

## Supplementary material

# Phenotypic, transcriptional, physiological and metabolic responses to carbon nanodots exposure in *Arabidopsis thaliana* (L.)

Jing Chen<sup>1, 2</sup>, Baofeng Liu<sup>1</sup>, Zhongzhou Yang<sup>1</sup>, Jiao Qu<sup>4</sup>, Hongwei Xun<sup>1</sup>, Runzhi Dou<sup>1</sup>, Xiang Gao<sup>\*1,3</sup>, and Li Wang<sup>\*1</sup>

<sup>1</sup> Key Laboratory of Molecular Epigenetics of MOE, Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China

<sup>2</sup> College of Life Science, Jilin Agricultural University, Changchun 130118, Jilin, China.

<sup>3</sup> National Demonstration Center for Experimental Biology Education, Northeast Normal University, Changchun, China

<sup>4</sup> School of Environment, Northeast Normal University, Changchun, China

Correspondence: X. Gao. Tel: +86 0431 85099360. Fax: +86 0431 85693965.

E-mail: gaoxiang424@163.com;

L. Wang. Tel: +86 0431 85099360. Fax: +86 0431 85693965.

E-mail: wanglee57@163.com

## Contents

### Materials and Methods

Characterization and Stability Testing of C-dots  
Transcriptomic Analysis and Quantitative Real-Time PCR Analysis  
H<sub>2</sub>O<sub>2</sub>, Lipid Peroxidation, and Antioxidant Defense System Measurement

### Results

Quantitative Analysis of C-dots in *Arabidopsis*

### Figures

Figure S1. High-resolution TEM image of synthesized C-dots.  
Figure S2. The stability of C-dots in ½ Murashige and Skoog medium.  
Figure S3. The linear equations between C-dots concentration and their absorbance values and the content of C-dots in the *Arabidopsis* roots and shoots.  
Figure S4. Venn diagrams of the genes with more than two fold expression changes and shared among 125 and 1000 mg/L exposure.  
Figure S5. Major gene ontology (GO) process categories of DEGs down/up-regulated (>2.0-fold changes) in *Arabidopsis* seedlings.  
Figure S6. Effects of C-dots on chlorophylls and carotenoids contents in *Arabidopsis* rosette leaves.

### Tables

Table S1. List of primers used for qRT-PCR amplification of selected genes.  
Table S2. The number list of differentially expressed genes that had more than two-fold differences in *Arabidopsis* affected by C-dots.  
Table S3. Gene ontology (GO) process categories of genes up-regulated (>2.0-fold change) in *Arabidopsis* roots.  
Table S4. Gene ontology (GO) process categories of genes up-regulated (>2.0-fold change) in *Arabidopsis* shoots.  
Table S5. Gene ontology (GO) process categories of genes down-regulated (>2.0-fold change) in *Arabidopsis* roots.  
Table S6. Gene ontology (GO) process categories of genes down-regulated (>2.0-fold change) in *Arabidopsis* shoots.  
Table S7. Annotation and relative expression quantity of 54 genes involved in chloroplast part (GO:0044434) in *Arabidopsis* shoots.  
Table S8. Primary metabolites detected in *Arabidopsis* roots exposed to different concentrations of C-dots.  
Table S9. Primary metabolites detected in *Arabidopsis* shoots exposed to different concentrations of C-dots.

## Materials and Methods

### *Characterization and Stability Testing of C-dots*

The morphology and zeta potential of C-dots in ½ Murashige and Skoog (MS) medium without agar (½ MS basal salts, 1% glucose, 0.7% agar, pH 5.8) were estimated separately by a JEOL JEM-2100F highresolution transmission electron microscopy (TEM) (JEOL Ltd, Tokyo, Japan) and a Zeta sizer Nano ZS (Malvern Instruments, UK), respectively. UV-visible absorption spectra of C-dots in ½ MS medium without agar was recorded by a Thermo NanoDrop 1000 spectrophotometer (Nanodrop, Wilmington, DE) before and after autoclaving. In addition, the stability of C-dots in ½ MS medium without agar was monitored for 7 days by a Cary Eclipse Fluorescence spectrometer (Varian Inc., Palo Alto, CA). The fluorescence intensities were excited at 460 nm and measured at 540 nm emission, and fluorescence of C-dots dispersed in DI-water at 0 minutes was set as 100%.

### *Uptake and Translocation Observation of C-dots*

To quantify the content of C-dots absorbed and further transferred by *Arabidopsis* seedlings, 100 mg of fresh rosette leaves exposed to 0-1000 mg/L C-dots were separately ground in 1 mL of DI-H<sub>2</sub>O using a mortar and pestle. Then, the extracts were centrifuged at 10 000 g for 20 min at room temperature. A certain quantity of C-dots was added to the supernatant of the unexposed seedlings to yield concentrations of 62.5, 125, 250, 500, 1000 and 2000 mg/L and then was recorded by the measurement of UV-visible absorbance at 400 nm to obtain standard curves using a Thermo NanoDrop 1000 spectrophotometer (Nanodrop, Wilmington, DE). Then, the supernatants of 62.5, 125, 250, 500 and 1000 mg/L exposed *Arabidopsis* rosette leaves were measured and quantified according to the standard curves under the same conditions.

### *Transcriptomic Analysis and Quantitative Real-Time PCR Analysis*

After 7 days of exposure to C-dots at 0, 125 and 1000 mg/L, the *Arabidopsis* roots and shoots (100 mg) were immediately frozen in liquid nitrogen. Total RNA from the frozen tissues of the control and C-dots exposed *Arabidopsis* seedlings was isolated using an RNAiso Plus (TaKaRa) kit according to the manufacturer's instructions. Contaminating DNA was removed by DNaseI treatment (TaKaRa). The integrity of the extracted RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Transcriptome libraries of *Arabidopsis* roots and shoots were separately constructed and then were sequenced using a HiSeq 2500 Illumina Genome Analyzer (BGI, Shenzhen, China) with standard protocols. Two biological replicates were conducted for each samples and were sequenced independently.

To validate the results acquired by RNA-seq, eight genes, including two genes

significantly up-regulated and two genes significantly down-regulated in *Arabidopsis* roots, as well as two genes significantly up-regulated and two genes significantly down-regulated in *Arabidopsis* shoots, were selected to confirm their expression levels using quantitative real-time PCR (qRT-PCR). Sequences of 8 selected genes were retrieved from the National Center for Biotechnology Information (NCBI) and subsequently subjected to design gene-specific qRT-PCR primers using Primer Premier software, version 5. To equalize RNA loading into the qRT-PCR, the housekeeping gene ACTIN 2 (*AT3G18780*) was used as an internal reference. The primers employed in this assay were listed in Table S1. The same RNA, used for the transcriptomic analysis experiments, was reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega). qRT-PCR was performed with an ABI StepOne Plus Real-Time PCR System (USA) and SYBR Green PCR Master Mix (TOYOBO). The thermal cycling conditions were 95°C for 60 s, 40 cycles of 95°C for 5 s and 60°C for 60 s, followed by a melting temperature cycle, with constant fluorescence data acquisition from 60 to 95°C.

#### *H<sub>2</sub>O<sub>2</sub>, Lipid Peroxidation, and Antioxidant Defense System Measurement*

The H<sub>2</sub>O<sub>2</sub> suspension were ground in 4 mL of 100mM potassium phosphate buffer (pH 6.8) by mortar and pestle with 500 mg fresh roots and leaves of *Arabidopsis*. Then, the suspension was centrifuged at 10 000g for 20 min, and the supernatant was mixed with 25 mM H<sub>2</sub>SO<sub>4</sub>, 150 μM xylenol orange, and 250 μM ferrous iron in a volume of 2 mL. Finally, with XO/Fe<sup>2+</sup> as blank, the absorbance at 560 nm was measured after incubation above reaction mixture in the dark for 30 min.

Lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARS). Simply, 500 mg fresh roots and leaves of *Arabidopsis* were separately ground in 2 mL 0.1% (w/v) trichloroacetic acid using mortar and pestle. Then, after centrifuged at 10 000g for 20 min, the supernatant (1 mL) was mixed with 1 mL TCA (20%) which contained 0.5% (w/v) thiobarbituric acid (TBA) and 100 μL of butylated hydroxytoluene (BHT, 4% in ethanol). The mixture was further heated at 95°C for 30 min, quickly cooled in an ice bath and centrifuged at 10 000g for 15 min. Finally, using 0.25% TBA in 10% TCA as blank, the absorbance at 532 and 600 nm was measured separately (extinction coefficient 155 mM<sup>-1</sup> cm<sup>-1</sup>).

