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ORIGINAL ARTICLE

The abundance of certain metabolites responds to drought stress in the highly drought tolerant plant *Caragana korshinskii*

Jiwei Zhang[1,2] · Guoxiong Chen[1] · Pengshan Zhao[1] · Qin Zhou[1] · Xin Zhao[1,3]

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Abstract Metabolomics offers opportunities for studying the systematic response of an organism to a genetic and/or an environmental change. Here, the metabolic consequences of drought stress were characterized in the highly drought tolerant plant *Caragana korshinskii*. The time-of-flight mass spectrometry platform employed identified several hundred metabolites in extracts of the leaf, stem, root collar, and root of plants which had been either subjected to drought stress or were well-watered. Each of the four organs harbored a number of potential metabolite markers for the drought response. An increased abundance of various small carbohydrates and soluble amino acids in each of the four organs was induced by the stress; these compounds may act as compatible solutes or antioxidants. Across the whole plant, there was a fall in the content of several Krebs cycle and glycolysis intermediates, as well as in that of the amino acids glutamic acid and aspartic acid. Pathway analysis suggested that most of the potential metabolite markers were involved in energy metabolism and amino-acid metabolism. The implication was that energy metabolism and photosynthesis are compromised during the adaptation of *C. korshinskii* to drought stress. Given the different spectrum of metabolites associated with the drought response in the four organs, it was concluded that each organ employs a distinct strategy to cope with drought stress.

Keywords Metabolomics · *Caragana korshinskii* · Drought stress · Metabolite markers · Organs · Energy metabolism

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✉ Xin Zhao
zhaox@lzb.ac.cn

Jiwei Zhang
zhangjiwei@lzb.ac.cn

Guoxiong Chen
guoxiong@lzb.ac.cn

Pengshan Zhao
zhaopengshan@lzb.ac.cn

Qin Zhou
zhouqin@lzb.ac.cn

[1] Key Laboratory of Stress Physiology and Ecology in Cold and Arid Regions, Gansu Province, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China

[2] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[3] Shapotou Desert Research and Experiment Station, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China

Introduction

The perennial xerophytic shrub *Caragana korshinskii* is widely distributed across the arid and semi-arid zones of north-western China and Mongolia (Wang et al. 2007; Zhang et al. 2009), where it is frequently used as a soil stabilizer (Li et al. 2003; Wang et al. 2004). Its tolerance to various abiotic stresses, and especially drought, makes it attractive as a model for the study of the plant response to stress (Li et al. 2004). Seedlings are able to maintain a level of photosynthetic activity even when exposed to fairly severe moisture stress (Fang et al. 2011). Mature plants

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react to strong drought conditions by shedding their leaves to reduce evapotranspirative water loss, while their stems retain photosynthetic capacity (Xu et al. 2012). The root system remains active even after the aerial part of the plant has become dehydrated, allowing rapid regrowth in response to rainfall (Fang et al. 2007). The ecophysiological responses to drought have been well characterized (Xu et al. 2012; Wang et al. 2007), but a little research has been directed at the molecular level, beyond the demonstration that starch hydrolysis and mobilization occur in the root system once shoots have been removed (Fang et al. 2006). More recently, Li et al. (2016) have taken a transcriptomic approach to reveal the identity of genes expressed in seedlings exposed to either drought or salinity stress. However, the metabolic consequences of drought stress have not been characterized in *C. korshinskii*.

The analysis of the metabolome has begun to have a positive impact on elucidating some of the complex molecular interactions which are integral to all biological systems (Angelcheva et al. 2014; Satou et al. 2014; Niinemets 2016). A range of technologies, such as gas chromatography (GC), liquid chromatography (LC), high-resolution nuclear magnetic resonance (NMR), and mass spectrometry (MS), have been harnessed in recent years to quantify important metabolites. The GC–MS approach is both powerful and sensitive, and can be adapted to generate data related to the response to abiotic stress in a high-throughput mode (Sanchez et al. 2012; Lu et al. 2013). GC–MS-based metabolite profiling has been used to characterize the metabolic response and to identify metabolite markers for tolerance to drought stress in a number of plant species (Obata et al. 2015). Since Gargallo-Garriga et al. (2014) have shown that the shoots and roots of the grasses *Holcus lanatus* and *Alopecurus pratensis* generate non-identical metabolomes when the plants are exposed to drought, metabolomic studies need to consider the response of individual plant organs, rather than the focusing on the whole plant. A metabolomic contrast derived from *Pinus pinaster* plants exposed to both a moderate and a prolonged drought episode showed that some metabolites respond similarly in many organs, while the changes to the concentration of others vary from organ to organ (de Miguel et al. 2016). The present study sets out to characterize the metabolomic response of *C. korshinskii* to extreme drought at the organ level. The aims were to explore what differences there were in individual metabolite concentrations generated by drought stress with a view to identifying plausible markers for tolerance; to reveal any organ-to-organ variation (leaf, stem, root collar, and root) in the content of the more abundant metabolites; and to discover what pathways likely confer drought tolerance.

