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Identification for the capability of Cd-tolerance, accumulation and translocation of 96 sorghum genotypes



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ABSTRACT

Cadmium (Cd) pollution is a worldwide environmental problem which heavily threatens human health and food security. Sorghum, as one of the most promising energy crop, has been considered to be the source of highquality feedstock for ethanol fuel. Ninety-six sorghum genotypes were investigated under hydroponic conditions to compare their capabilities of Cd-tolerance, accumulation and translocation for their potential in remediation of Cd contamination. Different genotypes varied largely in the tolerance to Cd stress with tolerance indexes ranked from 0.107 to 0.933. Great difference was also found in Cd uptake and accumulation with concentrations ranging from 19.0 to 202.4 mg/kg in shoots and 277.0–898.3 mg/kg in roots. The total amounts of Cd ranked from 6.1 to 25.8 µg per plant and the highest translocation factor was over 4 times higher than the lowest one. The correlation analysis demonstrated that Cd concentration in shoot reflected the ability of Cd translocation and tolerance of sorghum, and the path coefficient analysis indicated that root biomass could be taken as a biomarker to evaluate Cd extraction ability of sorghum. The results in this study can facilitate the restoring of Cd contaminated areas by sorghum.

1. Introduction

Cd pollution is becoming increasingly severe in recent years due to anthropogenic activities, such as mining, metallurgical industry and the application of phosphorus fertilizers and pesticides (Zhang et al., 2015). As one of the most toxic heavy metals, Cd can greatly influence the growth and development of plants, resulting in severe reduction in crop yield and quality. Worse still, Cd can accumulate in human body through food chain and induce many diseases such as prostate, lung cancers and bone disorders (Bertin and Averbeck, 2006; Dias et al., 2013). Accordingly, there is an urgent need to remediate the Cd pollution in the environment. Phytoremediation as an environmentfriendly and cost-effective green remediation technology has been paid much attention in the past years (Doty, 2008). However, most of the plants used for phytoremediation were hyperaccumulators with small biomass, slow growth rate and low economic benefit, so it is difficult to apply these plants to a large scale of fields (Liu et al., 2011). Recently, high biomass plants especially energy plants have been proposed to restore heavy metal contaminated soils, such as switchgrass, sorghum

and king grass (Chen et al., 2011; Metwali et al., 2013; Zhang et al., 2014).

Sorghum (Sorghum bicolor (L.) Moench) is a C4 plant with high photosynthetic efficiency. It has been identified as one of the critical herbaceous bioenergy crops by the United States Department of Energy (DOE), which can be used to produce bioethanol with seeds, cellulose, hemicellulose or sugar in stems (Gnansounou et al., 2005; Gill et al., 2014). Sorghum showed great tolerance to heat, salt and drought stress and was widely cultivated in many tropical, subtropical, and temperate regions (Soudek et al., 2014; Muratova et al., 2015). Several recent researches have shown the potential of sorghum in absorbing heavy metals. It is more tolerant to cadmium than wheat, maize and jack-bean (Metwali et al., 2013; Zancheta et al., 2015). Soudek et al. (2014) compared the performance of five sorghum cultivars under different Cd concentration and found the addition of glutathione significantly increased the accumulation of cadmium in the roots as well as in the shoots at the highest cadmium concentration applied. Jia et al. (2016) showed that sweet sorghum cultivar 'M-81E' kept almost normal growth when exposed to 10 µM cadmium for 30 days. Padmapriya et al.

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(2016) investigated the performance of millet (*Eleusine coracana*), mustard (*Brassica juncea*), sorghum, black gram (*Vigna mungo*), pumpkin (*Telfairia occidentalis*) in heavy metal contaminated soils and found that sorghum showed no significant change in biomass and biochemical parameters against control. Another report found that sorghum had higher bioaccumulation capability of Cd from soil to plant and higher transfer capability of Cd from roots to shoots under high Cd stress (Wang et al., 2017). Additionally, sorghum also exhibits tolerance to Cu (copper), Pb (lead), Ni (nickel), Cr (chromium), Zn (zinc) and Cs (cesium) stress and can be used in the phytoremediation of combined heavy metal pollution (Bonfranceschi et al., 2009; Salman et al., 2013; Metwali et al., 2013; Al Chami et al., 2015; Blanco et al., 2016; Wang et al., 2016).