To assess the effects of C-dots on the activity of antioxidant enzymes, fresh roots and leaves (200 mg) were ground in 4 mL of 100 mM potassium phosphate buffer (pH 6.8). After centrifugation at 10 000 g for 20 min, the supernatant was used for the enzyme activities analysis. The catalase (CAT) activity was determined by monitoring the degradation of H<sub>2</sub>O<sub>2</sub> and one unit of CAT was defined as the amount of enzyme required to degrade 1 μmol of H<sub>2</sub>O<sub>2</sub> per minute at 240 nm (extinction coefficient 39.4 mM<sup>-1</sup> cm<sup>-1</sup>). One unit of the ascorbate peroxidase (APOX) activity was defined as 1 μmol of ascorbate (extinction coefficient 2.8 mM<sup>-1</sup> cm<sup>-1</sup>) oxidized per minute at 290 nm. The guaiacol peroxidase (GPOX) activity was measured through monitoring the formation of guaiacol dehydrogenation product (extinction coefficient 6.39 mM<sup>-1</sup>

cm<sup>-1</sup>) at 420 nm, and one unit of GPOX is defined as 1 μmol of guaiacol oxidized per minute. The superoxide dismutase (SOD) activity was measured by monitoring the inhibition of nitroblue tetrazolium reduction at 560 nm. One unit of SOD activity is the amount of enzyme that decreased the rate of nitroblue tetrazolium reduction by 50%. The glutathione reductase (GR) activity was determined by the decrease in nicotinamide adenine dinucleotide phosphate (NADPH, extinction coefficient 6.2 mM<sup>-1</sup> cm<sup>-1</sup>) at 340 nm, and one unit of GR activity was defined as 1 μmol of NADPH oxidized per minute. The dehydroascorbate reductase (DHAR) activity was estimated by monitoring the formation of ascorbate (extinction coefficient 14 mM<sup>-1</sup> cm<sup>-1</sup>) at 265 nm. One unit of DHAR was equal to 1 μmol of ascorbate formed per minute. All of the enzyme activity assays were performed at 25°C using a Beckman Du-640 Spectrometer (Beckman Coulter, Brea, CA, USA).

## Results

### *The linear equations between C-dots and UV-vis absorbance*

To quantify the content of C-dots absorbed and further transferred by *Arabidopsis* seedlings, different concentrations of C-dots were added to the supernatant of the unexposed seedlings and were recorded by UV-visible absorbance at 400 nm. With the increase of C-dots, the values of UV-visible absorbance rose linearly. Upon multiple linear regression analysis, linear equations were established between C-dot concentrations and the values of UV-visible absorbance in *Arabidopsis* roots and shoots, respectively ( $y=0.0009x+0.0171$ ,  $R^2=1$ ;  $y=0.0009x+0.0136$ ,  $R^2=0.9995$ ) (Fig. S3).

## Figures

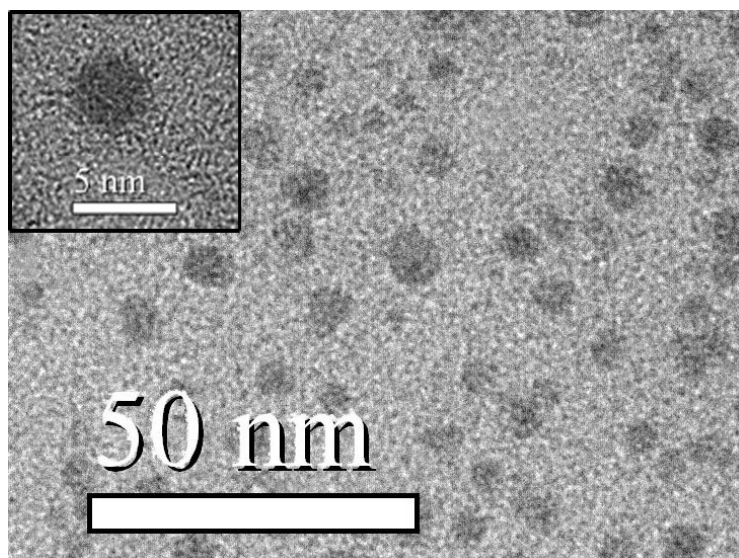


Fig. S1. High-resolution TEM image of synthesized C-dots (inset: a high-resolution TEM image of a single C-dot).

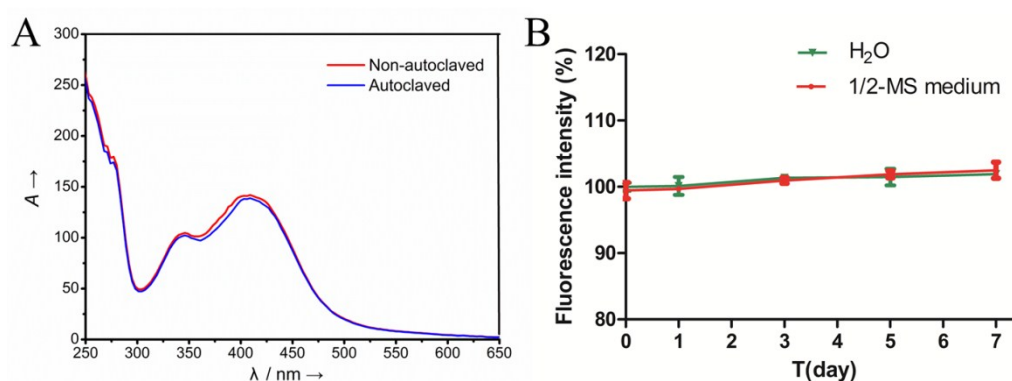


Fig. S2. The stability of 250 mg/L C-dots in  $\frac{1}{2}$  Murashige and Skoog medium. A) UV-visible absorption spectra of C-dots diluted in  $\frac{1}{2}$  Murashige and Skoog medium before and after autoclaving. B) The changes of C-dots fluorescence intensity in  $\frac{1}{2}$  Murashige and Skoog medium and DI-H<sub>2</sub>O. The fluorescence intensities were measured at 540nm emission following excitation at 460 nm, and fluorescence of C-dots dispersed in DI-water at 0 minute was set as 100%. The values were given as mean  $\pm$  SD of triplicate samples.

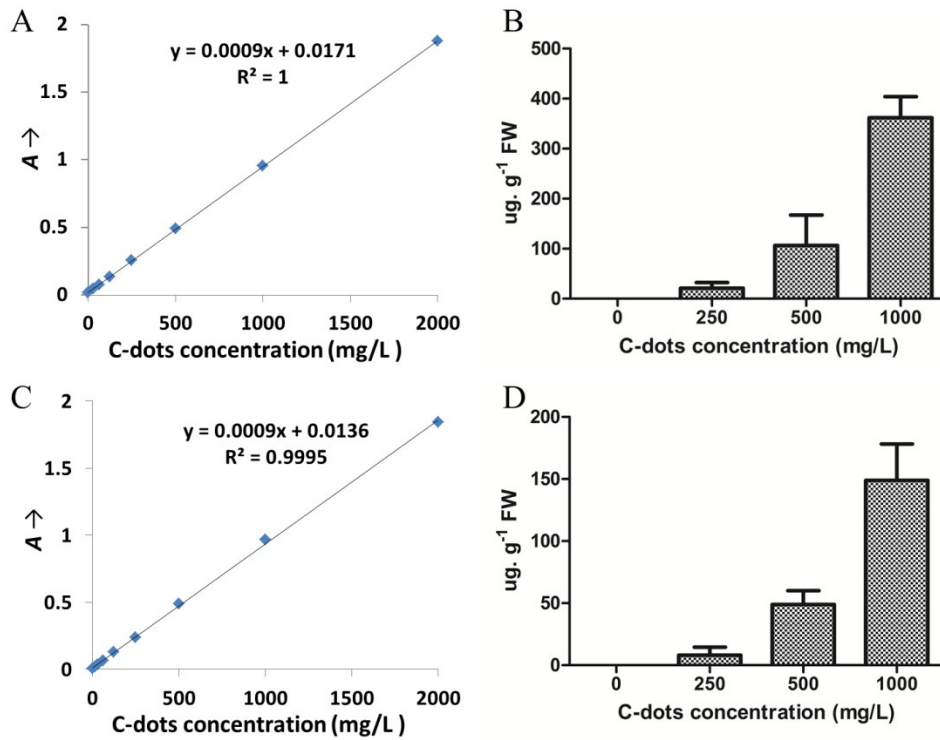


Fig. S3. The linear equations between C-dots concentration and their absorbance values and the content of C-dots in the *Arabidopsis* roots and shoots. A) The linear equations between C-dots concentration and their absorbance values at 400 nm in *Arabidopsis* roots recorded by UV-visible absorbance. B) The content of C-dots in the *Arabidopsis* roots exposed with 0, 250, 500 and 1000 mg/L C-dots, calculated by the left linear equations. C) The linear equations between C-dots concentration and their absorbance values at 400 nm in *Arabidopsis* shoots recorded by UV-visible absorbance. D) The content of C-dots in the *Arabidopsis* shoots exposed with 0, 250, 500 and 1000 mg/L C-dots, calculated by the left linear equations.

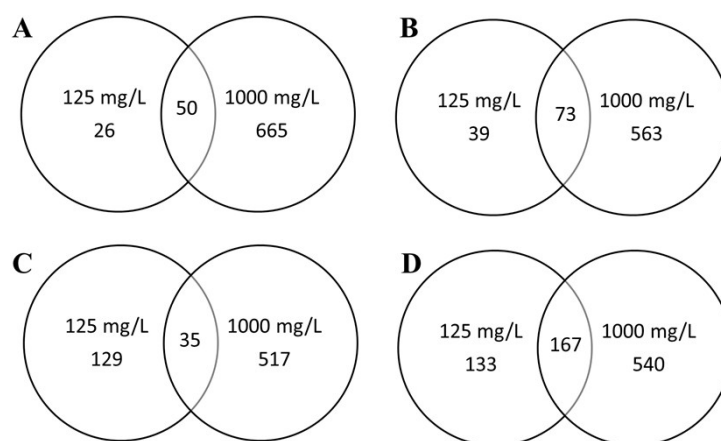


Fig. S4. Venn diagrams of the genes with more than two folds expression changes and shared among 125 and 1000 mg/L exposure. A) Up-regulated genes in *Arabidopsis* roots; B) Up-regulated genes in *Arabidopsis* shoots; C) Down-regulated genes in *Arabidopsis* roots, D) Down-regulated genes in *Arabidopsis* shoots. (A–D) were two

ways comparison, and overlapped areas were shared genes while non-overlapped areas were specific/unique genes for individual treatment.