Materials and methods

Plant growth and treatments, and the measurement of relative water content

Six individual 2-year-old *C. korshinskii* plants were raised in 4 L pots filled with three parts sand to one part commercial compost, and were subsequently transferred to a greenhouse, where three of the pots were drought-stressed by withholding watering, while the other three were kept well-watered. After 40 days, the plants were harvested, and then separated into leaf, stem, root collar, and root. Both the soil water content (SWC) and leaf relative water content (LRWC) were measured from three replicate samples per pot (Fig. 1), following Barrs and Weatherley (1962) and Jian et al. (2014). The leaf material was weighed to obtain its fresh mass (FM), then immersed in water overnight in the dark, and reweighed to obtain its turgid mass (TM). After baking at 80 °C for 24 h, its dry mass (DM) was recorded. LRWC was calculated from the expression $(FM - DM)/(TM - DM) \times 100\%$. The soil was sampled both from the top 10 cm and between 10 and 25 cm, using a steel cylinder of diameter 1.2 cm and length 5 cm. After removal of roots and stones, the soil was weighed (FM), baked at 105–110 °C for 72 h, and then reweighed to obtain its DM. SWC was calculated from the expression $(FM - DM)/DM \times 100\%$.

Sample preparation

Metabolites and their derivatives were extracted from each of the four organs following a protocol modified from those given by Kreuzwieser et al. (2009) and Erxleben et al. (2012). Approximately 50 mg of frozen powdered tissue was extracted per sample by adding 500 µL of a 3:1 mixture of chloroform and methanol. After homogenization, the material was centrifuged (14,000 $\times g$, 4 °C, 10 min). A 40 µL aliquot of the supernatant was dried under vacuum, and the resulting residue methoximated by the addition of 60 µL of 20 mg/mL methoxyamine hydrochloride dissolved in anhydrous pyridine, followed by incubation at 37 °C for 2 h with shaking (1400 rpm). The trimethylsilylation step was achieved by the addition of 80 µL *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide, followed by a 1 h incubation at 70 °C for 1 h with shaking (1400 rpm). A standard chloroform-based mixture of fatty acid methyl esters was added after cooling to ambient temperature. After a brief vortexing, the samples were centrifuged (14,000 $\times g$, 25 °C, 2 min) and a 100 µL aliquot of the supernatant was transferred to an amber GC–MS vial (Agilent Technologies, Palo Alto, CA, USA).

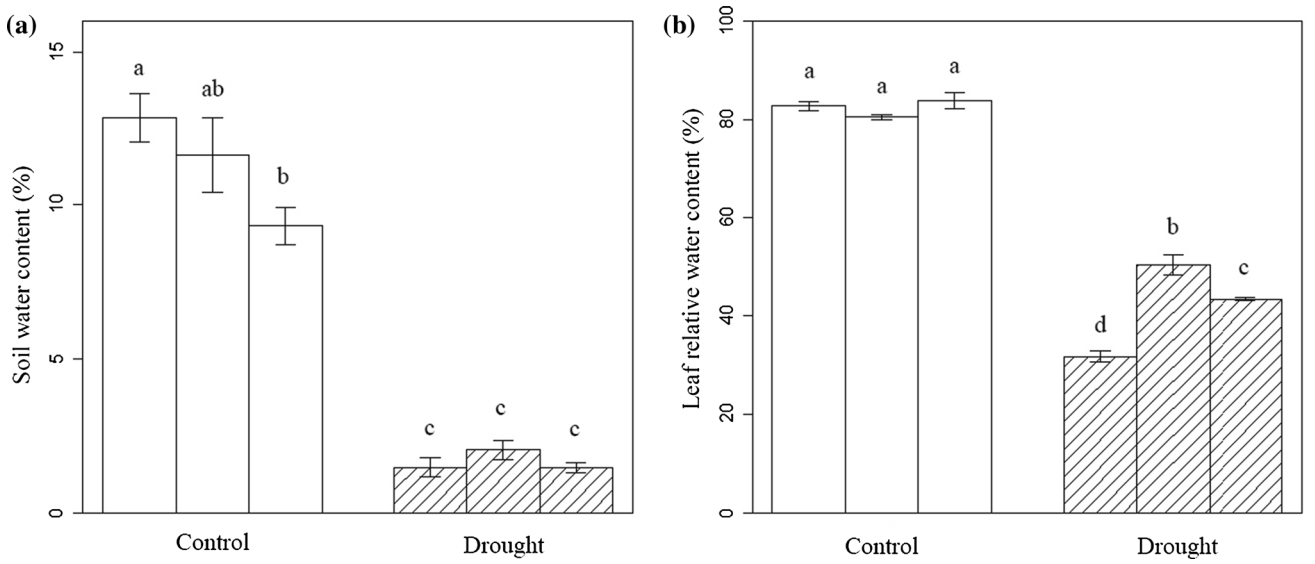

Fig. 1 Level of drought stress applied to 2-year-old *C. korshinskii* plants. Three plants were subjected to drought stress by withholding water for 40 days (*Drought*), while the other three were kept well-watered (*Control*). Soil water content (**a**) and leaf relative water content (**b**) were measured from three replicate samples per pot and per plant, respectively. *All bars* represent mean ± SE ($n = 3$). *Different letters* above *error bars* indicate statistically significant differences (Student's *t* test, $P < 0.05$)

GC–MS analysis and metabolite detection

The 7890 gas chromatograph system coupled with mass spectrometer (Agilent Technologies) was used for *metabolite* detection in this study. The running conditions were in accordance with Angelcheva et al. (2014) and Erxleben et al. (2012). Reference was made to the LECO/Fiehn Metabolomics Library (www.leco.com/component/edocman/leco-fiehn-metabolomics-library-209-207) to identify the compounds represented in the samples; on the basis of similarity values (which specify the accuracy of the identification), only those with a value greater than 600 were considered as reliable. Compounds associated with a value in the range 200–600 were given a putative annotation.

