Supporting Information

Humic acid acts as a natural antidote of graphene by regulating nanomaterial translocation and metabolic fluxes *in vivo*

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Number of tables: 2

Characteristics of graphene

Single-layer graphene was prepared by thermal exfoliation reduction and hydrogen reduction. The elements of graphene included carbon and minimal oxygen. XPS measurements revealed that the atomic concentration ratio of C1s to O1s was 96.49: 3.51, as described in Figure S1. The layer structure was very neat and tidy, with a thickness of approximately 0.3 nm, as shown in the FETEM image in Figure S2. The graphene exhibited a nanosheet morphology with a width of 0.5-2 μm and a thickness of 0.8 nm, as demonstrated in an AFM image (Figure S3). The Brunauer-Emmett-Teller (BET) surface area was 500-1000 m^2/g, and the electrical resistivity was less than 0.30 $\Omega.cm^{-1}$. The hydrodynamic diameter, *Zeta* potential, and RS images are presented in the section "Interactions of graphene with HA".

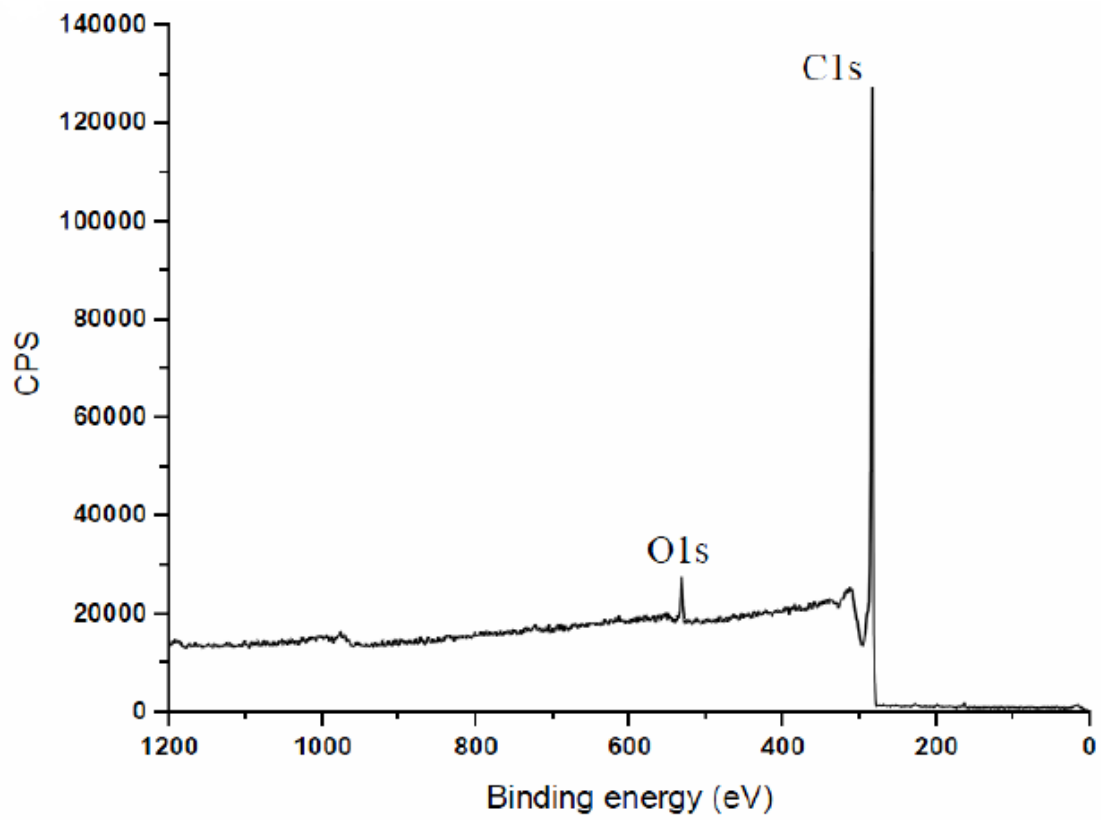


Figure S1. Wide scan X-ray photoelectron spectroscopy spectra of graphene