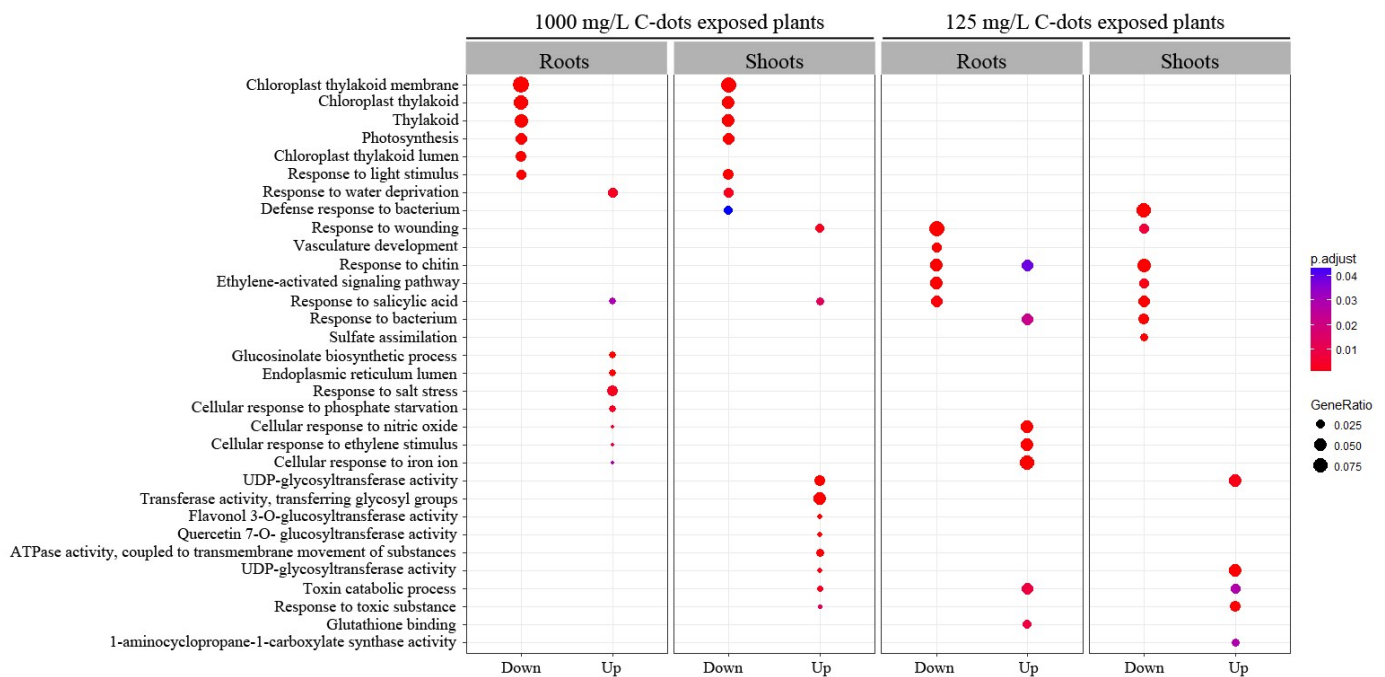


Fig. S5. Major gene ontology (GO) process categories of DEGs down/up-regulated (>2.0-fold changes) in *Arabidopsis* seedlings. The distribution of genes into GO categories was performed using the clusterProfiler package of R to draw. The depth of the color indicates the significance of the enriched pathway. The size of the circle indicates the size of input-genes/reference-genes ratio at different enrichment pathway.



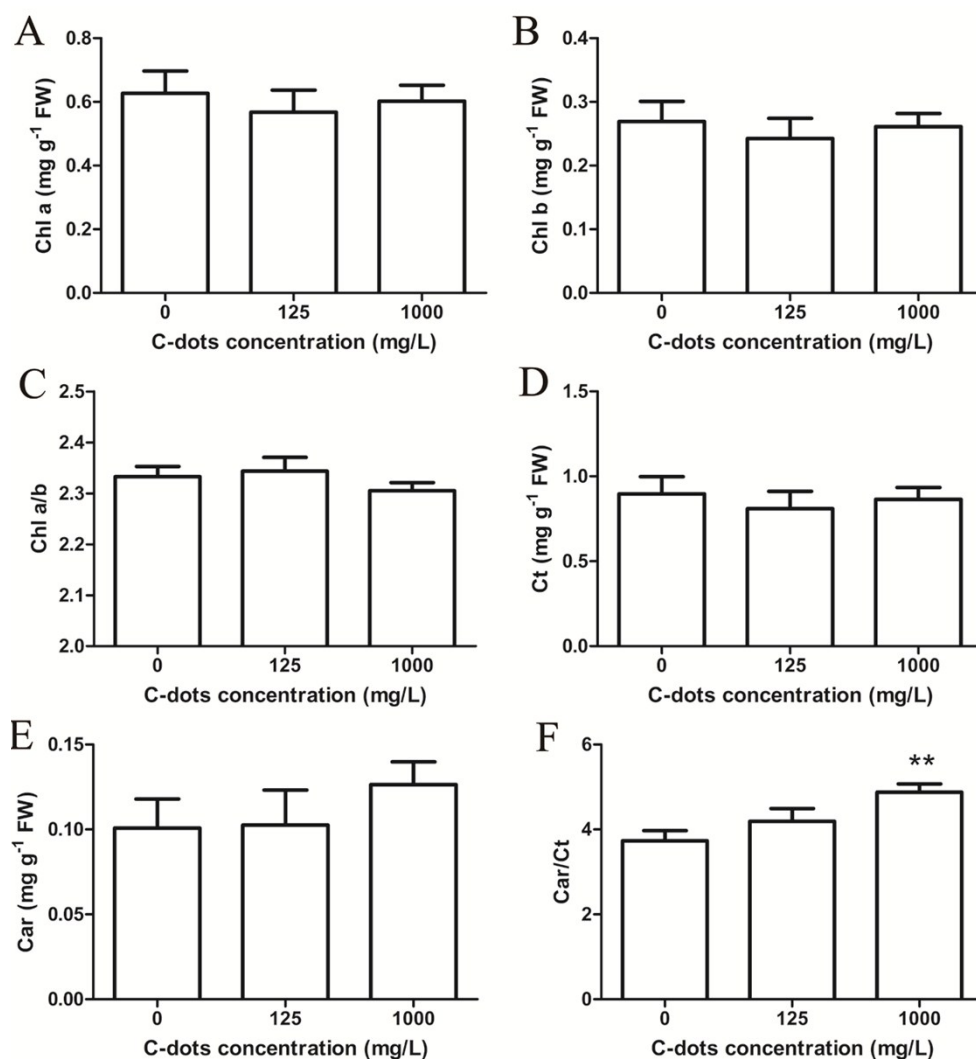


Fig. S6. Effects of C-dots on chlorophylls and carotenoids contents in *Arabidopsis* rosette leaves. A)Chlorophyll a, B)Chlorophyll b, C)Chlorophyll a/b ratio, D)total chlorophylls, E)carotenoids and F)carotenoids/total chlorophylls ratio in rosette leaves of 2-week-old *Arabidopsis* plants treated with 125, 1000mg/L C-dots, and without C-dots for 7 days. The values were given as mean  $\pm$  SD of triplicate samples, and \*\* indicated significance at  $p \leq 0.01$ .

## Tables

**Table S1. List of primers used for qRT-PCR amplification of selected genes.**

TAIR ID	Primer Sequence	
AT1G09080	Forward	5'- TAGAAGGCGAAGAGCAGAAAC-3'
	Reverse	5'- TGCAACCCAAGATGGTGTAA-3'

AT1G73120	Forward	5'-CAACACTCTCTTCCAGACACTT-3'
	Reverse	5'-CTTCATGTGGCACCCAACTA-3'
AT2G34420	Forward	5'-TCAACCATGGCTTTGTCTC-3'
	Reverse	5'-CAGTCTTCCTCATGGTCACAC-3'
AT3G13840	Forward	5'-GTATGCAATGGCCCACTTTG-3'
	Reverse	5'-GAAAGGTATGTCTGCGGTTAGA-3'
AT5G14565	Forward	5'-AGCGAGACCAATGACGTTAC-3'
	Reverse	5'-CTTCATCCTCGTCCGTTTCTT-3'
AT2G47015	Forward	5'-AGACAAAGCGGTAATGAGAGAG-3'
	Reverse	5'-CAGTGCATGGGTAGAGACAAA-3'
AT5G02810	Forward	5'-GAGGCGTCAAATGGGATACA-3'
	Reverse	5'-CACAAGAGACCGATACCAGATAAG-3'
AT5G63160	Forward	5'-GGCGGATCATCGAAGAAAGT-3'
	Reverse	5'-TCCGTGACACTCGGAGAATA-3'
At-act II	Forward	5'-CAACAATTGGGATGACATGGAG-3'
	Reverse	5'-GGTGCCTCGGTAAGTAGAATAG-3'

**Table S2. The number list of differentially expressed genes that have more than two-fold differences in *Arabidopsis* affected by C-dots.**

Concentration of C-dots	Tissue	Number of up regulated genes (% a)	Number of down regulated genes (% a)	Number of total affected genes	Percentage of total affected genes in genome
125 mg/L	Root	76 (31.67)	164 (68.33)	240	0.88
125 mg/L	Shoot	112 (27.18)	300 (72.82)	412	1.5
1000 mg/L	Root	715 (56.43)	552 (43.57)	1267	4.62
1000 mg/L	Shoot	636 (47.36)	707 (52.64)	1342	4.9