Statistical analysis

Chroma TOF 4.3X software (www.leco.com) and the LECO/Fiehn Rtx5 database (version Rtx5; LECO) were used for metabolite identification (Guo et al. 2015; Kind et al. 2009). A retention time index (RI) was used for peak identification, allowing a tolerance of 5000. Missing values were replaced by a value set at 50% of the minimum. The internal standard normalization was set using ribitol. The resulting data (peak number and normalized peak area) provided the input for both a principal component (PCA) and a partial least squares discriminant (PLS-DA) analysis, as implemented within the SIMCA-P 11.5 software package (Umetrics, Umeå, Sweden). Statistical significance relating to differences between the metabolite contents of the droughted and well-watered plants was assigned using Student's *t* test and an analysis of variance, implemented within PASW Statistics v18.0 software (SPSS Inc., Chicago, IL, USA). Metabolic pathways were constructed with the aid of the KEGG pathway database (www.genome.jp/kegg/pathway.html) and Metaboanalyst software (www.metaboanalyst.ca).

Results

The phenotypic effects of drought

There was a clear phenotypic difference between the droughted and the well-watered plants at the end of the 40 day period: the former plants exhibited extensive leaf chlorosis and some abscission, while the leaves of the latter remained green and had grown longer and wider. The SWC in the three droughted pots fell during the treatment period to an average of 1.65% by day 40, a level which was 12–17% that of the soil in the three well-watered pots (Fig. 1). The LRWCs decreased gradually during the course of the 40 days; at the end of the period, the well-watered plants' LRWC was 82.4%, whereas that of the droughted plants was 41.8% (Fig. 1).

Metabolite content

Leaf

In all, 664 putative metabolites were identified in the leaf extracts (Table S1). The PCA discriminated the well-

watered plants from the droughted ones (Fig. 2a). A total of 45 potential drought tolerance marker metabolites were identified, on the basis that their concentration was either increased (27 metabolites) or decreased (18) by at least twofold (P value < 0.05) (Table S2). Of those which were induced by the drought treatment, ten were sugars/glycosides, six were amino acids, and five were organic acids; the most prominent in each class was, respectively, *N*-acetyl-β-D-mannosamine (51.2-fold increase), asparagine (6.7-fold), and indoleacetate (10.2-fold). Among the 18 metabolites reduced in concentration by the drought treatment, the most prominent were aspartic acid and isocitric acid (Table S2).

Stem

A total of 560 putative metabolites were identified in the stem extracts (Table S3); once again, the PCA was able to separate the well-watered from the droughted plants (Fig. 2b). In all, 30 of the metabolites were considered as potential tolerance markers (Table S4): 25 showed an increase in abundance in the stressed plants and five a decrease. The latter were citric acid, linolenic acid (both falling to 24% of the abundance in the well-watered plants), valine, aconitic acid, and 3-hydroxy-3-methylglutaric acid (all falling to 26%), while the most prominent of the 25 up-regulated metabolites were lactobionic acid (19.2-fold increase), gentiobiose (15.6-fold), and raffinose (10.4-fold).

Root collar

Of the 498 putative metabolites detected in the root collar extracts, 15 were considered as potential marker metabolites (Tables S5 and S6). The PCA successfully distinguished the well-watered from the droughted plants (Fig. 2c). In contrast to the other organs, 10 of the 15 potential marker metabolites exhibited a decrease rather than an increase in abundance as a result of the stress. The most prominent metabolites to respond positively to the stress were sorbose (4.4-fold increase), fructose (4.7-fold), and raffinose (3.1-fold), while both 2,4-diaminobutyric acid and pimelic acid were detected in extracts of the droughted plants, but not in those of the well-watered plants. Among the down-regulated metabolites were citramalic acid (falling to 37% of the abundance in the well-watered plants), D-glyceric acid (40%), galactose (42%), and lactic acid (49%). Both adenine and *N*-acetyl-β-alanine were present in extracts of the well-watered plants, but not in those prepared from the droughted ones.