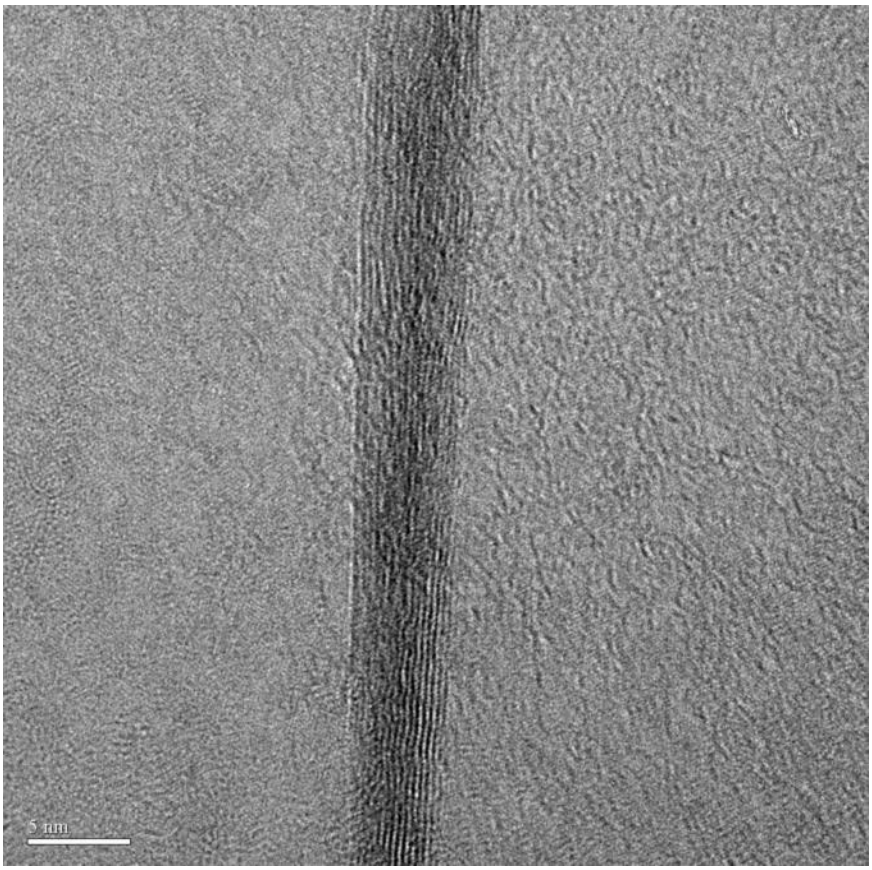


Figure S2. Field emission transmission electron microscopy image of graphene

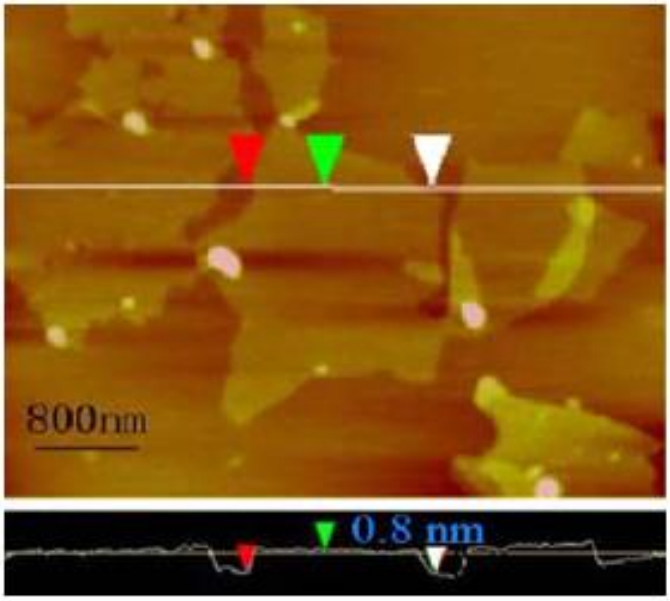


Figure S3. Atomic force microscope image of graphene

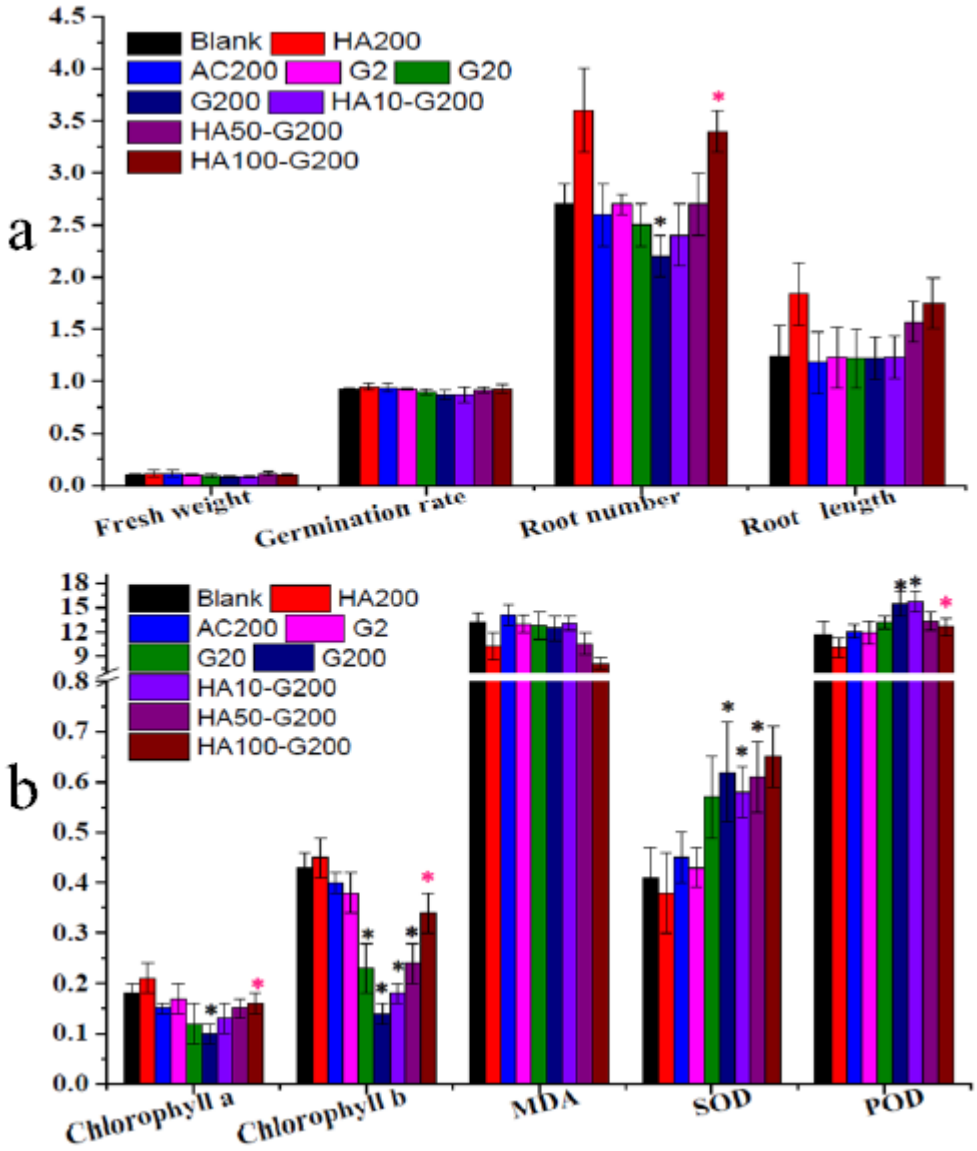


Figure S4. Phytotoxicity of graphene, graphene-HA, humic acid, and active carbon. (a) Graphene influences the growth and development of wheat. (b) Graphene alters chlorophyll synthesis and antioxidase activity. The number after the letter is the concentration (mg/L); thus, G2 and HA 100 represents graphene 2 mg/L and humic acid 100 mg/L. Black and pink "*" symbols indicate that the differences are significant compared to the control and graphene 200 mg/L samples, respectively. Significant level, $P < 0.05$. Units of the biochemical parameters: germination rate, 0-1 representing 0-100%; fresh weight of wheat, g; C_a, mg/g; germinal average length, cm;

root average length, cm; MDA, mg/g; SOD, U/mg/protein; CAT, U/g/protein; POD, U/mg/protein. HA, humic acid; AC, active carbon; MDA, malondialdehyde; SOD, superoxide dismutase; POD peroxidase.