Cultivating sorghum in Cd-contaminated soils not only provides feedstock for ethanol production, but also achieves the goal of phytoremediation. Furthermore, the fibrous residues derived from sorghum can be burned to produce electricity or process heat, after which cadmium left in ash can be recycled (Woods, 2001; Li, 2013). Through this process, sorghum accumulated cadmium is used to produce fuel ethanol instead of taking as food, which avoids the essential harm to human beings.

Plant species differ greatly in the capacity of uptake and tolerance to heavy metals. Barzanti et al. (2011) reported that *Alyssum* species showed variation in cadmium tolerance and accumulation. Yang et al. (2015) compared 24 willows (*Salix spp.*) clones to find out wide variations in manganese (Mn) tolerance and accumulation capability among them. Shi et al. (2015b) reported for the differences in arsenic (As) and Cd concentrations in grains and straws among 12 wheat cultivars. Sorghum cultivars also exhibited a great difference in the translocation ability of Cd to shoots (Soudek et al., 2014; Tsuboi et al., 2017). Therefore, screening germplasms with high ability of extracting heavy metals for phytoremediation is of great importance and value in practice.

In this study, we investigated the performance of absorption and transport of Cd among 96 sorghum genotypes under Cd stress in a hydroponic condition and screened out sorghum genotypes with the highest or lowest Cd uptake and translocation ability for further practice in the fields, which will provide valuable approaches for restoring Cd contaminated soils with sorghum plants.

2. Materials and methods

2.1. Experimental design

Ninety-six genotypes of sorghum obtained from Plant Genetic Resources Conservation Unit (http://www.ars-grin.gov/npgs/index. html), the United States Department of Agriculture, Griffin, United States of America, were used in this study. For convenience, genotypes were termed as numbers from 1 to 96 in our work, and their corresponding accessions and plant IDs are listed in Table 1. Their background and basic productivity are provided in Table S1. Seeds were soaked in deionized water at room temperature for 12 h, and then germinated in a saucer covered by filter paper. Three-day-old healthy plants with uniformed sizes were transplanted to 96-well plates with bottom cut off and cultivated hydroponically in containers filled with tap water for one week, then the tap water were changed with 1/2Hoagland solution, meanwhile Cd (as CdCl₂) was added to containers. Based on our previous study (Jia et al., 2016), we took 10 µM Cd as the treatment concentration, compared with the control under a 1/2 Hoagland solution free of Cd. These seedlings were grown in a greenhouse with a day/night temperature regime of 25/20 °C, photoperiod of 16 h, and relative humidity of \sim 60%. The nutrient solution was renewed once a week. The seedlings were harvested for further analysis after treatment of 10 µM Cd for two weeks, when most of the genotypes exhibited obvious phenotypes with stunted growth compared to control. Three independent hydroponic experiments were performed and five plants from each genotype were analyzed in every independent experiment. The 1/2 Hoagland solution contained 2.5 mM Ca(NO₃)₂,

Table 1

The information of 96 genotypes of sorghum examined in this study.