Root

The root extracts contained 479 putative metabolites, of which just seven were considered as potential markers (Tables S7 and S8). The seven compounds were 3-cyanoalanine (6.9 fold), 2-furoic acid (15.4 fold increase), malonamide (7.8 fold), asparagine (4.7 fold), sophorose (3.0 fold), coniferyl alcohol (not present in the

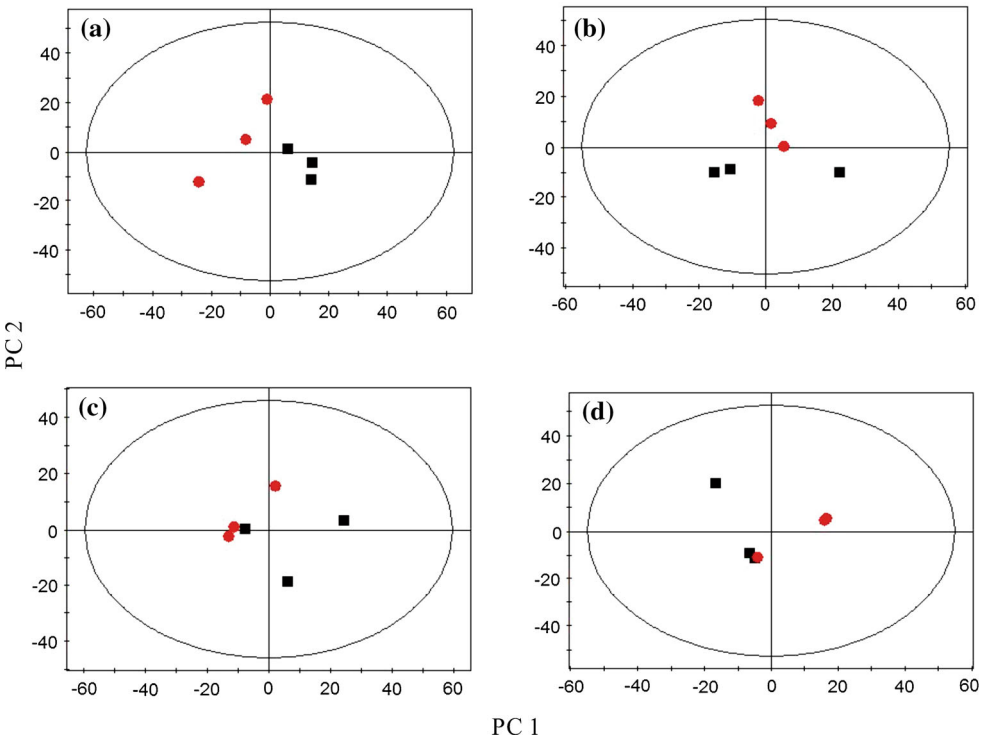

Fig. 2 Principle component analysis (PCA) of metabolites in *C. korshinskii* plants using PC1 versus PC2. 2-year-old *C. korshinskii* plants were subjected to drought stress by withholding water for 40 days. GC–MS-based metabolites were isolated from (**a**) leaves, (**b**) stems, (**c**) root collars, and (**d**) roots. *Black squares* and *red circles* denote well-watered and drought-stressed plants, respectively. *PC1* and *PC2* are the first two principal components

extracts of well-watered plants), and histidine (falling to 20% of the abundance in the well-watered plants). Further details are given in the Supplementary Data section.

Major changes in plant metabolism induced by drought stress

Reference to the LECO-Fiehn Rtx5 database allowed for the annotation of 320 the metabolites present in at least three of the four organs sampled (Table S9), the identity of 169 of these was established. The application of the PLS-DA allowed broad patterns of change in the concentrations of the set of 320 metabolites to be characterized. A clear and statistically significant separation of the samples into three groups was achieved (Fig. 3). The leaf profiles were distinct from those of the other three organs, while the well-watered plant profiles were distinguished from those of the droughted ones. Although no clear differentiation between the root, root collar, and stem metabolite profiles was possible, the droughted plant profiles could still be distinguished from those of the well-watered ones. A number of metabolites whose abundance was altered by the stress by at least twofold in at least two of the four organs were recognized; some were amino acids, some were organic acids, some were sugars/glycosides, and some were alcohols/aldehydes, leaving a residual group of miscellaneous compounds. The latter group included eight non-identified compounds. A complete list of these metabolites is given in Table 1, which also shows the extent of their response to the stress, their molecular mass-to-charge ratio, their RI, and their KEGG ID. The data were visualized by assembling a heat map (Fig. 4). The abundance of about 80% of the amino acids was increased in both the leaf and stem, as was the case for about 50% of them in the root collar and root. Among the organic acids, the majority increased in abundance in the leaf and stem, but in the root collar and root, it was the minority. With respect to the effect of the stress on the leaf, stem, and root contents of sugars/glycosides, a higher number of metabolites was increased rather than decreased in abundance; this was not the case for the root collar. The abundance of most of the alcohols/aldehydes was reduced in all of the organs except for the leaf. Finally, with respect to the remaining non-classified metabolites, three of the organs (leaf, stem, and root) displayed an increased abundance. The most strongly increased metabolites appearing in at least in two organs were the three amino acids phenylalanine, methionine, and 3-cyanoalanine, lactobionic acid, the sugars raffinose and gentiobiose, and one unclassified compound. The most strongly decreased ones were threonic acid, fumaric acid, fluorene, and phytol. Serine, valine, tryptophan, and urea were all reduced in abundance in the below ground organs but were accumulated in the aerial ones.

