The Accumulation and Physio-Biochemical Characteristics of Three Cassava Cultivars under As Stress

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Abstract: Cassava is one of the most important crops in tropical regions. Due to multiple mining activities, arsenic (As) pollution becomes a serious problem in planting lands. In this study, a hydroponic experiment was carried out to ascertain the influence of As on three cassava cultivars (sc12, 8229 and sc6068). The three cassava cultivars showed different accumulation and response characteristics to As. The 8229 was apt to accumulate As in the root. Low As concentration promoted the growth of 8229 but inhibited that of sc12 and sc6068. This indicated that 8229 is more tolerant to As than the other two cassava cultivars. The malondialdehyde (MDA) of the three cassava cultivars, both in roots and leaves, were elevated with increasing As concentration. This study could be a reference for cassava planting in safety.

1 INTRODUCTION

Cassava (*Manihot esculenta* crantz) is a woody perennial root crop belonging to the *Euphorbiaceae* family. It is an adaptable crop that can survive in infertile soil or marginal environments (Nassar and Ortiz, 2010). It has being cultivated abundantly in the tropics and subtropics (Hannah et al., 2017). Cassava tuber is organically rich in starch and carbohydrates, and is a staple food crop in some areas of Africa, South America and Southeast Asia (Okudoh et al., 2014). In China, cassava is one of the most important crops in tropical regions like Hainan, Guangdong, Guangxi and Yunnan provinces (Yao and Zhang, 2017). However, there are a number of mine fields located in these regions, which caused a serious problem of toxic element pollution. Arsenic (As) is one of the toxic trace elements, the report on China's soil pollution stated that 2.7% of soil is polluted by As (MEP and MLR of P. R. China, 2014). Cassava is apt to accumulate toxic metals. When cultivated in Au mining area in Ghana, it accumulated zinc and lead which far exceeded the recommended levels for daily consumption (Zango et al., 2013). As primary soil pollutant in China, As has threatened the safety of agricultural products. To understand the accumulating characteristics of As in crops is very important for food safety.

Cultivars have a great impact on accumulation ability of toxic trace metals, and the breeding of cultivars is a widely applied technique for selecting low accumulating varieties of crops such as wheat and maize for ensuring food safety (Fu et al., 2011; Xing et al., 2016). But there is little research reporting about the accumulation ability of toxic elements by different cassava cultivars so far. In order to understand the accumulation ability for As by different cassava cultivars and give a reference for cassava safe planting, a hydroponic experiment was conducted to ascertain the influence of As concentration on the growth of different cassava cultivars. Specifically, the aims of this study including (1) the accumulation characteristics for As of three cassava cultivars and (2) the influence of As on the growth and physiological metabolism of the three cassava cultivars.

2 MATERIALS AND METHODS

2.1 Hydroponic Experiments

Stem cuttings (about 15 cm tall, 0.8 cm in diameter) of three cassava cultivars (sc12, 8229, sc6068) were selected for propagation and cultivation. They were conducted in a pot with a modified Hoagland nutrient

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solution, the pH value was adjusted to 6.5 using diluted HCl or NaOH. After growing for 2 weeks, cuttings with new roots and leaves were transplanted to Hoagland nutrient solution containing different concentration of As. The sets with no As addition was the control treatments, denoted by CK. The concentration of As was set with a high level (0.5 mg/L) and a low (0.05 mg/L) level according to the V-level Quality Standard of surface water (GB 3838-2002) (0.1 mg/L). Cuttings were bond by sponge and fixed on cystosepiment, and were allowed to grow and observed for 4 weeks. Hoagland nutrient solution with As were renewed every 3 days to keep the As concentration unchanged. Each treatment was replicated 3 times. After 4 weeks, cassava were harvested, washed with tap water, and then rinsed thrice with deionized water. The plant height (distance from the top of the plant to the bottom of the root) was measured. The cassavas were segregated into shoot (including leaves and stems) and roots. Each part was placed in individually labelled paper bags and oven-dried at 60℃ for 7 days. After drying, each sample was weighted for biomass assessment.

2.2 Chemical Analysis

The plant samples were digested with 5 mL HNO_3 using microwave digestion system (Mars 6, CEM, US). Samples of certified standard reference materials GSV-2 from the China National Standard Materials Centre was analysed with the experimental samples. Arsenic concentrations were determined using an atomic fluorescence spectrometer (AFS-8220, Beijing Jitian Instrumental Co., Ltd., Beijing, China).