Item	Accession	Plantid	Item	Accession	Plantid
1	PI 22913	CHINESE AMBER	49	PI 175919	IS 12833
2	PI 52606	MN 2680	50	PI 176766	MN 2873
3	PI 92270	MN 2740	51	PI 179749	Juar
4	PI 144134	Inyangentombi	52	PI 180348	Juar
5	PI 144335	UFUTANE	53	PI 180487	Juar
6	PI 145626	Manyoble	54	PI 181080	HONEY
		-			SORGHUM
7	PI 145632	TEGEVINI	55	PI 181083	KAMANDRI
8	PI 145633	Tugela Ferry	56	PI 183001	GHAONLA
9	PI 147224	B. 35	57	PI 196049	IS 2131
10	PI 152593	ANKOLIB BLACK	58	PI 196583	MN 3080
11	PI 152727	Malwal Tonj	59	PI 196598	MN 3095
12	PI 152733	MERISSA (BARI)	60	PI 218112	IS 2352
13	PI 152751	NYTWAL	61	PI 251672	MN 4135
14	PI 152771	RAHMETALLA	62	PI 253986	MN 4138
		GALLABAT			
15	PI 152816	WAD FUR WHITE	63	PI 255239	CAXA
16	PI 152828	U.T. 23	64	PI 257599	NO. 5 GAMBELA
17	PI 152860	MERASI	65	PI 257600	NO. 6 GAMBELA
18	PI 152873	UMM EL TEIMAN	66	PI 266927	Co. 1
19	PI 152881	Lwel Kochung	67	PI 653411	M 81E
20	PI 152923	Duro El Jack	68	PI 273955	MN 4566
21	PI 152961	MALNAL	69	PI 273969	MN 4578
22	PI 651497	Theis	70	PI 302252	IS 13726
23	PI 152966	Ayuak	71	PI 303658	Nerum Boer
24	PI 152971	AWANLEK	72	PI 454500	ETS 3080
25	PI 153874	KATEMU	73	PI 455286	ETS 3488
26	PI 154750	Serere	74	PI 653616	WRAY
27	PI 154844	GRASSL	75	PI 501079	ZM-2476
28	PI 154846	KABIRI	76	PI 511355	SMITH
29	PI 154944	L31 Emiroit	77	PI 525013	MW 262
30	PI 154987	S. A. 1	78	PI 525041	MW 515
31	PI 155149	Dhurra No. 7	79	PI 562267	FAO 55049
32	PI 155516	MASAKA	80	PI 563295	RIO
33	PI 155760	Namuse	81	PI 583832	TOP 76-6
34	PI 155885	MN 1644	82	PI 586443	MN 818
35	PI 156178	MN 2014	83	PI 586541	TRACY
36	PI 156203	MN 2089	84	PI 641807	ATLAS
37	PI 156217	MN 2109	85	PI 641810	COLMAN (Y)
38	PI 156268	CHEDOMBA	86	PI 641815	EARLY FOLGER
39	PI 156393	MN 2277	87	PI 641817	EARLY SUMAC
40	PI 156463	Dobbs	88	PI 641821	HONEY DRIP
41	PI 156487	MN 2363	89	PI 641834	PLANTER
42	PI 156871	Rutobo	90	PI 641835	REX
43	PI 156890	Dura Huria	91	PI 641862	COLLIER
44	PI 157030	Andiwo III 57	92	PI 641909	Red Losinga
45	PI 157033	Ifube No. 18	93	PI 643008	MN 2751
46	PI 157035	Nyagwang No. 56	94	PI 643016	MN 2761
47	PI 157804	Feterita Abu Derega	95	PI 653617	KELLER
48	PI 170787	MN 2826	96	PI 651495	DALE

2.5 mM KNO₃, 0.5 mM KH₂PO₄, 1 mM MgSO₄, 0.01 mM Fe-EDTA, and micronutrients (0.715 mg/L H₃BO₄, 0.453 mg/L MnCl₂·4H₂O, 0.02 mg/L CuSO₄·5H₂O, 0.055 mg/L ZnSO₄·7H₂O, 0.005 mg/L H₂MoO₄).

2.2. Measurement of shoot length, root length and dry weight

The shoot length is the distance from the bottom of shoot to the highest junction of sheath and leaf while the longest root is measured as the root length. The roots and shoots of 96 sorghum seedlings were dried at 70 $^{\circ}$ C until constant weight, and then weighed as dry weight.

2.3. Cadmium determination

The roots and shoots of 96 sorghum genotypes after two weeks Cd treatment were dried at 70 °C until constant weight. Then the samples were grounded to fine powder and digested with a mixture of 6 mL nitric acid and 2 mL hydrogen peroxide using a microwave system (MARS; CEM Corporation, Matthews, NC, USA) based on the protocol described by Hansen et al. (2009). Thereafter Cd concentration was

determined using inductively coupled plasma-atomic emission spectrometer (Thermo Electron Corporation, 6300) according to Luo et al. (2009).

