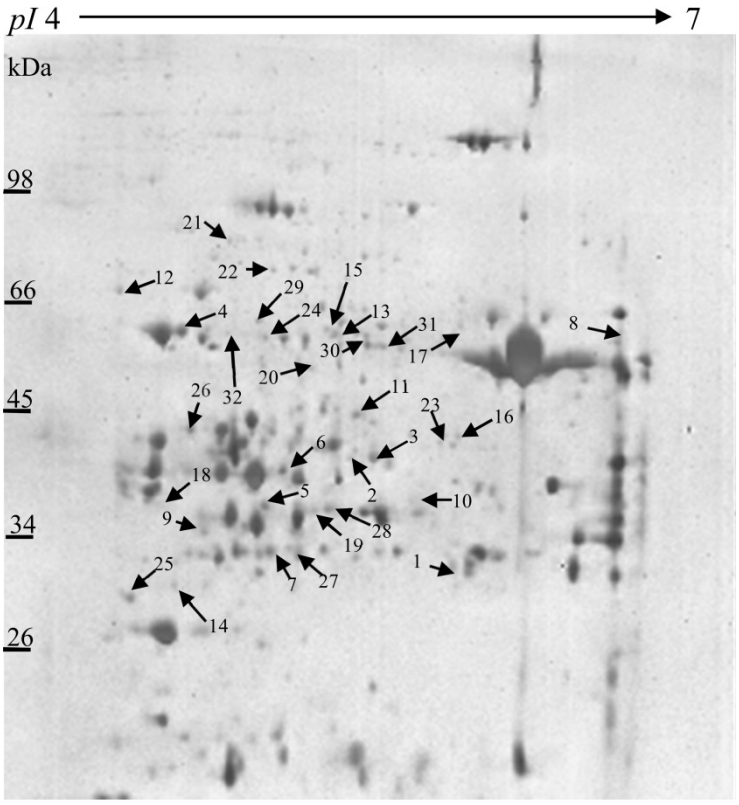

Fig. S5. Reference 2-DE gel of proteins in wheat leaves exposed to interaction of spring low temperature and water stress at the jointing stage. Differentially expressed protein spots in stress treatments (CL, DML, DSL, WSL, WLL) compared with CC were indicated with arrows and listed in Table S2. Abbreviations of treatments are explained in Fig. 1.

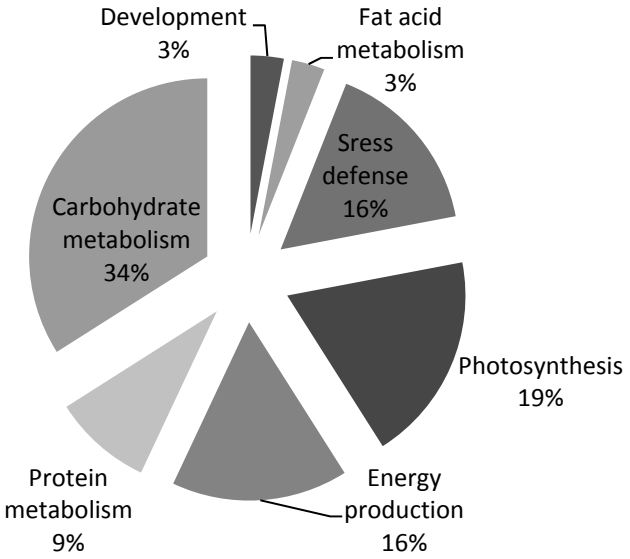

Fig. S6. Functional classification and distribution of all identified proteins based on analysis of sequence homology as listed in Table S2.