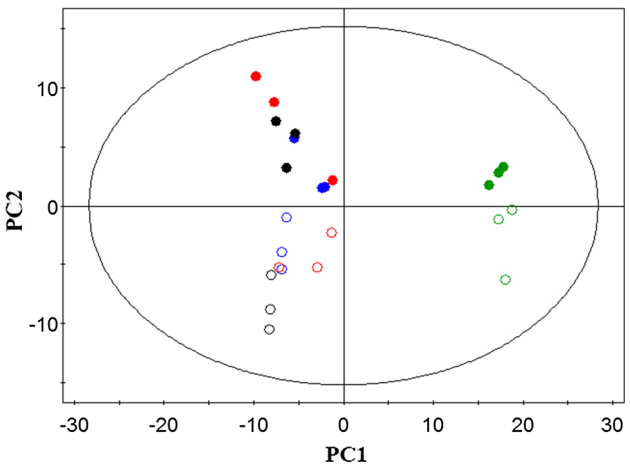

Fig. 3 Partial least squares discriminant analysis (PLS-DA) score *scatter plot* derived from the GC–MS-based metabolites of *C. korshinskii* plants. 2-year-old *C. korshinskii* plants were subjected to drought stress by withholding water for 40 days. *Green, blue, red,* and *black* denote leaves, stems, root collars, and roots, respectively. *Open* and *filled circles* indicate well-watered and drought-stressed plants, respectively

Discussion

Few studies to date have considered the interplay of different plant organs in determining a plant's overall function, thereby limiting our understanding of the mechanisms which underpin the strategies employed by plants to cope with environmental variation (Gordon and Jackson 2000; He et al. 2006; Reich and Oleksyn 2004; Wright et al. 2004). Here, the metabolic response to drought stress of four independent organs of *C. korshinskii* was investigated. The data support the idea that the ability of *C. korshinskii* to survive drought rests on the complementary activity of the various parts of the plant. The leaf, which is potentially the most drought sensitive part of the plant, harbored many more responsive metabolites than did any of the other three organs. The species is known to adapt to extreme drought stress by dropping its leaves, at the same time keeping its stem alive (Kreuzwieser et al. 2009). The stem metabolomes in the well-watered and droughted plants were very similar to one another, which imply that the stem has a strong level of tolerance to soil water deficits. Previously, it has been shown that the species is an effective accumulator of both carbohydrates (especially non-structural ones) and soluble amino acids in its stem during an episode of drought stress (Jansen et al. 2014; Sanchez-Martin et al. 2014). The present results are in agreement with this experience, since increases in the abundance of sorbitol, raffinose, and galactinol were noted in the stems of droughted plants. During a stress episode, the root collar

Table 1 Fold changes in the concentration of the most prominent metabolites present in the four organs of *C. korshinskii* as induced by drought stress

Metabolites	KEGG ID	RI	MS^n ions *m/z*	Fold (drought/control)			
				Leaf	Stem	Root collar	Root
Amino acids							
3-Cyanoalanine		11.733	141	5.58	3.88	4.79	6.87
Methionine	C00073	13.637	176	ND	22.31	6.51	6.29
Asparagine	C00152	13.449	100	6.69	4.5	4.13	4.72
Phenylalanine	C02265	14.928	218	14.3	10.2	1.94	4.04
Isoleucine	C00407	10.733	158	3.58	8.05	1.72	3.12
Leucine	C00123	10.398	158	9.17	7.77	1.33	1.7
Proline	C00148	10.811	142	5.6	2.79	0.9	1.42
Glycine	C00046	10.887	174	2.63	2.88	0.38	1.26
Tyrosine	C00082	18.269	218	7.47	3.74	1.07	1.14
Cycloleucine		11.676	156	4.21	1.43	1.69	1.1
Lysine	C00047	18.103	156	5.65	4.18	0.49	1.09
O-acetylserine		12.023	174	ND	1.92	3.11	1.07
Serine	C00065	11.586	204	2.38	2.38	0.59	1.06
L-Allothreonine	C05519	11.927	219	3.17	2.08	0.5	0.8
O-Phosphoryle-thanolamine		16.627	299	ND	0.58	0.39	0.65
Tryptophan	C00078	20.842	202	4.85	4.52	0.37	0.65
Aspartic acid	C00049	13.639	232	0.32	1.17	0.19	0.63
Glutamic acid	C00025	14.816	246	0.4	0.91	0.21	0.6
L-homoserine	C00263	11.569	146	ND	0.46	0.08	0.45
Canavanine degr prod		9.709	156	ND	0.79	0.15	0.44
N-Methyl-DL-alanine		8.993	130	2.89	4.21	1.27	0.41
Ornithine	C00077	14.77	142	ND	2.03	0.32	0.38
Valine	C00183	9.611	144	6.92	4.1	1.42	0.32
Histidine	C00135	18.062	154	ND	1.6	0.15	0.13
Acids							
Lactobionic acid		25.107	204	3.3	19.23	2.63	6.27
3-Hydroxybenzoic acid		14.21	290	ND	1.95	1.28	3.09
Indoleacetate	C02043	20.654	202	10.19	4.83	0.85	3
Glutaric acid		12.144	156	ND	2.94	1.59	2.93
Pyrrole-2-carboxylic acid		11.476	240	6.79	1.9	1.49	1.61
Fumaric acid		11.464	245	1.34	0.5	0.42	0.5
Malonic acid		9.427	147	1.14	2.16	0.21	0.44
Lactic acid		7.395	117	2.12	1.06	0.3	0.42
Threonic acid	C01620	14.098	292	0.84	0.48	0.41	0.39
Saccharic acid		18.964	292	ND	1.68	0.21	0.36
D-Glyceric acid		11.168	189	0.6	0.59	0.4	0.34
4-Hydroxy-3-methoxybenzoic acid		16.459	297	ND	0.93	0.64	0.29
Sugar/glycosides							
Raffinose	C00492	29.102	361	3.49	15.57	3.07	11.5
Gentiobiose		25.645	204	3.2	13.21	2.86	8.12
L-Threose		12.667	216	4.74	2.53	1.3	3.66
Galactinol		26.663	204	ND	1.75	0.71	2.67
Naringin	C09789	28.978	283	0.5	0.82	0.21	0.42
Arbutin	C06186	23.72	267	1.27	0.22	0.06	ND
Alcohol/aldehyde							