2.4. Calculation of cadmium tolerance index and translocation factor of sorghum

The modified membership function analysis (Ci et al., 2011; Shi et al., 2015a) was introduced in this study to evaluate Cd tolerance of sorghum genotypes on the basis of plant growth parameters (root and shoot length, root and shoot dry weight).

$$X(\mu) = \frac{X - X_{\min}}{X_{\max} - X_{\min}}$$

$$\overline{X}(\mu) = \frac{1}{n} \sum_{\mu=1}^{n} X(\mu)$$

Here, $X(\mu)$ is the membership function value of the μ th Cd-tolerant index ($\mu = 1, 2, 3, 4$, denoting relative shoot length (RSL), relative root length (RRL), relative shoot dry weight (RSDW) and relative root dry weight (RRDW), respectively) ranging from 0 and 1. X is the observed value of a growth indicator for each genotype, while Xmax, Xmin are the maximum and minimum value of the growth indicator, n is the number of indicators. The mean $X(\mu)$ (Cd-tolerant index) of each genotype was then obtained by averaging the membership function values of the four indexes. Classification of theses genotypes for different Cdtolerant groups followed the below criterion: Grade I ($\overline{X}(\mu) \ge 0.8$), Grade II (0.6 $\le \overline{X}(\mu) < 0.8$), Grade III (0.4 $\le \overline{X}(\mu) < 0.6$), Grade IV (0.2 $\le \overline{X}(\mu) < 0.4$), and Grade V ($\overline{X}(\mu) < 0.2$), which indicate the most Cd-tolerant genotypes, more Cd-tolerant genotypes, moderate Cd tolerant genotypes, more Cd-sensitive genotypes and the most Cd-sensitive genotypes, respectively (Liu et al., 2005; Ci et al., 2011).

Besides, the translocation factor (TF) that reflected the ability of Cd transport from root to shoot was introduced and expressed as follows: TF = (Cd concentration in shoot) / (Cd concentration in root) (Shi and Cai, 2009).

2.5. Statistical analysis

Correlation analysis and path-coefficient analysis was performed using SPSS 17.0 Program (SPSS Inc., Chicago, IL, USA). Graphical work was performed using GraphPad Prism 5.

3. Results and discussion

3.1. Toxicity of cadmium on 96 sorghum genotypes

After exposed to 10 µM Cd for two weeks, shoot length of all the genotypes as well as root length of most genotypes decreased. However, the root length of four genotypes increased, including No. 80 (Plant ID: 563295,RIO), 94 (Plant ID: 643016, MN 2761), 13 (Plant ID: 152751, NYTWAL) and 9 (Plant ID: 147224, B. 35) (Fig. S1). Previous study reported that plant height of sorghum cultivar 'M-81E' exhibited an obvious decrease when exposed to 10 uM Cd for 30 days compared to control (Jia et al., 2016). However, other reports showed that the growth of sorghum had no obvious change under low Cd stress. The shoot length of sorghum cv. 'Yajin No.1' was not significantly changed when the initial Cd in the soil was no more than 5 mg/kg (Tian et al., 2015). Low Cd stress (3 mg/kg) did not have significant influence on the plant height of sorghum cv. 'Nengsi 2#'and 'Cowley', whereas high Cd stress (15 mg/kg) decreased the plant height by more than 25% (Wang et al., 2017). The shoot biomass of 11 genotypes increased by 0.1-67.5% and the others decreased by 0.1-49.2% compared to controls, meanwhile, the root biomass of 62 genotypes increased by 0.6-168.1% and the rest decreased by 2.1-45.5% (Fig. S2). These results indicated that root biomass of most tested sorghum genotypes tended to exhibit an increase under 10 μ M Cd stress compared to control. Root is the first part of plants exposed to heavy metals and represents the ability of uptake and tolerance to metal stress (Huang et al., 2011). Thus, it is inferred that increase in root biomass suggested higher tolerance to cadmium stress, or absorption of higher amount of Cd. Similarly, Pinto et al. (2004) found that the sorghum biomass exhibited a significant increase with a range of 0.1 and 1 mg Cd L^{-1} . Jia et al. (2016) reported that the root dry weight of sorghum cv. 'M-81E' showed an insignificant increase when exposed to 10 µM Cd for 30 days compared to control. Wang et al. (2017) showed that stems of sorghum 'Cowley' exhibited a significant increase when grown in low Cd stress (3 mg/kg) for 100 days. This hormesis effect of Cd has also been observed in other plants, such as Indian mustard (Brassica juncea) (Singh and Tewari, 2003) and barley (Hordeum vulgare) (Aery and Rana, 2003), though the mechanism is unclear.