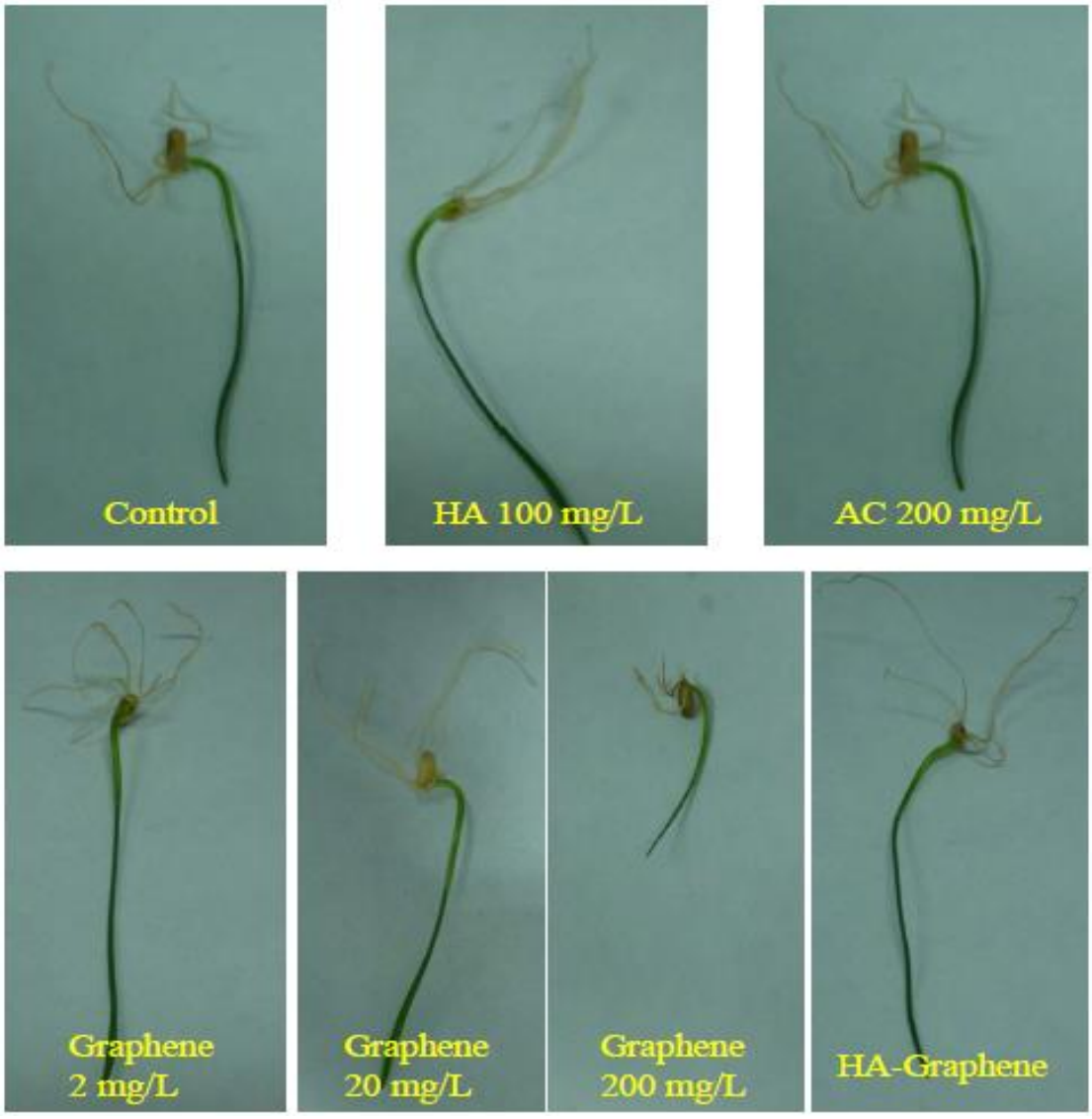


Figure S5. Images of wheat exposed to water (control), active carbon (AC) graphene and graphene-HA. The contents of HA and graphene are 100mg/L and 200 mg/L in HA-Graphene groups, respectively. HA, humic acid.

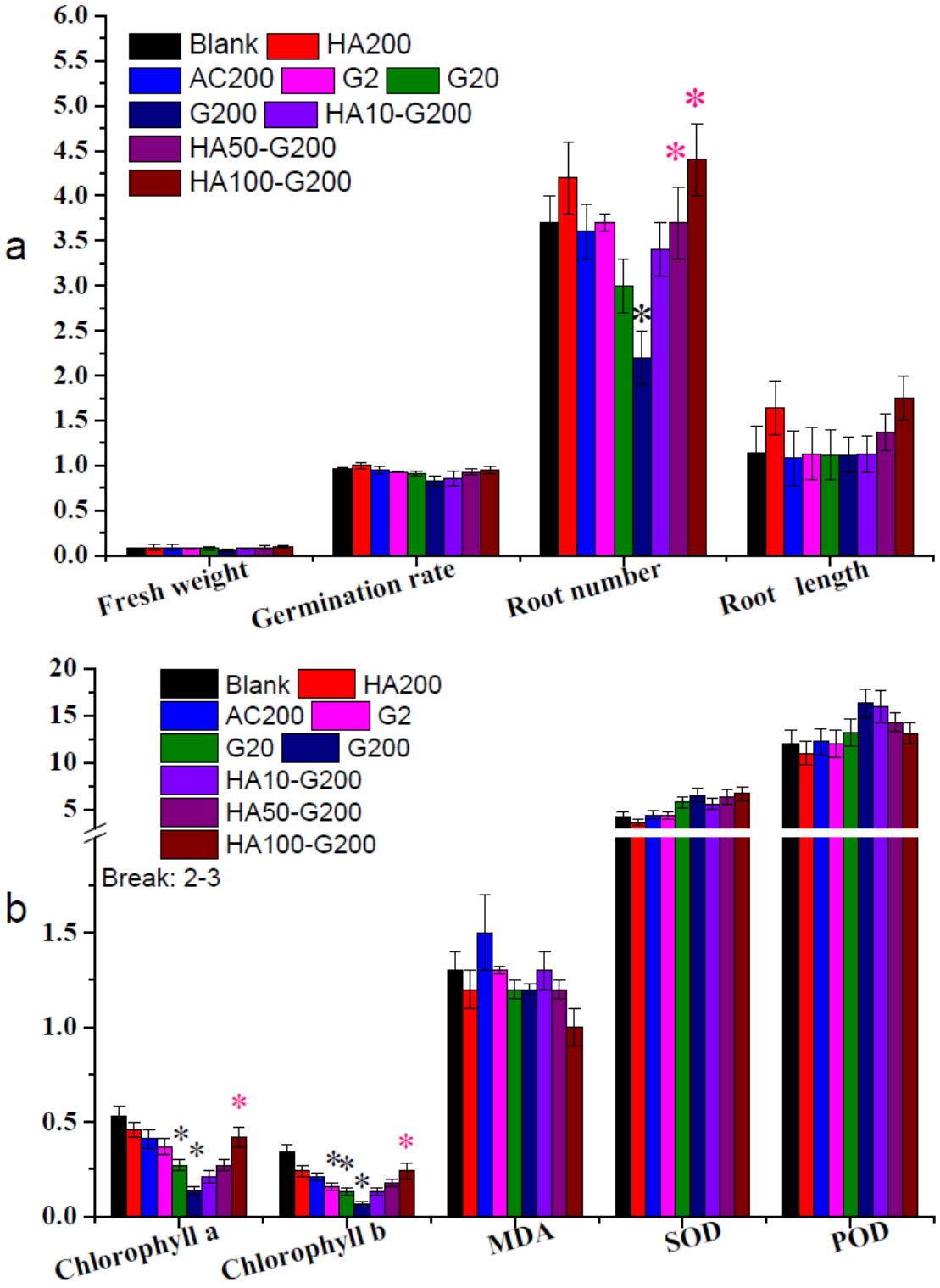


Figure S6. Phytotoxicity of graphene, graphene-HA, humic acid, and active carbon. (a) Graphene influences the growth and development of rice (*Oryza sativa L.*). (b) Graphene alters chlorophyll synthesis and antioxidase activity. The number after the

letter is the concentration (mg/L); thus, G2 and HA 100 represents graphene 2 mg/L and humic acid 100 mg/L. Black and pink "∗" symbols indicate that the differences are significant compared to the control and graphene 200 mg/L samples, respectively. Significant level, $P < 0.05$. Units of the biochemical parameters: germination rate, 0-1 representing 0-100%; fresh weight of rice, g; C_a, mg/g; germinal average length, cm; root average length, cm; MDA, mg/g; SOD, U/mg/protein; CAT, U/g/protein; POD, U/mg/protein. HA, humic acid; AC, active carbon; MDA, malondialdehyde; SOD, superoxide dismutase; POD peroxidase. The germination process of rice is same to that of wheat.