Table 1 continued

Metabolites	KEGG ID	RI	MS^n ions *m/z*	Fold (drought/control)			
				Leaf	Stem	Root collar	Root
Glutaraldehyde		8.777	86	ND	2.91	0.77	2.54
Vanillin		15.151	223	ND	0.94	0.54	0.29
Cortexolone		27.901	559	1.11	1.83	0.4	0.08
Octanal		18.246	316	0.42	0.29	0.03	0.02
Phytosphingosine	C12144	24.037	204	1.57	1.55	0.42	ND
Mannitol	C00392	18.163	319	1.57	0.6	0.25	ND
Phytol		20.474	143	0.67	0.65	0.44	ND
Othors							
2-Monoolein		24.836	95	ND	0.63	0.36	2.55
D-(glycerol 1-phosphate)		16.39	299	ND	2.43	5.79	2.41
N-Acetyl-beta-D-mannosamine	C00645	19.864	319	2.24	1.34	0.94	1.56
Spermidine	C00315	20.757	202	3.8	9.43	0.35	1.42
Pyridoxal phosphate		21.143	179	4.39	6.68	1.25	1.39
Putrescine	C00134	16.203	174	1.9	8.88	0.46	1.34
Gly-pro		18.731	174	ND	4.85	0.57	1.3
Urea	C00086	10.005	189	2.6	3.03	0.56	0.92
Maleamate	C01596	13.869	241	ND	1.24	0.33	0.5
Fluorene	C07715	15.047	165	0.87	0.86	0.27	0.45
Methionine sulfoxide		16.341	128	ND	0.87	0.21	0.38
Hydroxylamine	C00192	8.208	146	2.05	2.54	0.91	ND
Unknown compounds							
Unknown 29		7.830	146	15.62	20.11	5.50	6.40
Unknown 59		7.256	154	1.72	2.57	2.58	4.73
Unknown 83		7.799	154	1.69	2.42	3.21	4.33
Unknown 91		11.311	158	ND	2.51	2.34	2.51
Unknown 21		12.195	174	2.28	2.39	3.12	2.01
Unknown 28		28.068	259	ND	2.29	0.38	0.44
Unknown 110		27.150	235	2.04	0.83	0.35	ND
Unknown 142		30.466	204	1.87	0.59	0.49	ND

m/z molecular mass-to-charge ratio, *RI* retention time index. *Control* well-watered plants, *Drought* droughted plants, *ND* not detected

may act as a reservoir of essential metabolites which can be translocated to other parts of the plant (Gargallo-Garriga et al. 2014). Since carbohydrates stored in the root and root collar appear to be readily mobilized to the stem of a droughted plant, the capacity to transfer resources to the aerial part of the plants is likely to make an important contribution to the drought tolerance of *C. korshinskii* (Hao et al. 2015).

The abundance of the three amino acids phenylalanine, methionine, and 3-cyanoalanine, of the organic acid lactobionic acid, of the two sugars raffinose and gentiobiose, and of one unknown compound was notably induced by drought stress in at least two of the four organs sampled. Phenylalanine is a critical substrate in the phenylpropanoid pathway, through which low-molecular weight phenolics such as the phenylpropanoids, flavonoids, isoflavonoids, and anthocyanins are all synthesized (Boudet 2007). This compound was prominent in the leaf of droughted plants. Given that the leaf is the most drought sensitive of the four sampled organs, the present observations chime with the finding that in lentil seedlings, drought sensitivity can be associated with greater tissue phenylalanine content (Muscolo et al. 2015). The methionine content of the stem, root collar, and root (but not of the leaf) was boosted by the drought stress, indicating that methionine may be involved in amino-acid transport and storage in response to abiotic stress. The heightened content of 3-cyanoalanine, lactobionic acid, and gentiobiose in two or three of the four organs implies that they are likely important for the adaptation of *C. korshinskii* to drought stress. While 3-cyanoalanine has been suggested as a drought stress marker by at least two