These results suggest sorghum can tolerate moderate cadmium stress. However, for soils heavily contaminated by cadmium, the more tolerant genotypes should be chosen in order to ensure sorghum to complete their life cycles. To evaluate Cd tolerance of sorghum genotypes, tolerance index (TI), the average membership function values of the four indexes (RSL, RRL, RSDW and RRDW), was introduced in this study. TI values of 96 sorghum genotypes varied from 0.107 to 0.933 and were classified into 1, 5, 25, 46 and 19 genotypes, belonging to Grade I, II, III, IV and V, respectively. Among these genotypes, 32.3% of



Fig. 1. Cd tolerance indexes of 96 sorghum genotypes. Tolerance indexes were classified into grade I, II, III, IV and V marked on the corresponding genotypes.



Fig. 2. Cd concentrations and total Cd uptake in shoots and roots of 96 sorghum genotypes. (A) Cd concentrations in shoots and roots of each genotype. (B) Total cadmium uptake in shoots and roots. Values are means \pm SE (n = 3, five plants for each replicate). "S" and "R" represent "shoot" and "root", respectively.

ones were up to the criterion of Cd tolerant (Grade I to III) (Fig. 1), indicating for their potential of restoring Cd-polluted soils.

3.2. Cadmium accumulation and translocation capacity among 96 sorghum genotypes

Cd concentration in roots of all the 96 sorghum genotypes was much higher than that of shoots when exposed to 10 µM Cd for two weeks. The Cd concentration in roots ranked from 277.0 to 898.3 mg/kg among 96 genotypes (Fig. 2A), among which the highest was three times more than the lowest. Likewise, the Cd concentration in shoots ranked from 19.0 to 202.4 mg/kg among 96 genotypes (Fig. 2A), which showed more than ten times difference between the highest and the lowest. Significant differences in the amount of cadmium accumulation were also found in five other sorghum cultivars (Honey Graze, DSM 14-535, Nutri Honey, Sweet Virginia, Express and Sucrosorgho 506), in which the highest Cd concentration in roots or shoots was approximately two times higher than the lowest one when exposed to 200 µM Cd in hydroponic system for 28 days (Soudek et al., 2014). Tsuboi et al. (2017) compared the diversity of Cd accumulation in 106 sorghum landraces and the cultivar 'BTx623', and found that the highest Cd concentration in the fourth leaf was nearly 140 times of the lowest ones after four days of 5 μ M Cd treatment. In addition, the total Cd uptake per plant also showed a great difference varying from 6.1 to 25.8 μ g per plant (Fig. 2B), and Cd extracted by roots accounted for 63.5–89.5%, i.e., most portion of Cd was accumulated in sorghum roots, which is consistent with the previous studies (Soudek et al., 2014; Jia et al., 2016). Thus, promoting Cd transporting from root to shoot was a strategy to improve the efficiency of phytoremediation with sorghum.

Cd translocation factors of 96 sorghum genotypes was less than 1 and ranged from 0.052 to 0.22 with a maximum difference of more than four times among 96 sorghum genotypes (Fig. 3). A similar result was also found in another report that TFs of sorghum in different Cd levels were lower than one with a decreased trend as Cd treated concentration increasing. However, Wang et al. (2017) showed that TFs of sorghum cv. 'Nengsi 2#' was 0.65 and 1.48 under 3 mg/kg and 15 mg/kg Cd stress respectively, while 'Cowley' had the same trend with TFs of 0.69 and 1.46. These diversities may result from different growth conditions and genotypes and also indicates sorghum is more suitable to be used in soils contaminated by higher cadmium due to its high translocation ability. In summary, sorghum exhibited great genotypic differences in the uptake and translocation of Cd. Therefore, it is necessary to screen for genotypes with strong ability of extracting Cd in order to improve its efficiency of phytoremediation.



Fig. 3. Translocation factors of 96 sorghum genotypes. Translocation factor was calculated by the ratio of Cd concentration in shoot *versus* that in root. Values are means \pm SE (n = 3, five plants for each replicate).



Fig. 4. Correlation analysis between tolerance index, translocation factor, Cd concentration in root or shoot, and total Cd uptake in whole plant or shoot.