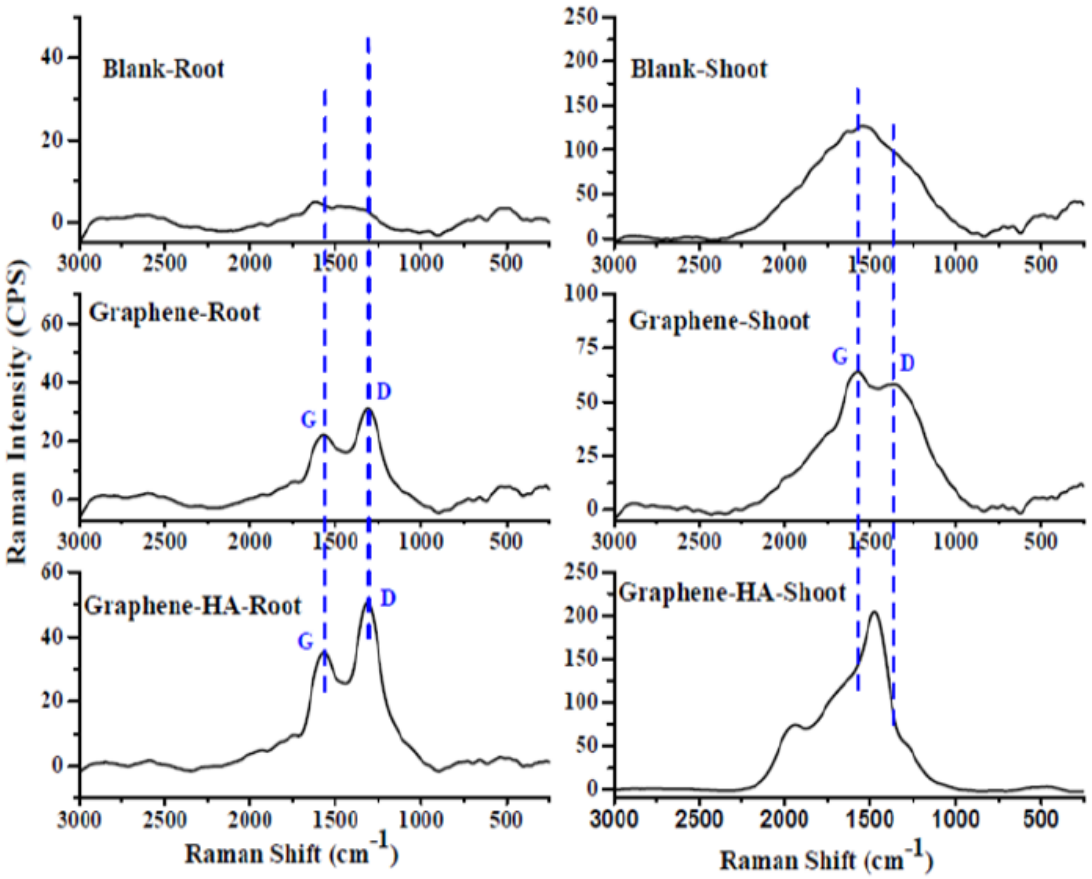


Figure S7. Raman spectra of wheat roots and shoots. G and D peaks indicate the specific band of graphene. Graphene, 200 mg/L; HA, 100 mg/L. HA, humic acid.