The ability of cassava to translocate As from roots to shoots was measured by calculating translocation factor (TF) using the following formula:

TF=As concentration in shoots/As concentration in roots.

The malondialdehyde (MDA) was measured according to Heath and Packer (Heath and Packer, 1968). In brief, 0.5g root or shoot of cassava was centrifuged at 3000 r/min for 10 min after being homogenized in 5 mL of 5% TCA. The collected supernatant (2 mL) was added to 2 mL of 0.67% TBA. The mixture was heated in water bath at 100℃ for 30 min, then cooled and re-centrifuged at 3000 r/min. The supernatant were measured at 450 nm, 532 nm and 600 nm.

2.3 Data Analysis

Variance analysis was conducted using the SPSS 20.0. Data are presented as mean values ± SE, and difference of means was subjected to the least significant difference (LSD) test at 0.05 probability level. All graphs were carried out with Origin Pro 2016 (OriginLab, USA).

3 RESULTS AND DISCUSSION

3.1 Accumulating Characteristics of As by Three Cassava Cultivars

The accumulated As in the shoot and root of 8229 cultivar were significantly (p<0.05) higher than the other two cultivars under both low and high concentration, but with lowest TFs. This indicated that 8229 was apt to accumulate As in root. Root is the main edible part of cassava, which means that a high risk of food pollution when 8229 is planted in As contaminated soils. In contrast, sc12 showed a low accumulating ability for As but had highest TFs, demonstrating that it was low-risk in As accumulation. To get a deep understanding of the risk of As contamination in cassava, the concentration of As in cassava were compared with their limit values according to the national standards for food safety (GB2762-2017) in Table 1. Only the concentration of As in roots of 8229 (0.802±0.021 mg/kg) under the treatment with 0.5mg/L As exceeds the limit value (0.5 mg/kg). Which again suggested 8229 has a high risk of As accumulation.

Its high accumulation of toxic elements had been reported by previous studies. Cassava accumulated up to 12.59 g/kg Hg and 18.99 mg/kg Au in its fibrous roots, thus it was considered as a suitable candidate for Hg and Au remediation (Hannah et al., 2017). Cassava could extract 30.58 g of Cd and 3174.69 g of Pb per hectare a year, suggesting a tremendous potential application in soil remediation (Shen et al., 2013).

Table 1: The accumulation characteristics of As by three cassava cultivars.

Concentration	Cultivars	Uptake by		Transfer factor (TF)	Limited value in food*
		Shoot(mg/kg)	Root(mg/kg)		
0.05 mg/L	sc12	0.05(3.0E-03)b	0.125(1.0E-03)b	0.42(0.03)a	0.5 mg/kg
	8229	0.07(6.0E-03) a	0.300(1.5E-02)a	0.22(0.01)c	
	sc6068	0.05(1.0E-03)b	0.137(2.0E-03)b	0.38(0.01)b	
0.5 mg/L	sc12	0.08(1.1E-03)b	0.236(1.0E-01)c	0.32(0.03)a	
	8229	0.10(9.0E-03)a	0.802(2.1E-02)a	0.13(0.002)c	
	sc6068	0.08(2.8E-03)b	0.309(3.4E-02)b	0.26(0.03)b	
* Limited values were obtained from the contaminate limits in food GB2762-2017.					
Different letters within each row indicated significant difference at $p<0.05$.					

3.2 The Height and Weight of Cassava under As Stress

To figure out how As influences the cassava growth, we measured their height and weight under different concentration of As (Figure 1). Under the stress of 0.05 mg/L As, only the weight of sc12 was significantly ($p<0.05$) lower than that of its control. While under the stress of 0.5 mg/L As, the height of sc12 and sc6068 were significantly lower than their controls, the weight of three cassava cultivars significantly ($p<0.05$) decreased compared with their controls. This indicated that sc12 was more sensitive to As than 8229 and sc6068. However, 8229 was most tolerant to As.

In this study, high concentration of As showed inhibition to cassava growth, while low concentration had no significant effect on growth. The inhibition of inorganic pollutants to crops has been reported by other studies. The weight of *Sedum aizoon* was promoted by 2 mg/L Cd but inhibited by 5 mg/L Cd (Guo et al., 2018). Low Pb concentration (1000 mg/L) had promotional effect on height and biomass of *Pogonatherum crinitum*, but high Pb concentration (2000 mg/L and 3000 mg/L) had inhibition effect on its shoot height and leaf biomass (Han et al., 2018).