3.3. Cadmium accumulation in shoots reflecting the capacity of Cd tolerance and translocation in sorghum genotypes

Cd tolerance indexes had a significantly negative relationship with Cd concentration in shoot (r = -0.276, p < 0.01) but no significant correlation with Cd concentration in root (r = -0.107, p = 0.301) (Fig. 4A and B). These results suggested that the tolerant sorghum genotypes accumulated much less Cd in its shoot than the non-tolerant ones, which was decided upon the intrinsic characteristics of genotypes on the ability of Cd transportation from root to shoot. However, a recent research reported that tolerance index of another heavy metal As of different wheat cultivars had a significantly positive relationship with As concentration in root but no significant correlation with As

concentration in shoot (Shi et al., 2015a), which was contrary to our results. These diverse results may be related to the difference of heavy metals and plant materials. In addition, tolerance index had a significant positive relationship with the total Cd extracted by sorghum (Fig. 4C), i.e. the Cd tolerant genotypes of sorghum possessed the high ability of phytoremediation to a large extent.

Likewise, translocation factor showed a significantly positive relationship with Cd concentration in shoot (r = 0.780, p < 0.01) but no significant correlation with Cd concentration in root (r = 0.147, p = 0.154) (Fig. 4D and E). Shi et al. (2015a) reported that translocation factor had significant correlation with As concentration in both roots and shoots of wheat. Translocation factor also exhibited a significant relationship with the total Cd uptake in shoot (r = 0.399, p < 0.01) (Fig. 4F), which indicated



Fig. 5. Correlation analysis between total Cd uptake per plant against dry weight of shoot (A), dry weight of root (B), Cd concentration in shoot (C) and Cd concentration in root (D).

sorghum with higher translocation factor extracted more Cd in shoots. From these results we inferred that Cd concentration in shoot could reflect the translocation ability of sorghum.

3.4. Root biomass as a biomarker for evaluating cadmium extraction ability of sorghum genotypes

The root/shoot biomass and Cd concentration in root/shoot collectively determined the total Cd extracted by sorghum. To investigate the contributions of these factors on total Cd uptake per plant, path coefficient analysis combined with correlation analysis was performed respectively. Path coefficient analysis can reflect the direct and indirect effects of different factors and provide a clearer picture of their interrelationships (Dewey and Lu, 1959; Akanda and Mundt, 1996; Lamboro et al., 2014). Results showed that the correlation coefficients of the total Cd uptake with shoot biomass, root biomass, Cd concentration in root, and Cd concentration in shoot were 0.697, 0.657, 0.484, and 0.232, respectively, all of which were significant at p < 0.01or 0.05. This may suggest that the total Cd uptake might be more dependent on the shoot biomass and then on root biomass (Fig. 5), however, path coefficient analysis showed that the root biomass had the strongest direct effect on the total Cd uptake, followed by Cd concentration in root. Although there was a positive relationship between shoot biomass and the total Cd uptake with a correlation coefficient of 0.697, the direct effect of shoot biomass on the total Cd uptake was only 0.195, and most of effect was indirect through the root biomass with a

Table 2

Path coefficient analysis of the total cadmium uptake per plant.^a

	1 1 1			
	DW of shoot	DW of root	Cd concentration in root	Cd concentration in shoot
DW of shoot	0.195	0.602	-0.040	-0.060
DW of root	0.158	0.742	-0.183	-0.060
Cd concentration in root	-0.013	-0.223	0.609	0.111
Cd concentration in shoot	-0.073	-0.275	0.418	0.162

^a The total Cd uptake per plant was taken as dependent variable while dry weight (DW) of shoot or root, as well as Cd concentration in root or shoot were taken as independent variables. The data in red is the direct path coefficient of the parameter on the total Cd uptake per plant.

path coefficient of 0.602 (Table 2). Therefore, the root biomass was a more important index to reflect sorghum's ability of extracting Cd compared to other factors. Collectively, root biomass can be considered as an important parameter to predict sorghum's capacity of phytoremediation.

4. Conclusions

Through germplasm screening, we found that sorghum genotypes varied greatly in Cd tolerance, uptake and translocation. The highest total Cd uptake per plant and Cd translocation factor of 96 sorghum genotypes was both 4.2 times higher than the lowest. Correlation analysis showed that Cd concentration in shoots could reflect the Cd translocation and tolerance of sorghum genotypes. Path coefficient analysis indicated that root biomass could be taken as a leading factor to evaluate Cd extracting ability of sorghum genotypes. In this study, valuable genotypes were screened out for further research on Cd extraction mechanism and practical application in the phytoremediation of Cd-polluted soils. These results also provide valuable references for restoring Cd contaminated soils with sorghum plants.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.07.002.

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