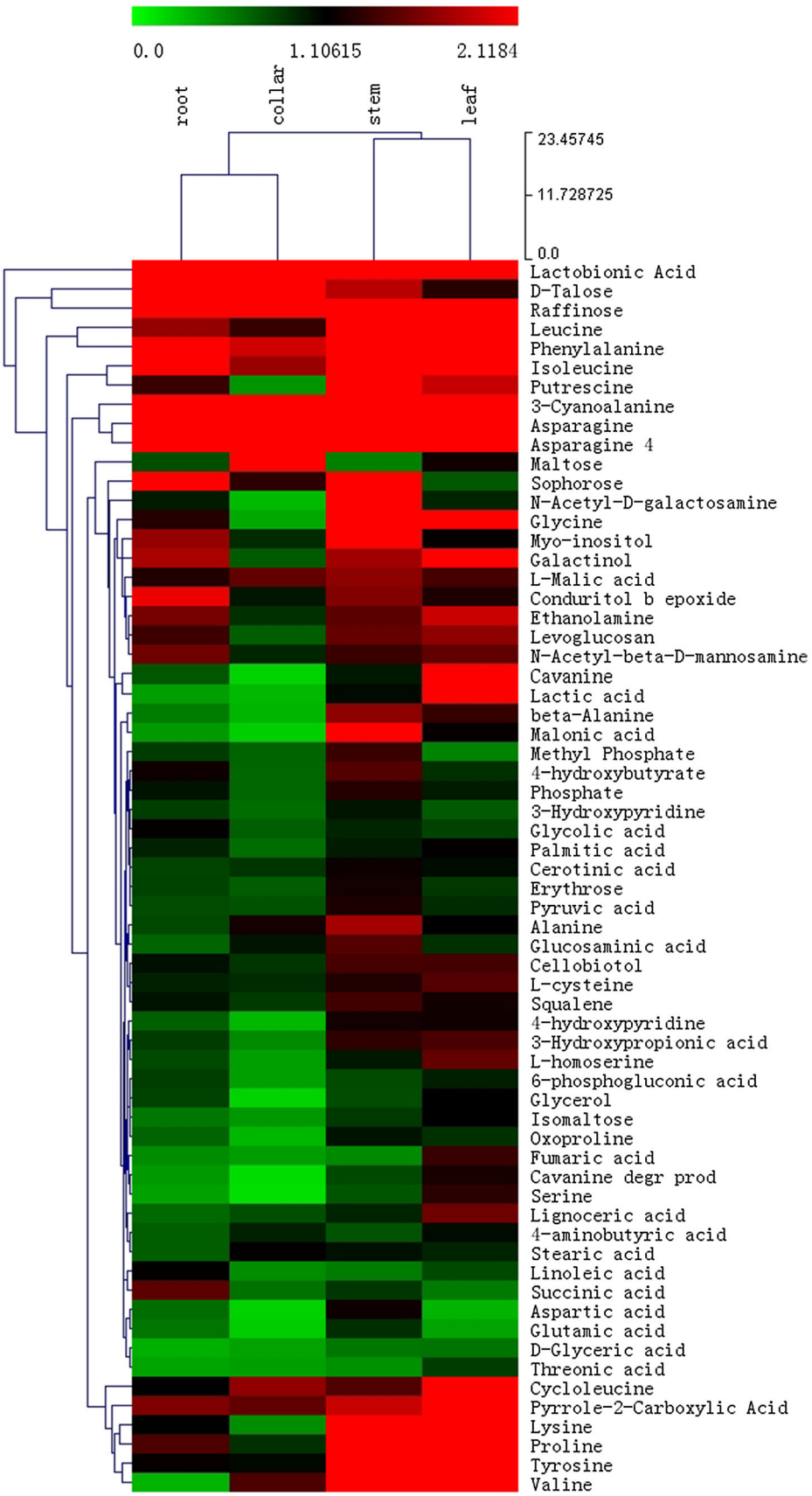

Fig. 4 Heat map and hierarchical clustering of the 64 metabolites in the four organs of 2-year-old *C. korshinskii* plants. The abundance of these metabolites changed over two times at least in three organs in plants subjected to drought stress by withholding water for 40 days. The concentration of each metabolite was normalized against that in well-watered plants: *blue* denotes a reduced relative concentration induced by drought and *red* a heightened one. The *color intensity* reflects the extent of the alteration in concentration