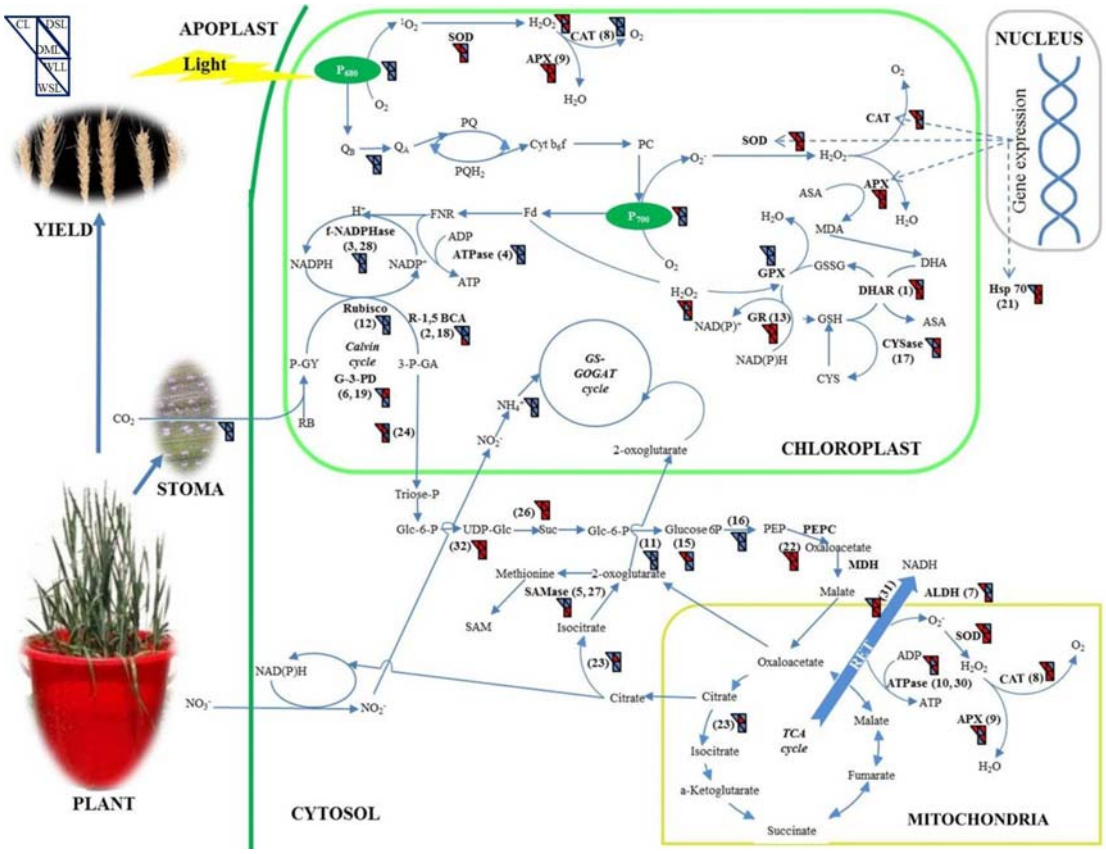

Fig. S7. Global presentation on response of winter wheat cv. Yannong 19 to the combination of low temperature and water stress. The numbers in parentheses indicates the protein spots in Table S2. The up- and down- regulation of pathways in different stress treatments as compared with the CC were indicated by red and blue triangles, respectively. APX, ascorbate peroxidase; AsA, Ascorbic acid; ATPase, ATP synthase; CAT, catalase; CYS, cysteine; CYSase, cysteine synthase; Cyt b_6f, cytochrome; DHA, dehydroascorbate; DHAR, monodehydroascorbate reductase; Fd, ferredoxin; FNR, ferredoxin-$NADP^+$ reductase; Glc-6-P, Glucose-6-phosphate; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; G-3-PD, glyceraldehyde-3-phosphate dehydrogenase; MDA, monodehydroascorbate; MDH, malate dehydrogenase; PC, plastocyanin; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; P-GY, 3-phosphoglycerate; PQ, plastoquinone; P_{680} and P_{700}, PSII and PSI reaction center pigments; Q_A and Q_B, PSII primary and secondary plastoquinone electron acceptors; RB, Ribulose bisphosphate; RET, respiratory electron transport; R-1, 5 BCA, ribulose-1,5-bisphosphate carboxylase activase; SAM, S-adenosyl-L-methionine; SAMase, S-adenosyl-L-methionine synthesis; SOD, superoxide dismutase; Suc, sucrose; Triose-P, Triosephosphate; UDP-Glc, Uridine diphosphate glucose; $^1O_2$, singlet oxygen. Abbreviations of treatments are explained in Fig. 1.