**Table S3. Gene ontology (GO) process categories of genes up-regulated (>2.0-fold change) in *Arabidopsis* roots.**

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0016144	P	S-glycoside biosynthetic process	10	32	2.00E-08	9.30E-06
GO:0019758	P	glycosinolate biosynthetic process	10	32	2.00E-08	9.30E-06
GO:0019761	P	glucosinolate biosynthetic process	10	32	2.00E-08	9.30E-06

GO:0019760	P	glucosinolate metabolic process	11	47	1.10E-07	2.50E-05
GO:0016143	P	S-glycoside metabolic process	11	47	1.10E-07	2.50E-05
GO:0019757	P	glycosinolate metabolic process	11	47	1.10E-07	2.50E-05
GO:0016138	P	glycoside biosynthetic process	12	59	1.60E-07	3.10E-05
GO:0016137	P	glycoside metabolic process	13	78	5.50E-07	9.50E-05
GO:0031669	P	cellular response to nutrient levels	11	58	1.10E-06	0.00016
GO:0009267	P	cellular response to starvation	10	51	2.40E-06	0.00033
GO:0031668	P	cellular response to extracellular stimulus	11	69	6.30E-06	0.00071
GO:0071496	P	cellular response to external stimulus	11	69	6.30E-06	0.00071
GO:0042594	P	response to starvation	10	57	6.80E-06	0.00071
GO:0016036	P	cellular response to phosphate starvation	8	35	7.20E-06	0.00071
GO:0031667	P	response to nutrient levels	11	71	8.30E-06	0.00077
GO:0006790	P	sulfur metabolic process	16	153	1.60E-05	0.0014
GO:0042221	P	response to chemical stimulus	77	1548	2.70E-05	0.0022
GO:0009991	P	response to extracellular stimulus	11	82	3.40E-05	0.0026
GO:0044272	P	sulfur compound biosynthetic process	11	83	3.80E-05	0.0027
GO:0006811	P	ion transport	23	302	5.30E-05	0.0037
GO:0006950	P	response to stress	81	1706	7.70E-05	0.0051
GO:0050896	P	response to stimulus	127	2998	0.00019	0.012
GO:0006812	P	cation transport	19	251	0.00024	0.014
GO:0019748	P	secondary metabolic process	24	362	0.0003	0.017
GO:0009607	P	response to biotic stimulus	29	482	0.0004	0.022
GO:0033554	P	cellular response to stress	20	290	0.00056	0.029
GO:0000041	P	transition metal ion transport	7	49	0.0006	0.031
GO:0050801	P	ion homeostasis	9	81	0.00071	0.035
GO:0030001	P	metal ion transport	14	173	0.00077	0.035
GO:0055066	P	di-, tri-valent inorganic cation homeostasis	7	51	0.00077	0.035
GO:0022857	F	transmembrane transporter activity	53	835	6.40E-07	0.0004
GO:0022891	F	substrate-specific transmembrane transporter activity	44	660	1.40E-06	0.00045
GO:0022892	F	substrate-specific transporter activity	48	772	3.40E-06	0.00059
GO:0005215	F	transporter activity	62	1098	3.80E-06	0.00059
GO:0004568	F	chitinase activity	6	19	1.40E-05	0.0017
GO:0001871	F	pattern binding	5	17	0.00011	0.0084
GO:0030247	F	polysaccharide binding	5	17	0.00011	0.0084
GO:0015075	F	ion transmembrane transporter	30	462	8.90E-05	0.0084

		activity				
GO:0016491	F	oxidoreductase activity	55	1078	0.00016	0.011
GO:0008324	F	cation transmembrane transporter activity	23	341	0.00031	0.02
GO:0003700	F	transcription factor activity	68	1469	0.00048	0.027
GO:0016765	F	transferase activity, transferring alkyl or aryl (other than methyl) groups	11	115	0.00069	0.036
GO:0020037	F	heme binding	9	83	0.00084	0.041
GO:0015144	F	carbohydrate transmembrane transporter activity	10	102	0.00098	0.044
GO:0005788	C	endoplasmic reticulum lumen	5	7	4.70E-07	0.00012

**Table S4. Gene ontology (GO) process categories of genes up-regulated (>2.0-fold change) in *Arabidopsis* shoots.**

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0019748	P	secondary metabolic process	40	362	9.30E-14	1.20E-10
GO:0042398	P	cellular amino acid derivative biosynthetic process	20	172	4.40E-08	2.80E-05
GO:0009698	P	phenylpropanoid metabolic process	17	132	9.70E-08	4.20E-05
GO:0050896	P	response to stimulus	132	2998	1.50E-07	4.70E-05
GO:0006575	P	cellular amino acid derivative metabolic process	22	223	1.90E-07	4.80E-05
GO:0009699	P	phenylpropanoid biosynthetic process	15	111	2.80E-07	5.10E-05
GO:0006725	P	cellular aromatic compound metabolic process	25	287	3.20E-07	5.10E-05
GO:0042221	P	response to chemical stimulus	78	1548	2.60E-07	5.10E-05
GO:0019438	P	aromatic compound biosynthetic process	18	185	2.70E-06	0.00034
GO:0009404	P	toxin metabolic process	9	46	2.90E-06	0.00034
GO:0009407	P	toxin catabolic process	9	46	2.90E-06	0.00034
GO:0006869	P	lipid transport	13	105	4.60E-06	0.00049
GO:0010876	P	lipid localization	13	119	1.80E-05	0.0018
GO:0044283	P	small molecule biosynthetic process	32	541	3.40E-05	0.0031
GO:0006979	P	response to oxidative stress	19	249	4.70E-05	0.004
GO:0009605	P	response to external stimulus	21	295	5.30E-05	0.0042
GO:0009267	P	cellular response to starvation	8	51	5.50E-05	0.0042
GO:0071555	P	cell wall organization	12	118	7.60E-05	0.0054

GO:0031668	P	cellular response to extracellular stimulus	9	69	8.60E-05	0.0055
GO:0071496	P	cellular response to external stimulus	9	69	8.60E-05	0.0055
GO:0042594	P	response to starvation	8	57	0.00012	0.0076
GO:0031669	P	cellular response to nutrient levels	8	58	0.00014	0.0082
GO:0071554	P	cell wall organization or biogenesis	15	191	0.0002	0.011
GO:0016137	P	glycoside metabolic process	9	78	0.00022	0.012
GO:0016036	P	cellular response to phosphate starvation	6	35	0.00029	0.015
GO:0009991	P	response to extracellular stimulus	9	82	0.00033	0.016
GO:0009808	P	lignin metabolic process	6	36	0.00033	0.016
GO:0031667	P	response to nutrient levels	8	71	0.00058	0.026
GO:0006519	P	cellular amino acid and derivative metabolic process	25	465	0.00086	0.038
GO:0008194	F	UDP-glycosyltransferase activity	27	183	9.00E-13	5.00E-10
GO:0035251	F	UDP-glucosyltransferase activity	18	80	3.30E-12	9.30E-10
GO:0046527	F	glucosyltransferase activity	20	117	4.70E-11	8.60E-09
GO:0016757	F	transferase activity, transferring glycosyl groups	36	434	4.00E-09	5.60E-07
GO:0016758	F	transferase activity, transferring hexosyl groups	26	274	3.50E-08	3.90E-06
GO:0080044	F	quercetin 7-O-glucosyltransferase activity	7	16	8.40E-08	7.80E-06
GO:0016491	F	oxidoreductase activity	61	1078	1.00E-07	8.00E-06
GO:0004364	F	glutathione transferase activity	9	48	4.20E-06	0.00029
GO:0016209	F	antioxidant activity	14	124	6.00E-06	0.00037
GO:0042626	F	ATPase activity, coupled to transmembrane movement of substances	15	148	1.10E-05	0.00055
GO:0043492	F	ATPase activity, coupled to movement of substances	15	148	1.10E-05	0.00055
GO:0016820	F	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	15	150	1.30E-05	0.00059
GO:0003824	F	catalytic activity	248	6896	2.00E-05	0.00087
GO:0022857	F	transmembrane transporter activity	44	835	2.50E-05	0.001
GO:0019825	F	oxygen binding	17	201	3.20E-05	0.0012
GO:0016684	F	oxidoreductase activity, acting on peroxide as acceptor	11	99	6.60E-05	0.0022
GO:0004601	F	peroxidase activity	11	99	6.60E-05	0.0022
GO:0022804	F	active transmembrane transporter activity	29	491	7.60E-05	0.0024

GO:0005215	F	transporter activity	52	1098	8.20E-05	0.0024
GO:0005199	F	structural constituent of cell wall	6	31	0.00014	0.0039
GO:0016829	F	lyase activity	20	302	0.00021	0.0055
GO:0080043	F	quercetin 3-O-glucosyltransferase activity	5	22	0.00023	0.0059
GO:0015405	F	P-P-bond-hydrolysis-driven transmembrane transporter activity	15	195	0.00025	0.0059
GO:0016765	F	transferase activity, transferring alkyl or aryl (other than methyl) groups	11	115	0.00025	0.0059
GO:0015399	F	primary active transmembrane transporter activity	15	196	0.00027	0.0059
GO:0016840	F	carbon-nitrogen lyase activity	5	23	0.00029	0.0062
GO:0016740	F	transferase activity	92	2465	0.0013	0.027
GO:0004553	F	hydrolase activity, hydrolyzing O-glycosyl compounds	17	293	0.0024	0.048