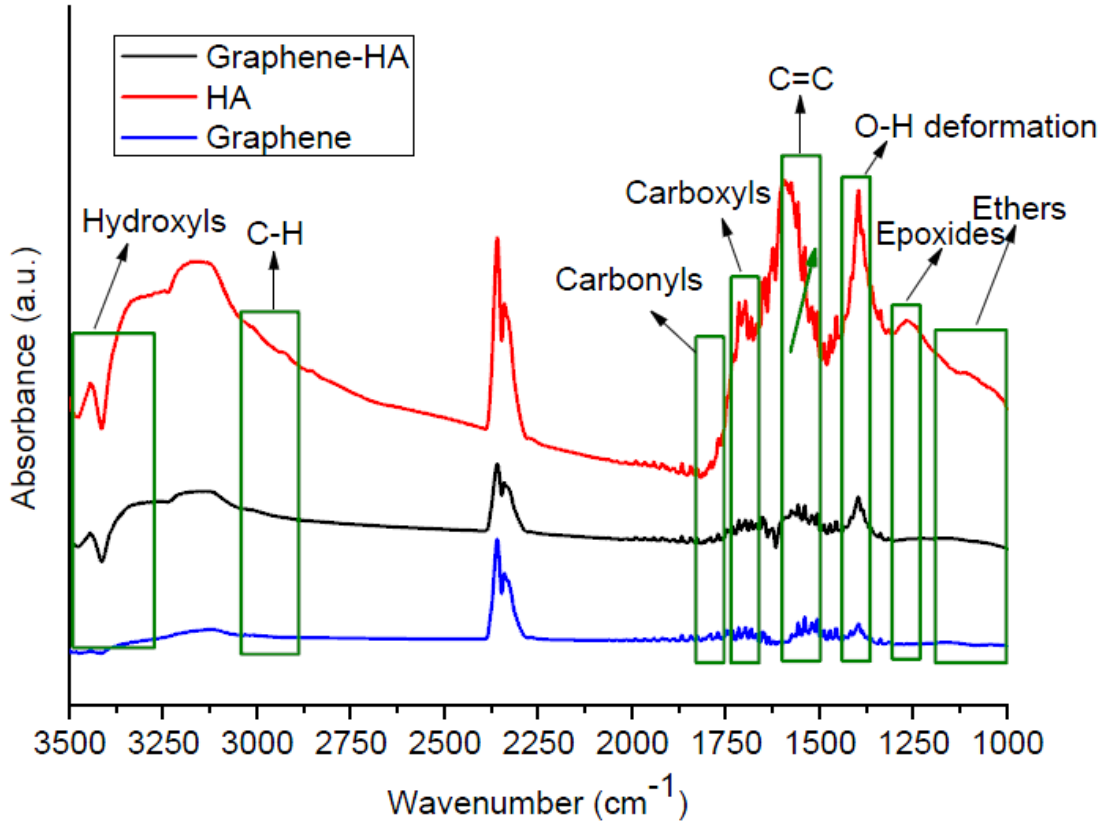
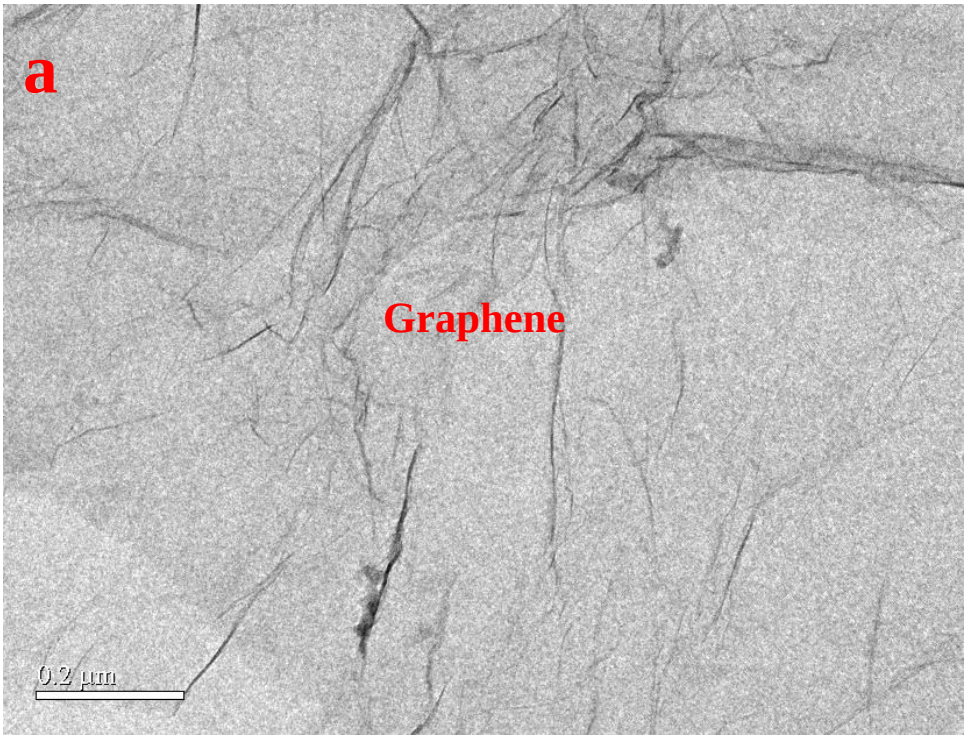
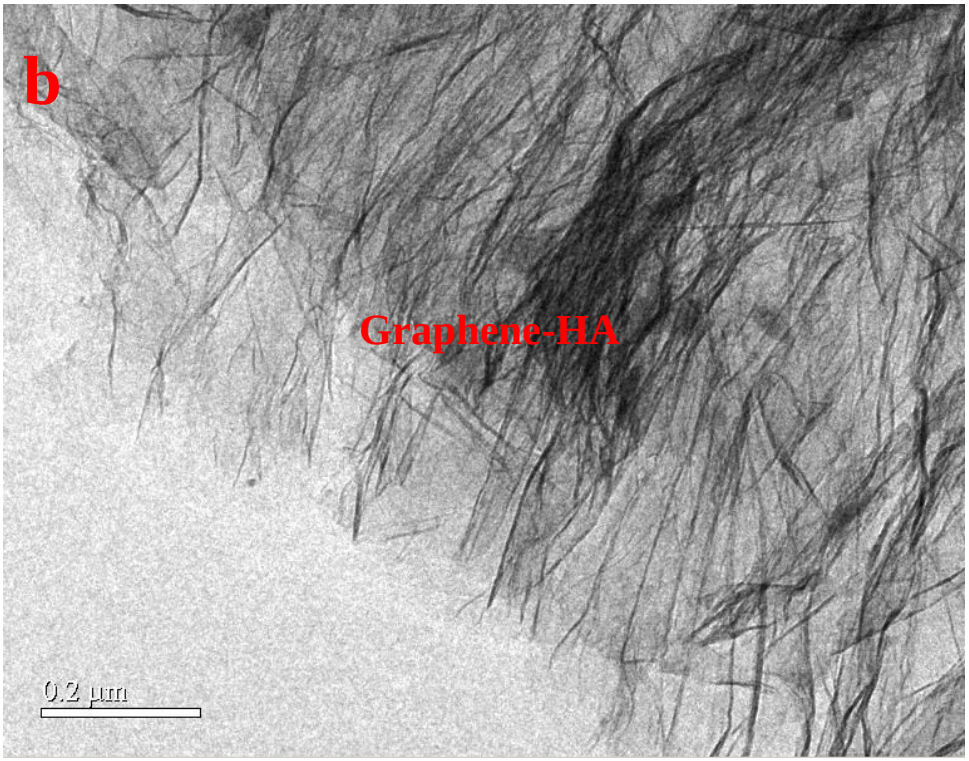


Figure S8. Fourier transform infrared spectra of graphene, HA, and graphene-HA. The green arrow indicates the blue shift of the C=C groups of graphene-HA by approximately 40 cm^{-1} compared with the spectrum of HA. HA, humic acid.