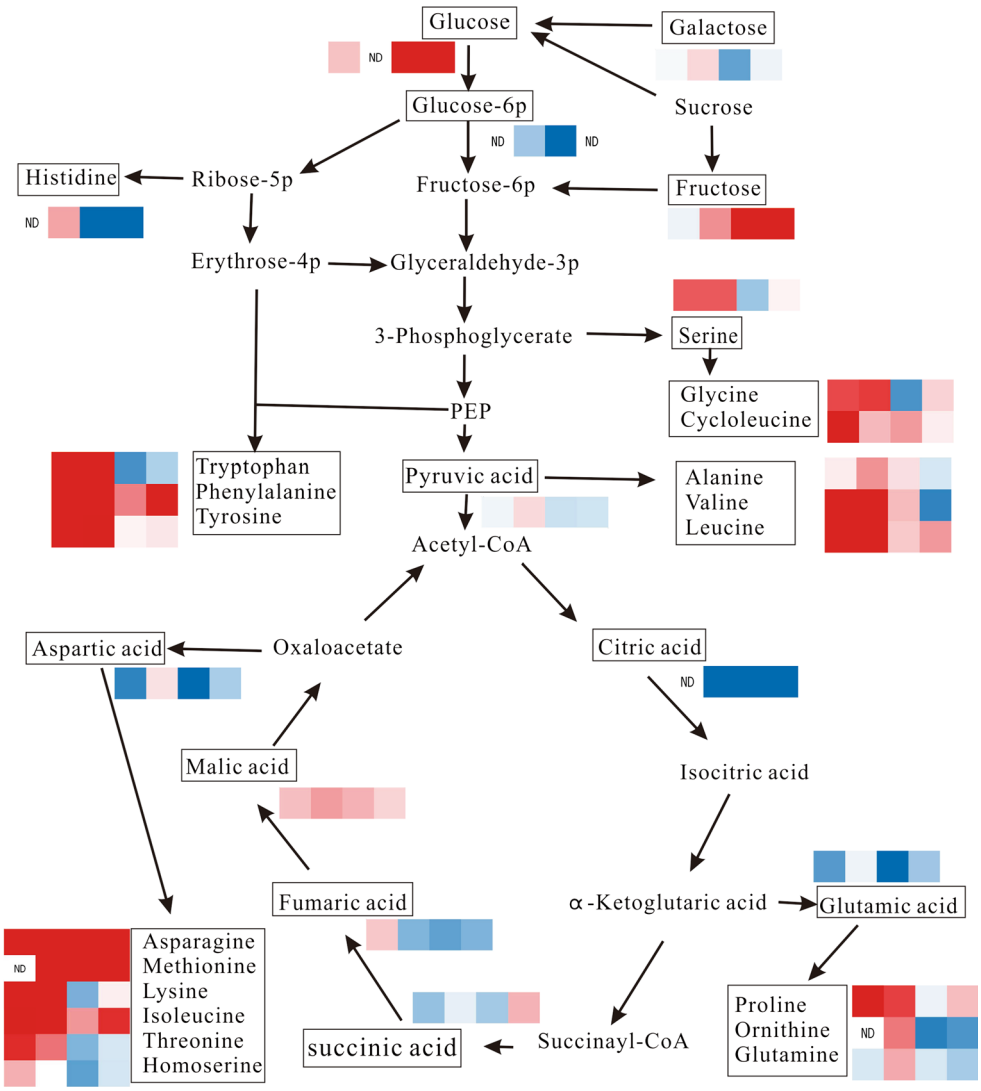

Fig. 5 Simplified metabolic map for each of the four *C. korshinskii* organs, based on GC–MS metabolite profiling. The *boxed* metabolites were detected in the present research. The *squares* are ordered from *left* to *right*: leaf, stem, root collar, and root. The *squares' color* (*blue* or *red*) indicates, respectively, a decrease or an increase in the metabolite's concentration induced by the drought stress. Increasing color intensity indicates higher induced changes. *ND* not detected

sets of authors (van der Graaff et al. 2006; Watanabe et al. 2013), the drought responsiveness of neither of the other two has been noted previously, suggesting that they could represent a *C. korshinskii*-specific biomarker for drought tolerance. According to Takahashi et al. (2014), gentiobiose is used by plants as a signaling molecule rather than as an energy source, so the drought responsiveness of this rare disaccharide is probably related to signaling in *C. korshinskii*. Raffinose was particularly strongly represented in the stem and root of stressed *C. korshinskii* plants; this sugar is known to accumulate in droughted plants, and is thought to contribute to desiccation tolerance (Blackman et al. 1992; Brenac et al. 1997), both because of its ability to stabilize membranes and its anti-oxidation properties (van den Ende 2013). The non-identified compound #29 was the only metabolite which was increased in abundance in all four organs as a result of the drought stress, so its identification clearly merits further effort. This compound might be used as a *C. korshinskii*-specific biomarker for drought tolerance.

Most of the metabolites detected here are associated with generic biochemical pathways, notably glycolysis, the tricarboxylic acid cycle (TCA), and amino-acid metabolism (Fig. 5). The abundance of the TCA cycle intermediates citric and fumaric acid was materially reduced by the drought stress, implying that both the TCA cycle and glycolysis were inhibited by an inadequate supply of moisture. In contrast, almost all of the various amino-acid synthesis pathways (except for those leading to aspartic acid and glutamic acid) appeared to have been enhanced. The suggestion is that high levels of soluble sugar and amino acid are important for the roots to express drought stress tolerance, and that an active catabolism is required to allow the plant to successfully tolerate drought stress (Sanchez et al. 2008; Widodo-Patterson et al. 2009).

In conclusion, the abundance in *C. korshinskii* of many metabolites is raised by drought stress, particularly in its leaves, which are the most drought sensitive part of the plant. The metabolome of the root collar was not much affected by the stress, which is consistent with the idea that this is the most drought tolerant organ of the plant. The metabolic response to drought stress of four independent organs might help us to gain a better understanding of the ability of *C. korshinskii* to survive drought. The research has identified some novel drought stress responsive metabolites, some of which could prove to be informative as metabolic markers. The variation in the abundance of gentiobiose and some of the other drought-responsive metabolites provides some clues as to how *C. korshinskii* is able to survive in regions where drought stress is commonplace. These adaptations may be exploited in developing strategies to enhance the drought tolerance of important crop, horticultural and arboricultural species.

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