**Table S5. Gene ontology (GO) process categories of genes down-regulated (>2.0-fold change) in *Arabidopsis* roots.**

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0015979	P	photosynthesis	20	107	6.10E-13	6.10E-10
GO:0019684	P	photosynthesis, light reaction	13	63	1.70E-09	8.60E-07
GO:0006091	P	generation of precursor metabolites and energy	16	181	5.10E-06	0.0017
GO:0009765	P	photosynthesis, light harvesting	5	15	1.50E-05	0.0038
GO:0009767	P	photosynthetic electron transport chain	6	27	2.70E-05	0.0055
GO:0009813	P	flavonoid biosynthetic process	7	53	0.00019	0.033
GO:0022900	P	electron transport chain	7	55	0.00025	0.036
GO:0016168	F	chlorophyll binding	7	25	1.10E-06	0.00035
GO:0046906	F	tetrapyrrole binding	11	109	4.30E-05	0.0071
GO:0019825	F	oxygen binding	15	201	7.10E-05	0.0079
GO:0015035	F	protein disulfide oxidoreductase activity	6	41	0.00031	0.026
GO:0030599	F	pectinesterase activity	8	80	0.00049	0.032
GO:0009579	C	thylakoid	44	301	7.60E-22	2.10E-19
GO:0034357	C	photosynthetic membrane	37	221	9.70E-21	8.00E-19
GO:0031976	C	plastid thylakoid	38	237	1.40E-20	8.00E-19

GO:0044436	C	thylakoid part	39	247	8.20E-21	8.00E-19
GO:0009534	C	chloroplast thylakoid	38	237	1.40E-20	8.00E-19
GO:0031984	C	organelle subcompartment	38	239	1.90E-20	9.00E-19
GO:0055035	C	plastid thylakoid membrane	32	190	2.80E-18	8.80E-17
GO:0042651	C	thylakoid membrane	33	203	2.60E-18	8.80E-17
GO:0009535	C	chloroplast thylakoid membrane	32	190	2.80E-18	8.80E-17
GO:0044434	C	chloroplast part	54	604	1.60E-16	4.60E-15
GO:0044435	C	plastid part	54	658	5.60E-15	1.40E-13
GO:0031978	C	plastid thylakoid lumen	14	70	6.30E-10	1.40E-08
GO:0009543	C	chloroplast thylakoid lumen	14	70	6.30E-10	1.40E-08
GO:0031977	C	thylakoid lumen	14	76	2.00E-09	4.00E-08
GO:0009521	C	photosystem	10	40	1.70E-08	3.30E-07
GO:0030076	C	light-harvesting complex	8	25	5.60E-08	9.80E-07
GO:0031090	C	organelle membrane	39	633	6.20E-08	1.00E-06
GO:0009507	C	chloroplast	92	2160	8.40E-08	1.30E-06
GO:0009536	C	plastid	92	2298	1.00E-06	1.50E-05
GO:0009523	C	photosystem II	6	25	1.70E-05	0.00024
GO:0030095	C	chloroplast photosystem II	5	18	4.10E-05	0.00054
GO:0010287	C	plastoglobule	7	57	0.00031	0.0039

**Table S6. Gene ontology (GO) process categories of genes down-regulated (>2.0-fold change) in *Arabidopsis* shoots.**

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0015979	P	photosynthesis	34	107	1.90E-25	2.40E-22
GO:0019684	P	photosynthesis, light reaction	18	63	2.40E-13	1.50E-10
GO:0050896	P	response to stimulus	163	2998	9.90E-12	4.10E-09
GO:0009628	P	response to abiotic stimulus	75	1060	3.30E-11	1.00E-08
GO:0009416	P	response to light stimulus	42	434	5.50E-11	1.40E-08
GO:0009266	P	response to temperature stimulus	36	339	9.10E-11	1.90E-08
GO:0009314	P	response to radiation	42	448	1.50E-10	2.60E-08
GO:0006950	P	response to stress	99	1706	2.00E-09	3.10E-07
GO:0006952	P	defense response	45	577	1.10E-08	1.50E-06
GO:0006091	P	generation of precursor metabolites and energy	22	181	3.00E-08	3.70E-06
GO:0009408	P	response to heat	18	125	3.70E-08	4.20E-06
GO:0009639	P	response to red or far red light	19	155	2.20E-07	2.30E-05
GO:0009767	P	photosynthetic electron	8	27	7.80E-07	7.50E-05

		transport chain				
GO:0010218	P	response to far red light	9	41	2.60E-06	0.00023
GO:0010114	P	response to red light	10	53	3.10E-06	0.00026
GO:0010200	P	response to chitin	15	127	6.10E-06	0.00048
GO:0009743	P	response to carbohydrate stimulus	19	197	8.10E-06	0.00059
GO:0042221	P	response to chemical stimulus	78	1548	1.10E-05	0.00079
GO:0009617	P	response to bacterium	18	192	2.00E-05	0.0013
GO:0022900	P	electron transport chain	9	55	3.20E-05	0.002
GO:0006955	P	immune response	21	265	5.40E-05	0.0032
GO:0002376	P	immune system process	21	266	5.70E-05	0.0032
GO:0048511	P	rhythmic process	8	47	6.50E-05	0.0034
GO:0007623	P	circadian rhythm	8	47	6.50E-05	0.0034
GO:0009607	P	response to biotic stimulus	31	482	6.90E-05	0.0034
GO:0055114	P	oxidation reduction	13	126	0.0001	0.0049
GO:0009409	P	response to cold	18	220	0.00012	0.0054
GO:0080134	P	regulation of response to stress	9	67	0.00016	0.0067
GO:0009637	P	response to blue light	8	53	0.00016	0.0067
GO:0031347	P	regulation of defense response	8	54	0.00018	0.0072
GO:0050832	P	defense response to fungus	10	84	0.00019	0.0072
GO:0010033	P	response to organic substance	51	995	0.00019	0.0072
GO:0045087	P	innate immune response	19	249	0.00019	0.0072
GO:0009642	P	response to light intensity	9	70	0.00022	0.008
GO:0042742	P	defense response to bacterium	13	138	0.00025	0.009
GO:0009620	P	response to fungus	12	121	0.00027	0.0092
GO:0050776	P	regulation of immune response	6	33	0.00037	0.012
GO:0002682	P	regulation of immune system process	6	33	0.00037	0.012
GO:0051707	P	response to other organism	27	448	0.00051	0.016
GO:0009814	P	defense response, incompatible interaction	10	95	0.00052	0.016
GO:0006457	P	protein folding	15	209	0.0016	0.047
GO:0010035	P	response to inorganic substance	18	276	0.0017	0.05
GO:0016168	F	chlorophyll binding	11	25	4.70E-11	2.00E-08
GO:0046906	F	tetrapyrrole binding	12	109	9.90E-05	0.021
GO:0009579	C	thylakoid	37	301	6.80E-13	2.20E-10
GO:0034357	C	photosynthetic membrane	31	221	1.40E-12	2.30E-10



GO:0044434	C	chloroplast part	54	604	3.10E-12	3.40E-10
GO:0044435	C	plastid part	56	658	8.80E-12	7.20E-10
GO:0030076	C	light-harvesting complex	11	25	4.70E-11	3.10E-09
GO:0010287	C	plastoglobule	15	57	8.30E-11	4.50E-09
GO:0009521	C	photosystem	12	40	1.20E-09	5.50E-08
GO:0042651	C	thylakoid membrane	24	203	1.30E-08	5.20E-07
GO:0044436	C	thylakoid part	26	247	3.70E-08	1.30E-06
GO:0009535	C	chloroplast thylakoid membrane	22	190	7.20E-08	2.10E-06
GO:0055035	C	plastid thylakoid membrane	22	190	7.20E-08	2.10E-06
GO:0031976	C	plastid thylakoid	24	237	2.40E-07	6.10E-06
GO:0009534	C	chloroplast thylakoid	24	237	2.40E-07	6.10E-06
GO:0031984	C	organelle subcompartment	24	239	2.80E-07	6.60E-06
GO:0009523	C	photosystem II	8	25	4.00E-07	8.80E-06
GO:0009507	C	chloroplast	106	2160	1.60E-06	3.30E-05
GO:0009536	C	plastid	107	2298	1.20E-05	0.00024
GO:0009570	C	chloroplast stroma	18	210	6.50E-05	0.0012
GO:0009532	C	plastid stroma	19	240	0.00012	0.0021
GO:0030095	C	chloroplast photosystem II	5	18	0.00014	0.0023
GO:0044425	C	membrane part	50	1004	0.00041	0.0063
GO:0005576	C	extracellular region	16	205	0.00046	0.0068
GO:0031978	C	plastid thylakoid lumen	8	70	0.0011	0.015
GO:0009543	C	chloroplast thylakoid lumen	8	70	0.0011	0.015
GO:0031977	C	thylakoid lumen	8	76	0.0018	0.024
GO:0010319	C	stromule	5	32	0.0023	0.029

**Table S7. Annotation and relative expression quantity of 54 genes involved in chloroplast part (GO:0044434) in *Arabidopsis* shoots.**

Gene ID	Annotation	Relative Expression Quantity				logFC	P-value
		CK-1	CK-2	1000-1	1000-1		
	oxidoreductases, acting on NADH or NADPH, quinone or similar compound as acceptor						
<i>AT5G58260</i>	or NADPH, quinone or similar compound as acceptor	54.60119922	67.93502867	33.40551322	26.39392387	-1.212288388	0.002226225
<i>AT2G01870</i>	transmembrane protein	9.420120937	10.09434899	5.412383688	5.26106626	-1.059961511	1.03E-05
<i>AT4G02770</i>	photosystem I subunit D-1	1251.168596	1499.191709	662.5260661	637.4508568	-1.26361074	2.97E-08
<i>AT4G27700</i>	Rhodanese/Cell cycle control phosphatase superfamily protein	57.00527682	66.47631546	31.65021261	29.35276519	-1.201380689	4.84E-21
<i>AT1G14150</i>	PsbQ-like 2	73.53527629	77.86145358	36.30596252	29.60696242	-1.38754352	1.23E-12
<i>AT1G06680</i>	photosystem II subunit P-1	1412.202488	1733.395306	893.5057199	878.204031	-1.009561485	2.71E-07
<i>AT5G51720</i>	2 iron, 2 sulfur cluster binding protein	46.21028127	52.00079245	12.88335527	11.48151964	-2.197436785	3.09E-30
<i>AT4G12800</i>	photosystem I subunit I	1337.337127	1714.347263	783.9850025	798.0208204	-1.127176996	0.002974107