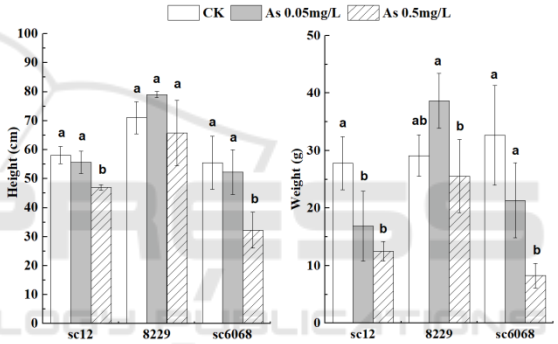

Figure 1: The height and weight of three cassava cultivars under the stress of As. Different letters indicated a significant difference at $p<0.05$ (same as in Figure 2).

3.3 Resistance Reaction of Cassava under As Stress

Malonaldehyde (MDA) is an index of cell membrane damage. Arsenic had a great impact on MDA in root and leaf of the three cassava cultivars (Figure 2). The roots MDA of 8229 and sc6068 under the stress of 0.05mg/L and 0.5 mg/L As were significantly ($p<0.05$) higher than their controls. But As had indistinctive impact on that of sc12, which might be related to its low accumulation ability for As (showing in Table 1). Under the stress of 0.5mg/L As, the leaf MDA of three cassava cultivars were all significantly ($p<0.05$) increased, as compared with their controls. However, under the stress of 0.05 mg/L As, only the leaf MDA of sc6068 was observably ($p<0.05$) increased than its control,

indicating sc6068 was more susceptive to As stress than sc12 and 8229 in leaf. The increased MDA contents followed the order of sc6068>8229>sc12, in the root of cassava under both As levels. These findings suggested that sc12 had the lowest MDA increment and strong tolerant for As. However, sc6068 had the highest increment for As, showing an intolerant ability.

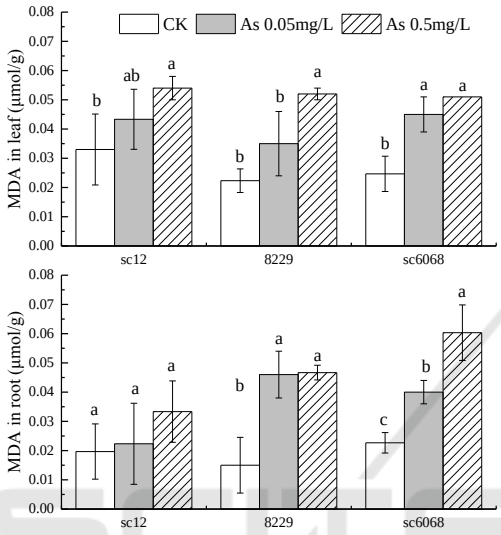

Figure 2: The malonaldehyde in root and leaf of three cassava cultivars under different concentration of As.

Malonaldehyde is one of the most important products of lipid peroxidation. Under the stress of toxic elements, plants are known to produce more "reactive oxygen species" (ROS), which subsequently cause the lipid peroxidation (Shahid et al., 2014). Malonaldehyde is often used as an indicator of the extent of oxidative stress. The content of MDA in each cassava cultivar increased along with increasing of As concentration, suggesting that cassava suffered oxidative stress by As. Similar increment of MDA has been observed in *Brassica napus* (Ali et al., 2015) and maize (Anjum et al., 2016). The varied MDA content is related with ROS scavenging ability. Compared between two maize cultivars, a lower MDA was observed in Dong Dan 80 than Run Nong 35 in the stress of Cd and Cd+As, which indicated that Dong Dan 80 exhibited more efficient ROS scavenging system (Anjum et al., 2016). In this study, sc12 had the lowest MDA increment for As. It could be speculated that it has a strong ROS scavenging ability.

4 CONCLUSIONS

Three cassava cultivars, sc12, 8229 and sc6068, showed different accumulation and response characteristics to As stress. The 8229 was apt to accumulate As in roots. Low As concentration had a promotion on the growth of 8229, showing a high accumulation capacity for As. Sc12 had a low accumulation capacity for As. The MDA of the three cassava cultivars, both in roots and leaves, were increased with increasing As concentration, suggesting that cassava has a resistant reaction at physio-biochemical level. These findings could be a reference for planting cassava in safety.

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