a
Graphene
0.2 μm


b
Graphene-HA
0.2 μm

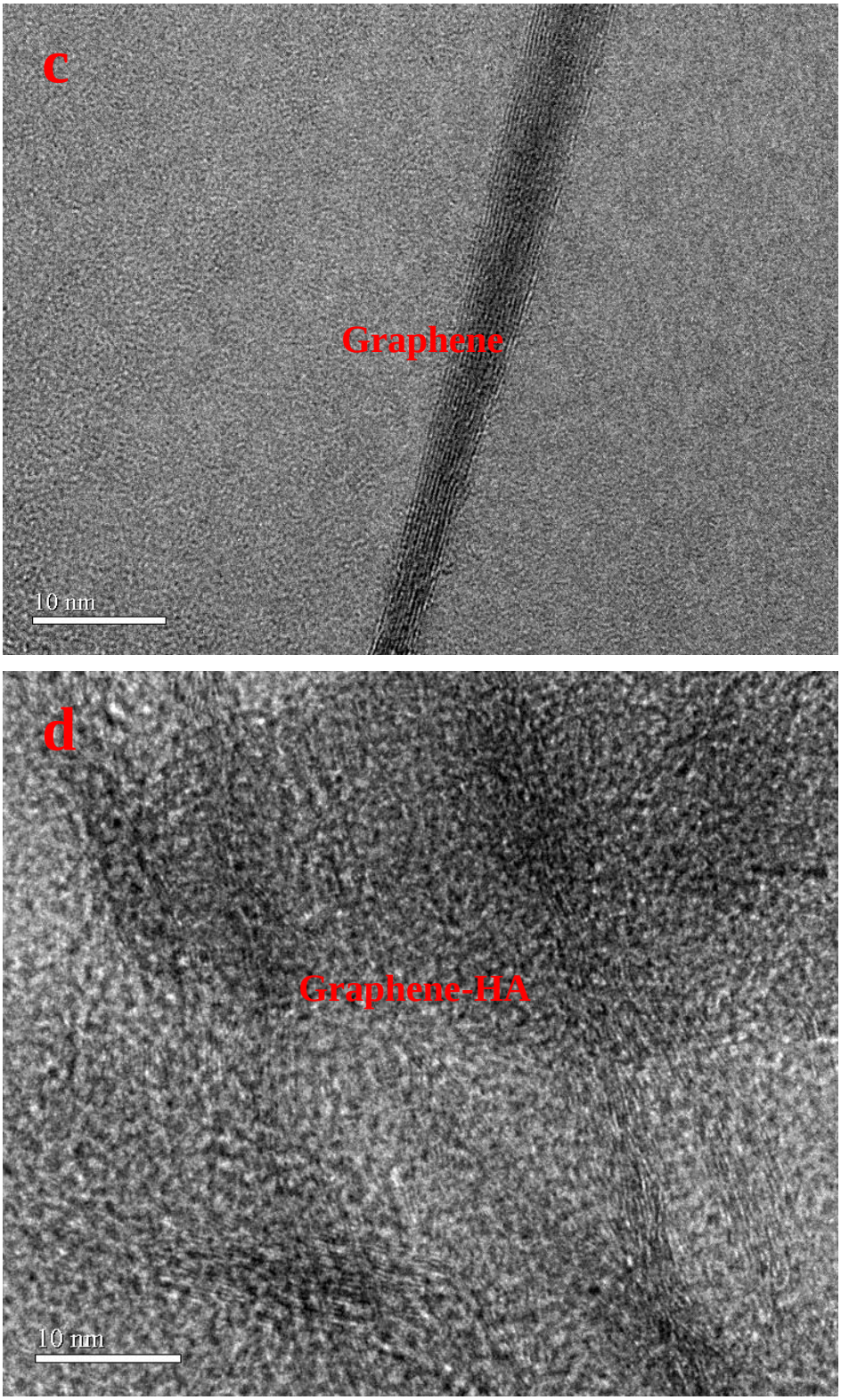


Figure S9. TEM images of graphene and graphene-HA. TEM, transmission electron microscope, HA, humic acid.

Table S1. Metabolites in the roots of wheat.

		Relative abundance of metabolites		
Name	Number	Control	Graphene	HA-Graphene
Ribose	1	1.800938138	3.142593	0.492334
Fructose	2	62.13598627	101.5645	167.7063
Arabinose	3	3.540866694	3.286051	ND
Mannose	4	274.050341	189.3264	155.887
Glucose	5	59.2095292	60.39128	67.24674
Glucopyranose	6	4.833353137	9.156474	36.33039
Galactose	7	0.433114106	0.228493	18.83264
Xylose	8	ND	6.06641	0.559071
Arabinofuranose	9	ND	ND	4.508779
Maltose	10	ND	3.906503	12.96023
Galactopyranose	11	ND	ND	0.75495
Glucopyranoside	12	460.7322652	78.50784	69.43295
Glycine	13	0.946958062	1.212161	2.215779
Leucine	14	3.151083257	3.558655	ND
Isoleucine	15	2.562325389	3.308296	7.239058
Alanine	16	2.278076203	2.707429	2.560955
Lysine	17	6.857184234	1.954249	15.16858
Tyrosine	18	ND	1.069791	3.810128
Serine	19	0.903396296	4.030179	3.838343
Threonine	20	0.585984705	2.176803	2.692619
Cysteine	21	ND	0.156726	0.121082
Valine	22	2.3267221	2.867631	3.079702
Phenylalanine	23	1.505172623	1.253824	3.421199
Proline	24	2.796965775	1.62165	13.73878
Aspartic acid	25	ND	ND	0.100849
Asparagine	26	2.751016008	3.500474	19.0289
Alkane (C_4, C_{16}, C_{17}, C_{21}, C_{22}, C_{25}, C_{26}, C_{28})	27	12.97705381	26.61543	0.860396
Tetradecanoic acid	28	2.742580971	0.334715	0.756053
Hexadecanoic acid	29	57.05108416	22.26746	21.58663
Octadecanoic acid	30	85.79861407	40.30665	24.98882
Octadecadienoic acid	31	3.522263394	ND	ND
Phosphate	32	ND	16.30656	19.33508

Propenoic acid	33	ND	3.002218	ND
Butanoic acid	34	ND	0.555485	0.270238
Propanoic acid	35	7.596232119	10.18495	2.318812
Phthalic acid	36	7.120865797	0	0.521116
Butenedioic acid	37	ND	0.453208	0.056889
Malic acid	38	ND	4.401314	0.833218
Aconitic acid	39	0.944724125	1.077422	1.465306
Gluconic acid	40	ND	4.115108	22.54645
Ribonic acid	41	ND	0.383375	0.214954
Glycerol	42	16.91948988	ND	ND
Glyceric acid	43	1.883555279	1.125781	ND
Cadaverine	44	3.085413221	4.775939	1.665072
Glutamine	45	ND	8.787409	7.620518
Urea	46	20.75770126	0.956784	0.281234
Inositol	47	5.038451635	4.306486	9.61061
Glucitol	48	6.888574897	2.327831	2.859736
Mannitol	49	2.111493855	5.165707	2.451698
Lanostane	50	2.234398889	1.684319	2.247696
Propanamine	51	ND	0.477115	ND
Butylamine	52	ND	0.784976	0.40248

ND, not detected. Graphene, 200 mg/L; graphene-HA, HA 100 mg/L plus graphene 200 mg/L. HA, humic acid.

Table S2. Metabolites in the shoots of wheat.

		Relative abundance of metabolites		
Name	Number	Control	Graphene	HA-Graphene
Ribose	1	2.22214	3.953672843	8.717604
Fructose	2	262.83667	238.8159647	391.7434
Mannose	3	383.51615	184.0922213	376.3576
Glucose	4	81.71887	95.23330579	141.5398
Glucopyranose	5	7.05386	11.97037828	15.17047
Galactose	6	9.26255	0.465774932	13.42377
Xylose	7	3.58748	ND	47.21339
Arabinofuranose	8	ND	5.344381957	0.195208
Maltose	9	1.22238	3.07256297	8.408563
Galactopyranose	10	7.24898	13.11454366	2.362288
Glucopyranoside	11	22.14167	23.65916068	108.2899
Glycine	12	1.98645	0.814907386	2.269704
Leucine	13	ND	1.49007224	3.812513
Isoleucine	14	0.77222	1.856611974	2.757308
Alanine	15	1.30329	0.825625884	2.522621
Lysine	16	9.71626	2.623263274	2.254828
Tyrosine	17	ND	ND	3.302777
Serine	18	1.01851	2.314565052	4.55951
Threonine	19	0.74128	1.661513381	2.77944
Cysteine	20	0.27084	0.075806188	ND
Valine	21	0.96996	1.777315192	3.020784
Phenylalanine	22	ND	2.386770994	3.077531
Proline	23	4.35347	0.505998996	12.46039
Aspartic acid	24	4.35347	6.175262007	0
Asparagine	25	15.91289	35.32258603	33.45899
Alkane	26	14.956	3.114678534	2.998964
Tetradecanoic acid	27	1.16169	0.177179603	ND
Hexadecanoic acid	28	70.60047	14.90850771	24.75275
Octadecanoic acid	29	65.17929	23.31712821	55.7001
Octadecadienoic acid	30	ND	1.375485741	ND
Phosphate	31	15.8445	23.17702017	32.45089
Butanoic acid	32	6.58528	9.694648361	10.53991
Propanoic acid	33	3.67446	0.558951848	0.210942
Phthalic acid	34	1.74541	ND	1.393582
Threonic acid	35	0.16303	0.257225675	ND