<i>AT1G52230</i>	photosystem I subunit H2	349.5755857	450.7568692	159.0546148	156.9162092	-1.519315324	2.45E-11
<i>AT4G19170</i>	nine-cis-epoxycarotenoid dioxygenase 4	15.35570694	19.74217666	7.349569823	8.789241644	-1.301709254	3.36E-17
<i>AT2G05100</i>	photosystem II light harvesting complex protein 2.1	121.5231506	157.1194092	31.24450796	29.20359781	-2.382114772	4.13E-07
<i>AT2G34420</i>	photosystem II light harvesting complex protein B1B2	5614.999775	5370.671637	2251.182301	2471.163723	-1.416049112	1.33E-05
<i>AT1G23740</i>	Oxidoreductase, zinc-binding dehydrogenase family protein	25.81028186	28.16685506	15.6827241	13.349558	-1.081070467	0.000105018
<i>AT2G26500</i>	cytochrome b6f complex subunit (petM)	506.6154109	885.1489462	309.2699164	276.2713573	-1.408329311	0.00410608
<i>AT1G15980</i>	NDH-dependent cyclic electron flow 1	77.17469085	85.01534409	39.45910454	32.28989663	-1.361865777	9.02E-12
<i>AT5G38420</i>	Ribulose biphosphate carboxylase (small chain) family protein	3844.432718	4173.087017	2300.337033	1943.100704	-1.104286084	1.24E-06
<i>AT1G20020</i>	ferredoxin-NADP[+]- oxidoreductase 2	419.1373375	465.7756298	196.7875919	187.5859008	-1.389858332	0.000207911
<i>AT5G53490</i>	Tetratricopeptide repeat (TPR)-like superfamily protein	77.3408258	129.4993557	56.14408456	57.85449748	-1.023476667	6.46E-07
<i>AT1G32080</i>	membrane protein	54.54498282	66.30639747	31.02129187	28.06444279	-1.213279099	5.07E-16
<i>AT1G54780</i>	thylakoid lumen 18.3 kDa protein	131.0302354	156.7722372	82.26780848	79.79978195	-1.011180553	0.00040162
<i>AT5G62720</i>	Integral membrane HPP family protein	119.9165968	144.1945745	38.46299572	61.00294353	-1.598511295	3.34E-13
<i>AT3G15840</i>	post-illumination chlorophyll fluorescence increase subunit NDH-M of	97.86837382	118.0720566	52.86159885	54.64992793	-1.189312415	5.72E-06
<i>AT4G37925</i>	NAD(P)H:plastoquinone dehydrogenase complex	66.84700087	75.20943362	37.36011194	34.8039563	-1.163173029	4.66E-16
<i>AT4G09650</i>	F-type H <sup>+</sup> -transporting ATPase subunit delta	370.2421012	433.0357571	239.855942	211.0778127	-1.015548745	1.16E-06
<i>AT2G04030</i>	Chaperone protein htpG family protein	101.6285791	120.2537604	65.49250311	57.30860981	-1.035313951	4.97E-11
<i>AT5G07020</i>	proline-rich family protein	102.8769888	122.3082729	65.49593587	61.94781202	-1.004084805	4.00E-12
<i>AT3G54890</i>	chlorophyll a-b binding protein 6	1339.693687	1261.780617	679.6239451	589.7999736	-1.230422791	1.47E-07
<i>AT1G67740</i>	photosystem II BY	885.2034278	1014.748368	478.9596858	463.1492093	-1.197196713	2.01E-07
<i>AT1G14345</i>	NAD(P)-linked oxidoreductase superfamily protein	67.98684912	77.71635399	36.67480403	30.7692248	-1.294690825	7.74E-08
<i>AT1G15820</i>	light harvesting complex photosystem II subunit 6	518.5386335	619.5977769	286.5568149	298.2168894	-1.144582386	7.27E-06
<i>AT5G21430</i>	Chaperone DnaJ-domain superfamily protein	61.28051691	63.96186267	36.71658699	33.66902622	-1.021559971	2.46E-09
<i>AT4G17090</i>	chloroplast beta-amylase	49.29345859	65.24538413	16.211112	19.79806243	-1.849486548	4.37E-11
<i>AT1G80380</i>	P-loop containing nucleoside triphosphate hydrolases superfamily	121.0110015	102.6086051	63.5042566	60.77573695	-1.050453002	0.002051589

	protein						
AT3G28220	TRAF-like family protein	1.701618016	1.322324073	0.669160505	0.546368894	-1.52166538	5.93E-05
AT3G01440	PsbQ-like 1	26.56192311	33.9728914	12.57077969	9.380462052	-1.638667826	1.79E-06
AT3G47470	light-harvesting chlorophyll-protein complex I subunit A4	1117.569286	1229.120232	628.031272	646.1375776	-1.069712003	1.76E-07
AT2G34430	light-harvesting chlorophyll-protein complex II subunit B1	2153.710174	1867.048128	746.3290508	682.2268931	-1.693901685	2.73E-11
AT5G66190	ferredoxin-NADP[+]-oxidoreductase 1	357.0372088	381.3346162	191.6308951	187.5953066	-1.150920226	5.69E-13
AT2G05070	photosystem II light harvesting complex protein 2.2	118.3024132	159.7819897	37.88342353	33.92039855	-2.127618957	8.89E-06
AT3G48420	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	54.35113327	58.72947431	26.0968898	24.53062265	-1.347662154	5.76E-05
AT3G50820	photosystem II subunit O-2	475.7644344	564.4041495	299.2893485	290.5293518	-1.001687807	5.67E-06
AT3G16250	NDH-dependent cyclic electron flow 1	56.59377766	69.3587478	28.95640005	24.3769338	-1.419458775	4.99E-15
AT3G62410	CP12 domain-containing protein 2	292.0221084	349.6578165	122.7531804	110.7203773	-1.640205994	2.81E-16
AT1G20340	Cupredoxin superfamily protein	2024.588399	2024.180401	1188.347947	1104.241	-1.013266538	0.000375279
AT4G04020	fibrillin	29.1876242	38.47893665	16.12524662	14.08400868	-1.339557078	6.21E-17
AT1G51110	Plastid-lipid associated protein PAP / fibrillin family protein	26.67054664	28.39566756	11.58840751	11.96541417	-1.415709056	7.03E-20
AT1G61520	PSI type III chlorophyll a/b-binding protein	1314.673057	1511.517822	764.4211945	766.0695276	-1.070441905	4.66E-06
AT5G02940	ion channel POLLUX-like protein, putative	14.70755609	17.54219959	6.961075763	7.593393245	-1.332289194	1.34E-13
AT3G54050	high cyclic electron flow 1 thylakoid lumenal protein	216.6625859	240.0070917	129.916241	125.6426038	-1.024773727	6.66E-13
AT1G77090	(Mog1/PsbP/DUF1795-like photosystem II reaction center PsbP family protein)	24.72780729	24.55827415	13.57496169	11.51899504	-1.165966276	5.40E-06
AT1G55480	protein containing PDZ domain, a K-box domain, and a TPR region	52.28236903	60.96242927	32.380986	29.5336477	-1.054465174	8.49E-06
AT3G13470	TCP-1/cpn60 chaperonin family protein	80.12207414	96.60347885	54.15564246	45.68956617	-1.003975048	1.35E-09
AT1G60950	2Fe-2S ferredoxin-like superfamily protein	1590.235855	1732.829928	923.0044946	836.0748109	-1.104920462	2.46E-11
AT3G47070	thylakoid soluble phosphoprotein	147.1026899	178.2231943	85.94994935	82.41808176	-1.132074436	9.91E-07

**Table S8. Primary metabolites detected in *Arabidopsis* roots exposed to different concentrations of C-dots**

Metabolites	Retention Time	0 mg/L C-dots	125 mg/L C-dots	1000 mg/L C-dots
<b>Amino Acid</b>				
L-Proline	14.869	8917980±7888490 <sup>ab</sup>	7634440±7149610 <sup>b</sup>	18252000±5827090 <sup>a</sup>
L-Tyrosine	21.83	4488610±391034 <sup>ab</sup>	4708580±494764 <sup>a</sup>	3853480±745613 <sup>b</sup>
Pidolic Acid	14.998	2648050±327861 <sup>a</sup>	2553040±507896 <sup>a</sup>	3379330±2521950 <sup>a</sup>
4-Oxoproline	15.625	1696680±813330 <sup>a</sup>	1966310±523564 <sup>a</sup>	1859180±782115 <sup>a</sup>
<b>Organic Acid</b>				
Pentanedioic acid	14.555	5278860±1324510 <sup>a</sup>	3821480±843460 <sup>a</sup>	4139850±1120300 <sup>a</sup>
3-Methyladipic acid	21.089	4742270±950723 <sup>a</sup>	3459910±975399 <sup>b</sup>	2208360±404149 <sup>c</sup>
Gluconic acid	17.734	4109770±374274 <sup>a</sup>	3924260±606438 <sup>a</sup>	4246690±934486 <sup>a</sup>
Carbamic acid	12.186	3782040±1413150 <sup>a</sup>	4477670±654099 <sup>a</sup>	5009650±776936 <sup>a</sup>
2-Ketoadipic acid	19.821	3526840±727478 <sup>a</sup>	3738880±550093 <sup>a</sup>	3673220±564604 <sup>a</sup>
Salicylic acid	9.244	3105810±440563 <sup>a</sup>	3253590±901468 <sup>a</sup>	3161560±876693 <sup>a</sup>
Malic acid	13.249	2713820±862439 <sup>a</sup>	2682470±493778 <sup>a</sup>	4184490±1456180 <sup>a</sup>
Dehydrodesmosterol acetate	21.051	2070870±73337.9 <sup>b</sup>	2678980±164080 <sup>a</sup>	2336500±389001 <sup>ab</sup>
Benzoic acid	15.067	1914210±576951 <sup>a</sup>	0±0 <sup>b</sup>	0±0 <sup>b</sup>
Ethanedioic acid	8.808	1348280±335286 <sup>a</sup>	1657540±487734 <sup>a</sup>	2219160±1060910 <sup>a</sup>
<b>Fatty Acid</b>				
Hexadecanoic acid	19.546	174184000±11874000 <sup>a</sup>	183501000±12844000 <sup>a</sup>	174331000±15002800 <sup>a</sup>
Octadecanoic acid	21.357	93879400±42182700 <sup>b</sup>	131048000±10393000 <sup>ab</sup>	135580000±9538600 <sup>a</sup>
Tetradecanoic acid	17.543	8809310±693741 <sup>a</sup>	8783360±1118930 <sup>a</sup>	8430680±736743 <sup>a</sup>
Octadecenoic acid	21.257	3430240±305408 <sup>a</sup>	2941110±477093 <sup>b</sup>	2014680±470205 <sup>b</sup>
<b>Sugar</b>				
Glucose-1-phosphate	38.551	571050000±43405000 <sup>a</sup>	561255000±36392100 <sup>a</sup>	544070000±82150500 <sup>a</sup>
Melezitose	23.007	253489000±51492800 <sup>a</sup>	288576000±23831400 <sup>a</sup>	436419000±53415300 <sup>a</sup>
d-Glucose oxime	16.84	97517000±30151200 <sup>a</sup>	60961100±21459300 <sup>b</sup>	42245800±14463000 <sup>b</sup>
d-Galactose oxime	16.565	90845600±32889200 <sup>a</sup>	69937700±23120300 <sup>ab</sup>	47984300±14400400 <sup>b</sup>
Ribulose-5-phosphate	23.068	20557700±2855050 <sup>b</sup>	31867100±4406140 <sup>a</sup>	32883300±2352110 <sup>a</sup>
Gulose	17.811	19785500±3225670 <sup>a</sup>	24142200±10306100 <sup>a</sup>	21052300±6715860 <sup>a</sup>
Maltose	23.183	9285400±1929160 <sup>a</sup>	9263610±1137840 <sup>a</sup>	9490900±815465 <sup>a</sup>
d-Glucopyranoside	28.769	7489710±3511420 <sup>a</sup>	5881800±1400750 <sup>ab</sup>	3411900±1035500 <sup>b</sup>
d-Xylofuranose	23.695	6044390±936774 <sup>a</sup>	5628210±800785 <sup>a</sup>	5948660±847641 <sup>a</sup>
d-Turanose	28.387	5742240±1989260 <sup>b</sup>	6728100±1073790 <sup>b</sup>	17371100±5306700 <sup>a</sup>
d-Glucopyranose	25.644	5380620±2493740 <sup>a</sup>	6729970±456424 <sup>a</sup>	4979090±965178 <sup>a</sup>
d-Glucose	16.978	4194590±1453870 <sup>a</sup>	3687680±390307 <sup>a</sup>	5087440±1546350 <sup>a</sup>
Talose	17.857	4189250±1401370 <sup>b</sup>	5030250±386624 <sup>b</sup>	8088450±1777320 <sup>a</sup>
Hexopyranose	24.612	2749370±232389 <sup>a</sup>	2809760±290225 <sup>a</sup>	2499160±261691 <sup>a</sup>
5-Ketofructose	15.908	2544340±736701 <sup>a</sup>	2113130±446122 <sup>a</sup>	2868010±685310 <sup>a</sup>
Galactinol	27.753	2436050±616350 <sup>a</sup>	1438700±187873 <sup>b</sup>	1814400±526283 <sup>ab</sup>
Levoglucofan	16.061	1671180±243759 <sup>ab</sup>	2156830±583449 <sup>a</sup>	1278420±271156 <sup>b</sup>

<b>Others</b>				
Glycerol	9.657	24934900±3808720 <sup>a</sup>	26673400±4153800 <sup>a</sup>	31327500±5727870 <sup>a</sup>
Inositol	18.415	21654900±3052190 <sup>b</sup>	28604400±4549870 <sup>a</sup>	34189000±5723950 <sup>a</sup>
d-Mannitol	17.054	21413300±6594100 <sup>a</sup>	15820200±4809590 <sup>a</sup>	13659500±4324200 <sup>a</sup>
1,3-Dipalmitin	37.565	16182100±6846370 <sup>a</sup>	23484500±10579700 <sup>a</sup>	22632400±5477840 <sup>a</sup>
Phloretin	22.274	10140000±4132470 <sup>a</sup>	10950400±2117650 <sup>a</sup>	14335900±1829020 <sup>a</sup>
Phosphoric acid	10.673	9730340±7492450 <sup>a</sup>	11254900±6543870 <sup>a</sup>	13276400±2325310 <sup>a</sup>
2-Monostearin	25.239	9280790±1690980 <sup>a</sup>	10535100±2477790 <sup>a</sup>	11837200±4643900 <sup>a</sup>
Arabitol	14.655	7698750±2231800 <sup>a</sup>	6950320±1838880 <sup>a</sup>	6667770±1478000 <sup>a</sup>
2-Monopalmitin	23.779	7575330±956010 <sup>b</sup>	8967850±667779 <sup>a</sup>	9329930±567109 <sup>a</sup>
Anhydrohexitol	16.137	4304480±756264 <sup>a</sup>	4421780±383063 <sup>a</sup>	3567110±721329 <sup>a</sup>
Monomyristin	22.495	3131720±588573 <sup>a</sup>	3423340±512162 <sup>a</sup>	3297480±451331 <sup>a</sup>
Monolaurin	20.883	2407120±658991 <sup>a</sup>	2583020±818727 <sup>a</sup>	1760470±355351 <sup>a</sup>
Phosphoglyceride	16.481	1948330±324088 <sup>a</sup>	1765480±415246 <sup>a</sup>	1783800±267004 <sup>a</sup>

The values were given as mean ± SD of six samples. Different letters represent significant differences between the treatment means ( $p < 0.05$ , LSD).

**Table S9. Primary metabolites detected in *Arabidopsis* shoots exposed to different concentrations of C-dots**

<b>Metabolites</b>	<b>Retention Time</b>	<b>0 mg/L C-dots</b>	<b>125 mg/L C-dots</b>	<b>1000 mg/L C-dots</b>
<b>Amino Acid</b>				
L-Proline	14.861	98427400±11763200 <sup>a</sup>	116835000±29729800 <sup>a</sup>	121860000±25694600 <sup>a</sup>
L-Valine	8.029	26694100±6082850 <sup>b</sup>	47478300±5985000 <sup>a</sup>	46317600±12428400 <sup>a</sup>
Serine	10.497	9658090±2932030 <sup>a</sup>	11600700±3566060 <sup>a</sup>	10102300±3410650 <sup>a</sup>
L-Ornithine	11.422	5995430±2200600 <sup>a</sup>	4786070±1542260 <sup>a</sup>	7387530±3387840 <sup>a</sup>
L-Aspartic acid	13.111	5141080±910296 <sup>a</sup>	5622660±1065840 <sup>a</sup>	4774060±1449440 <sup>a</sup>
L-Threonine	10.879	2737370±987722 <sup>a</sup>	3944190±574927 <sup>a</sup>	3686810±1547520 <sup>a</sup>
L-Homoserine	11.124	2405270±1288520 <sup>b</sup>	5084180±2354930 <sup>a</sup>	6658100±1474380 <sup>a</sup>
Glycine	6.806	1796280±550435 <sup>b</sup>	3144080±520259 <sup>a</sup>	2796130±899896 <sup>a</sup>
<b>Organic Acid</b>				
Octadecanoic acid	21.341	305842000±28502100 <sup>a</sup>	320903000±29953800 <sup>a</sup>	304929000±15987000 <sup>a</sup>
D-Galactopyranosiduronic acid	22.083	13229000±12924300 <sup>a</sup>	3755850±493650 <sup>a</sup>	4504700±2271850 <sup>a</sup>
2-hydroxyl-Butanedioic acid	13.226	12443700±3032060 <sup>a</sup>	10459100±1271480 <sup>a</sup>	6678190±2110500 <sup>b</sup>
Ethanedioic acid	8.806	10864800±1119620 <sup>b</sup>	16675200±4302670 <sup>a</sup>	15954900±2351490 <sup>a</sup>
2-Ketoadipic acid	19.782	9071710±1151210 <sup>b</sup>	11312000±1465980 <sup>a</sup>	12092300±1384960 <sup>a</sup>
Propanedioic acid	18.193	8275120±1996710 <sup>a</sup>	9882470±3662200 <sup>a</sup>	6409150±2171390 <sup>a</sup>
Glycerol phosphate	16.45	5948790±1043070 <sup>a</sup>	5801580±1203150 <sup>a</sup>	5974270±2076800 <sup>a</sup>