Butenedioic acid	36	0.08212	0.315533207	ND
Malic acid	37	1.22888	2.293420461	0.864783
Aconitic acid	38	1.96387	7.204027641	3.06536
Gluconic acid	39	2.13953	23.91772046	8.401047
Pyruvic acid	40	3.51974	6.969024802	ND
Glycerol	41	ND	0.529199564	0.649367
Glyceric acid	42	2.33689	0.529199564	3.261842
Cadaverine	43	2.33689	0.529199564	3.768561
Glutamine	44	18.60886	0.471851488	0.138383
Urea	45	2.9749	6.352057827	0.677637
Inositol	46	1.15836	5.062493686	15.75061
Glucitol	47	2.13953	6.023868934	5.647381
Mannitol	48	0.84149	ND	12.37134
Lanostane	49	1.88067	0.938997072	3.11857
Butylamine	50	ND	0.615011511	0.784941

ND, not detected. Graphene, 200 mg/L; graphene-HA, HA 100 mg/L plus graphene 200 mg/L. HA, humic acid.

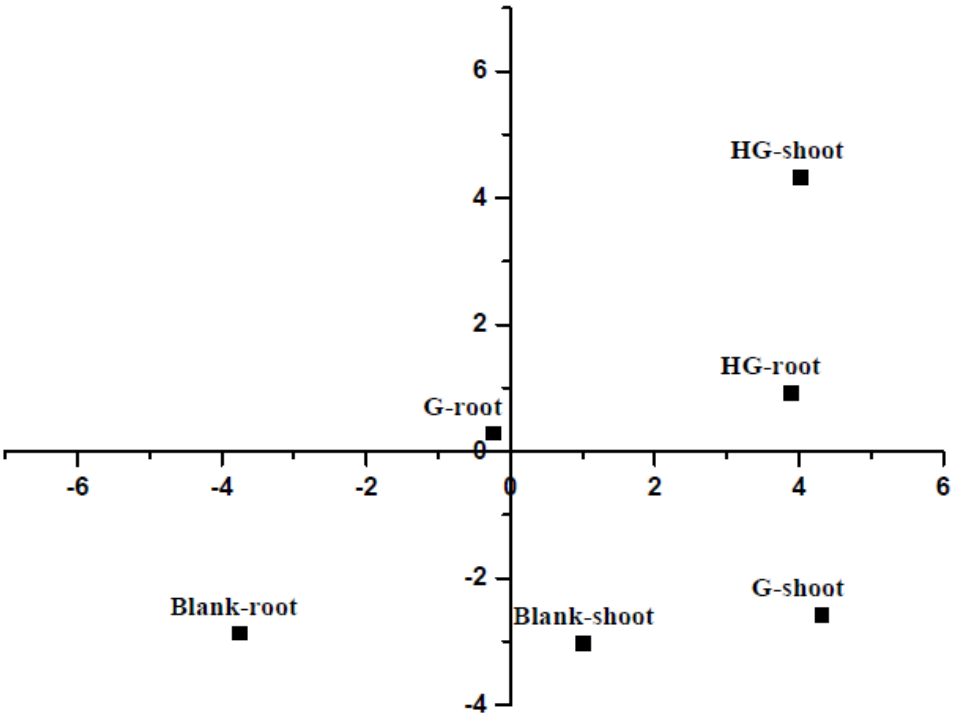


Figure S10. Metabolic cluster analysis of wheat using PCA scores plot. G, graphene; HG, humic acid plus graphene.

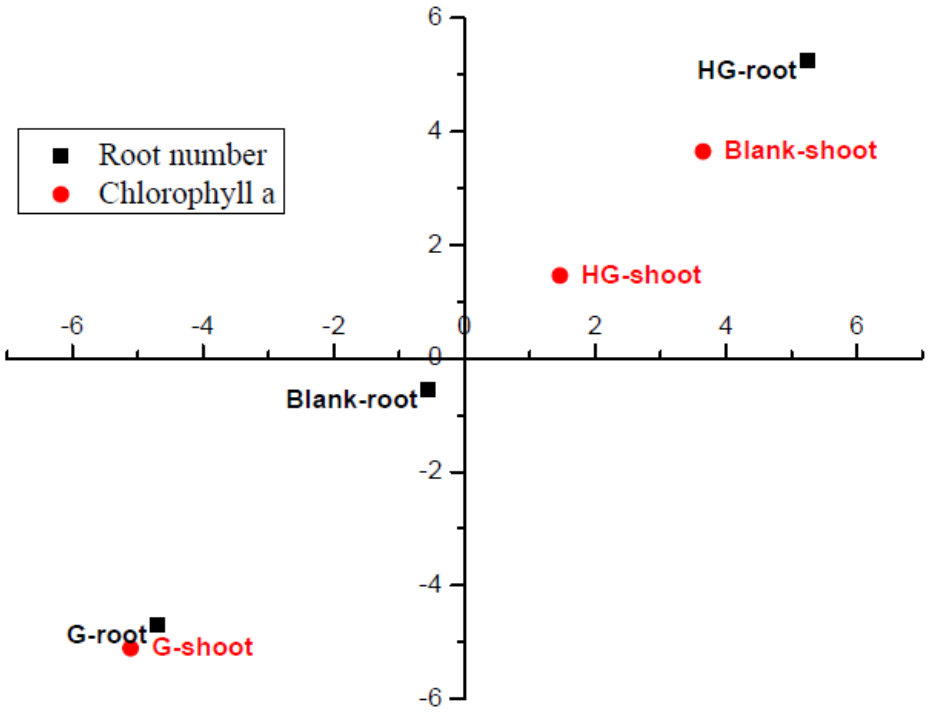


Figure S11. Metabolic cluster analysis of wheat using OPLS-DA scores plot. Metabolites in roots are used for root proliferation analysis. Metabolites in shoots are used for chlorophyll a analysis. G, graphene; HG, humic acid plus graphene.