Fumaric acid	11.567	5363250±1886990 <sup>a</sup>	4439720±1062450 <sup>a</sup>	1920850±777291 <sup>b</sup>
L-Threonic acid	13.623	5223840±2528460 <sup>a</sup>	3251120±1007930 <sup>ab</sup>	2736300±724693 <sup>b</sup>
Hexacosanoic acid	27.975	5189590±980359 <sup>b</sup>	7657570±1090750 <sup>a</sup>	6653040±2021120 <sup>ab</sup>
Fumaric acid	24.115	4686750±1066270 <sup>a</sup>	4847570±843257 <sup>a</sup>	4964640±1032820 <sup>a</sup>
2-Pentenedioic acid	16.328	4650460±570869 <sup>a</sup>	5066100±1253840 <sup>a</sup>	3782210±800988 <sup>a</sup>
Dodecanoic acid	15.358	4340590±804674 <sup>a</sup>	4401310±793453 <sup>a</sup>	4323010±728653 <sup>a</sup>
2,2-Dihydroxyacetic acid	12.179	3957130±756821 <sup>a</sup>	3601790±969620 <sup>a</sup>	3825550±1374500 <sup>a</sup>
Hexanedioic acid	13.972	3873910±1482440 <sup>a</sup>	4377680±672514 <sup>a</sup>	3129700±1542500 <sup>a</sup>
Pentadecanoic acid	18.521	3590110±1246150 <sup>a</sup>	3214530±850666 <sup>a</sup>	3977110±1669020 <sup>a</sup>
Terephthalic acid	22.319	3559320±953546 <sup>b</sup>	4616660±826994 <sup>ab</sup>	5653140±1335800 <sup>a</sup>
Hexenoic acid	10.434	3276070±1175260 <sup>a</sup>	3959370±3066310 <sup>a</sup>	4992720±1138500 <sup>a</sup>
Octacosanoic acid, synthetic	31.054	3086680±431992 <sup>b</sup>	3390930±226643 <sup>a</sup>	3733200±264232 <sup>a</sup>
Butyric acid	7.196	2659300±843639 <sup>b</sup>	2787520±1214960 <sup>b</sup>	5738700±3274210 <sup>a</sup>
Tetradecanoic acid	17.513	25782600±3145570 <sup>a</sup>	25241800±3630380 <sup>a</sup>	24640000±3223000 <sup>a</sup>
10,12-Docosadienedioic acid	28.158	2426950±902310 <sup>a</sup>	2614610±174020 <sup>a</sup>	2684460±1136510 <sup>a</sup>
Carboxylic acid	11.506	2153330±263655 <sup>b</sup>	3907640±774940 <sup>a</sup>	4307900±1166370 <sup>a</sup>
Eicosanoic acid	27.287	1241520±394254 <sup>a</sup>	1348880±235959 <sup>a</sup>	1505770±269500 <sup>a</sup>
<b>Fatty Acid</b>				
Hexadecanoic acid	19.523	332728000±40646800 <sup>a</sup>	347045000±34648900 <sup>a</sup>	336020000±22333500 <sup>a</sup>
Myristic acid	22.793	8187100±1915060 <sup>a</sup>	8658270±1114600 <sup>a</sup>	9636520±1258850 <sup>a</sup>
Oleic acid	21.242	7100630±947194 <sup>a</sup>	28211500±48871600 <sup>a</sup>	9698190±1749780 <sup>a</sup>
Sebacic acid	21.074	5929840±1393700 <sup>a</sup>	4674440±1008470 <sup>a</sup>	0±0 <sup>b</sup>
Octadecanoic acid	20.745	4475550±1126960 <sup>a</sup>	5748700±757632 <sup>a</sup>	5231280±899158 <sup>a</sup>
Cis-10-Heptadecenoic acid	27.592	2720010±614203 <sup>b</sup>	3768170±716816 <sup>a</sup>	3470880±202378 <sup>ab</sup>
9,12,15-Octadecatrienoic acid	33.996	2218880±315699 <sup>b</sup>	2763730±270787 <sup>a</sup>	2855770±470034 <sup>a</sup>
<b>Sugar</b>				
D-Glucopyranoside	22.961	114186000±20157700 <sup>b</sup>	171842000±29103900 <sup>a</sup>	192980000±46164700 <sup>a</sup>
D-Ribose	16.55	48965700±18449900 <sup>a</sup>	39540000±16154700 <sup>a</sup>	26807900±14214100 <sup>a</sup>
Gulose	17.795	34176900±9872880 <sup>a</sup>	46155300±16204600 <sup>a</sup>	42845900±14666500 <sup>a</sup>
Glucose	16.825	31603900±14330300 <sup>a</sup>	22990000±7321030 <sup>a</sup>	26704500±24612500 <sup>a</sup>
D-Fructose	16.687	31136200±7888570 <sup>a</sup>	26404600±10549000 <sup>a</sup>	19147400±10390400 <sup>a</sup>
Mannose	25.59	12775500±3209830 <sup>a</sup>	15231100±2345820 <sup>a</sup>	15324800±1946890 <sup>a</sup>
D-Erythro pentose	19.721	12583100±2079550 <sup>a</sup>	13580900±1417380 <sup>a</sup>	12873700±2758960 <sup>a</sup>
D-Turanose	25.086	10609100±1945210 <sup>a</sup>	13515600±879849 <sup>a</sup>	11053700±2127070 <sup>a</sup>
N-Acetyl glucosamine				
methoxime	18.078	9868990±2679890 <sup>a</sup>	12075600±2701470 <sup>a</sup>	7629430±3319820 <sup>a</sup>
Maltose	19.163	9737210±2817150 <sup>a</sup>	8202220±1477250 <sup>a</sup>	8455030±2039980 <sup>a</sup>
Mannofuranose	23.657	9250990±1550760 <sup>a</sup>	11312900±1560310 <sup>a</sup>	10887000±1480350 <sup>a</sup>
D-Xylofuranose	16.122	8937180±2451240 <sup>a</sup>	8905690±968645 <sup>a</sup>	8749180±1551320 <sup>a</sup>
Glycoside	23.519	5885070±792295 <sup>a</sup>	7966970±397229 <sup>a</sup>	5648100±1065330 <sup>a</sup>
Melibiose	19.859	5618890±1639440 <sup>b</sup>	8752240±2060860 <sup>ab</sup>	10036700±2544790 <sup>a</sup>

DL-Arabinopyranose	18.139	4089580±241499 <sup>a</sup>	2996300±559574 <sup>b</sup>	3113770±500527 <sup>b</sup>
Melezitose	30.053	3328160±1020790 <sup>a</sup>	4100590±631156 <sup>a</sup>	3447240±740773 <sup>a</sup>
Hexopyranose	19.094	2353070±417841 <sup>b</sup>	2652570±420171 <sup>b</sup>	3922900±913545 <sup>a</sup>
<b>Others</b>				
Myo-Inositol	18.392	15826400±6330370 <sup>a</sup>	18777700±4500090 <sup>a</sup>	19396600±4282220 <sup>a</sup>
Benzofuran	16.932	14826600±2043560 <sup>a</sup>	17325000±3579060 <sup>a</sup>	16656700±2978280 <sup>a</sup>
Xylitol	14.647	11507800±2551180 <sup>a</sup>	11127700±2572640 <sup>a</sup>	11001500±1926310 <sup>a</sup>
Glucitol	17.008	10882800±3718280 <sup>a</sup>	10485900±3106320 <sup>a</sup>	9510100±5841280 <sup>a</sup>
1-Monopalmitoylglycerol	24.024	161632000±21140200 <sup>a</sup>	173287000±12612000 <sup>a</sup>	185296000±29306000 <sup>a</sup>
1-Glycerol stearate	25.483	97887500±13751000 <sup>a</sup>	103308000±6982640 <sup>a</sup>	110401000±18352800 <sup>a</sup>
Phosphoric acid	10.643	95020900±22826500 <sup>a</sup>	76535700±9000740 <sup>ab</sup>	57093300±12942700 <sup>b</sup>
Glycerol	9.664	85103600±22742000 <sup>a</sup>	86108400±22533300 <sup>a</sup>	71243800±10044900 <sup>a</sup>
3,4-Octadecanetriol	15.847	16136100±3423360 <sup>a</sup>	15993500±2843860 <sup>a</sup>	15465100±4601200 <sup>a</sup>
2-Monopalmitin	23.741	13923300±1117560 <sup>b</sup>	17439800±1732370 <sup>a</sup>	18460700±1888080 <sup>a</sup>
1,4-Butanediamine	15.098	6561720±736956 <sup>a</sup>	6533860±900172 <sup>a</sup>	6354280±715217 <sup>a</sup>
Gulonic acid-lactone	16.045	6190290±2068680 <sup>a</sup>	8882730±890440 <sup>a</sup>	7989220±3326200 <sup>a</sup>
1,4-Benzenediol	14.425	4596690±532964 <sup>a</sup>	4162990±455045 <sup>a</sup>	4324310±766528 <sup>a</sup>
2,6-Di-tert-butylbenzoquinone	14.257	3371450±302040 <sup>a</sup>	3400080±778529 <sup>a</sup>	3486580±602244 <sup>a</sup>
Methyl -heptadecanoate	20.783	1546270±362835 <sup>b</sup>	2215490±331830 <sup>ab</sup>	2997140±1266640 <sup>a</sup>

The values were given as mean ± SD of six samples. Different letters represent significant differences between the treatment means ( $p < 0.05$ , LSD).