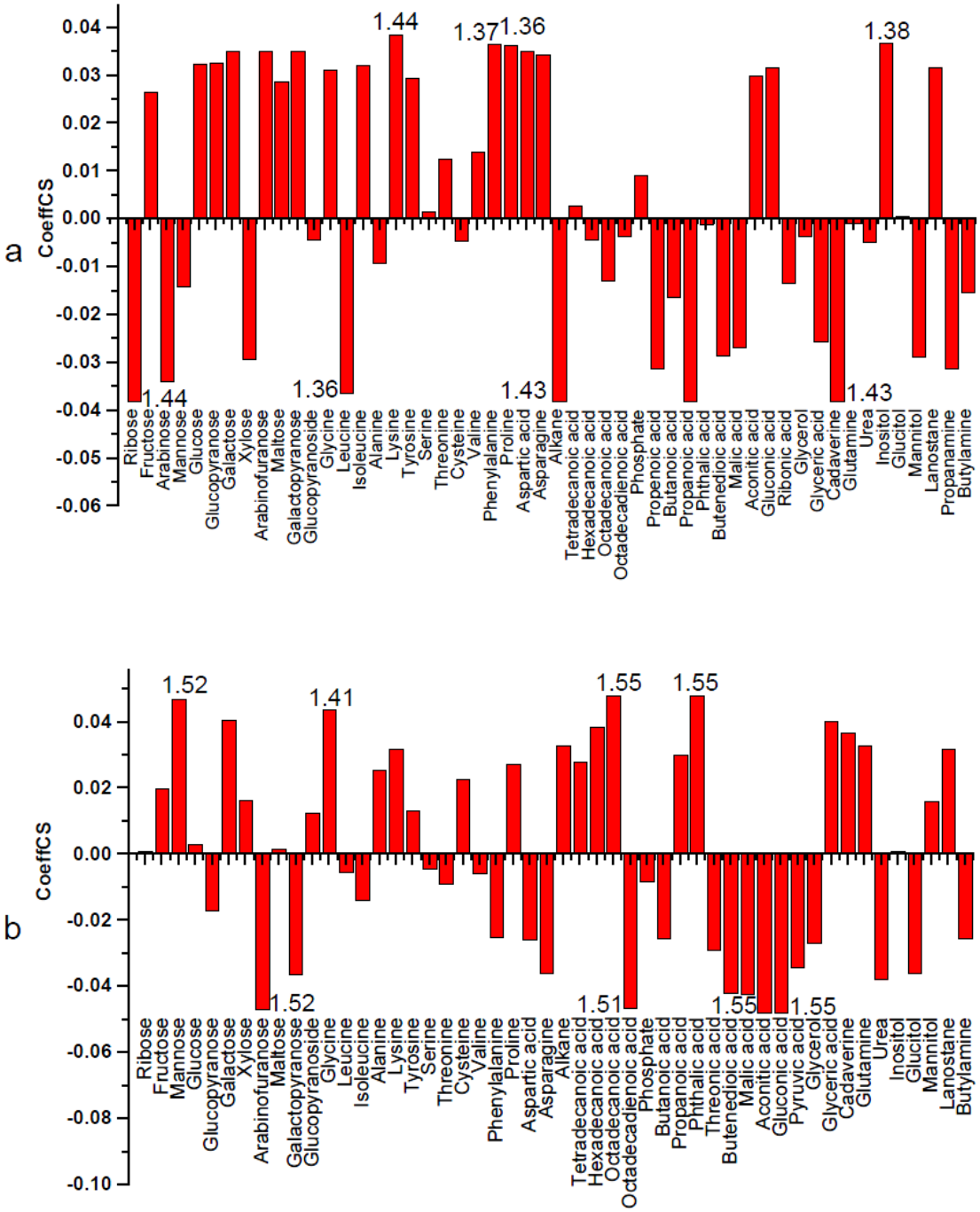


Figure S12. Coefficient plots of metabolites in roots (a) and shoots (b) using OPLS-DA model. The labeled numbers are the values of VIP.

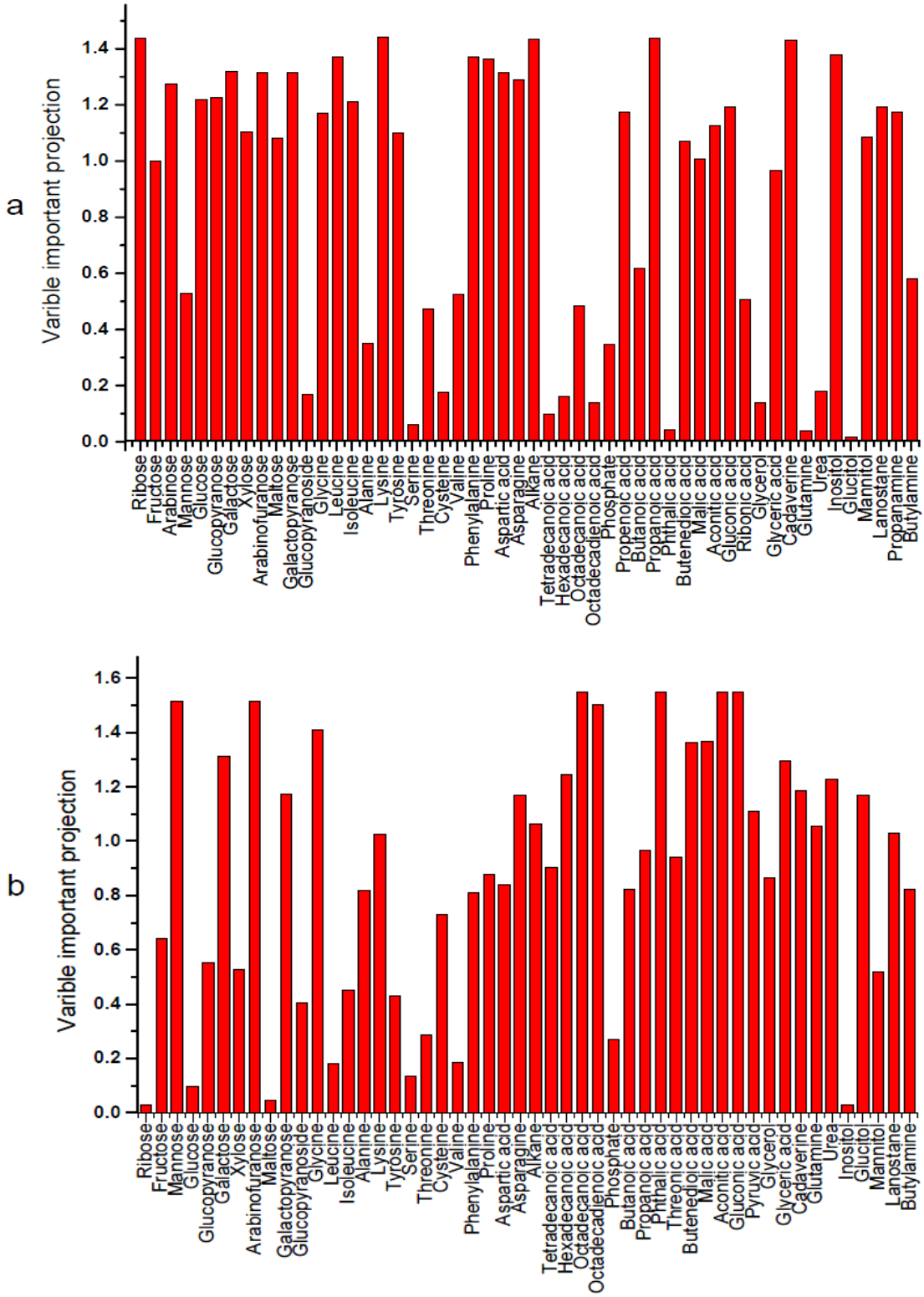


Figure S13. Variable important projection of metabolites in roots (a) and shoots (b) of wheat using OPLS-DA model.