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## Variation in arsenic accumulation and translocation among wheat cultivars: The relationship between arsenic accumulation, efflux by wheat roots and arsenate tolerance of wheat seedlings



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#### HIGHLIGHTS

- As(V) uptake and As(III) efflux were significantly differed among wheat cultivars.
- Relative As(III) efflux by wheat roots ranged from 56% to 83%.
- As(V) tolerance index was positively correlated with root As concentration.
- As(V) tolerance index was negatively correlated with relative As(III) efflux.

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#### ABSTRACT

Fifty-seven wheat cultivars were used to investigate the differences in arsenic (As) accumulation, efflux and translocation among wheat cultivars and their relationship with arsenate (As(V)) tolerance under hydroponic condition. The relationship between wheat root As accumulation, As(V) uptake, arsenite (As(III)) efflux and As(V) tolerance of 14 wheat cultivars were also investigated. The results showed there were significant (p < 0.001) differences in As(V) tolerance, As accumulation and translocation among 57 wheat cultivars. Arsenate tolerance of wheat seedlings was positively correlated with As(V) uptake (p < 0.05), root As concentration (p < 0.001), but negatively correlated (p < 0.05) with TFs and relative As(III) efflux. No significant correlation between As(III) efflux and As(V) tolerance was found (p = 0.442). 56–83% of total As taken up by roots was extruded to nutrient solution. Root As concentration was positively correlated with As(V) uptake (not significant, p = 0.100), negatively correlated (p < 0.001) with relative As(III) efflux, whereas not significantly correlated (p = 0.773) with As(III) efflux. The results indicated that As(V) tolerant wheat cultivars have much higher capacity of root As accumulation. Arsenic detoxification in root cells is important for wheat seedlings under As(V) exposure.

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#### 1. Introduction

Arsenic (As), a noxious, nonessential metalloid, is found in all environment media. Arsenic contamination in the environment is wide-spread due to both natural and anthropogenic reasons [1,2]. Over 150 million people world-wide are exposed to unsafe levels of As in the drinking water [3]. Increased concentrations of As in the edible part of crops is another principal pathway for human exposure to As [4].

Many studies have focused on As uptake and metabolism in rice during the past few decades [5–7]. Wheat is the dominant crop which is grown under aerobic conditions in temperate countries and with a total production similar to that of rice [8]. A few studies on As accumulation and translocation in wheat have also been reported in recent years [4,9,10]. The results of previous studies have shown that As in wheat grains was mainly existed as inorganic form [9–11]. Inorganic As, a class I carcinogen, is more toxic than pentavalent methylated As species to animals and human cells [12,13]. So, it will result in health risk for those people who consume wheat grain with elevated As. Understanding the mechanisms of As metabolism in wheat is important to human health.

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Studies have shown that the abilities of arsenic accumulation and translocation by plants depend on plant species and genotypes [14–17].

Under aerobic condition, the main form of As that plants take up is inorganic arsenate (As(V)) [18]. After taken up by roots, As(V) is rapidly reduced to As(III), then some of As(III) extrude to the external medium [19]. Generally, the efflux of As(III) by rice root was largely and rapidly [20,21]. Without the strong efflux of As(III), the amount of As accumulation in *Holcus lanatus* plant tissues would have been 10-fold higher [22]. However, whether As(III) efflux by plant roots is one of the detoxification mechanism is not clear so far. Little is known about the cultivar differences in arsenic accumulation, translocation and As(III) efflux, and their relationship with As tolerance of wheat seedlings.

The objectives of this study are to (1) investigate the differences in As accumulation, translocation, and arsenate tolerance among 57 wheat cultivars, (2) study the variation in As(V) uptake and As(III) efflux among 14 wheat cultivars, (3) analyze the relationship between As uptake, accumulation, efflux by wheat roots and arsenate tolerance of wheat seedlings.

#### 2. Materials and methods

#### 2.1. Wheat cultivars

Fifty seven cultivars of winter wheat (*Triticum aestivum* L.) used in the present study were listed in Table 1.

#### 2.2. Plant culture

Wheat seeds were surface sterilized in 3%  $H_2O_2$  (w/w) for 10-min, rinsed and soaked in distilled water overnight, and then germinated in moist quartz sand for 3-d. Twelve uniform seedlings were selected and transplanted to 2-L 0.5 mmol  $L^{-1}$  CaCl<sub>2</sub> solution containing 2 mmol  $L^{-1}$  MOPS-buffer (3-[N -morpholino] propane sulfonic acid). Solution pH was adjusted to 7.0 using HCl or NaOH. All pots were placed in a growth cabinet at 25 °C/20 °C day/night temperatures and a 12 h/12 h day/night period (350 µmol m<sup>-2</sup> s<sup>-1</sup>).

#### 2.3. Experimental design

#### 2.3.1. Preliminary experiment

Huaimai 29 wheat cultivar was used in this experiment. After growing in 0.5 mmol  $L^{-1}$  CaCl $_2$  solution (containing 2 mmol  $L^{-1}$  MOPS-buffer) for 1-d, wheat seedlings were treated with 0, 5, 10, 15, and 20  $\mu$ mol  $L^{-1}$  Na $_2$ HAsO $_4$ . Each treatment was replicated five times. After 72-h treatment, the elongation of root was recorded. Wheat seedlings were separated to roots and shoots, rinsed with deionized water, blotted dry, then dried at 60 °C to constant weight, and weighed. Relative root elongation (RRE), relative root dry weight (RRDW), and relative shoot dry weight (RSDW) were calculated by dividing the given parameter under As treatment by the one under the control.

## 2.3.2. Arsenate tolerance and As accumulation in wheat seedlings under As(V) exposure

Fifty-seven wheat cultivars were used in this study. After growing in  $0.5\,\mathrm{mmol}\,L^{-1}$  CaCl $_2$  solution (containing  $2\,\mathrm{mmol}\,L^{-1}$  MOPS-buffer) for 1-d, wheat seedlings were treated with 0 and  $10\,\mathrm{\mu mol}\,L^{-1}$  Na $_2$ HAsO $_4$ . Each treatment was replicated five times. After 72-h treatment, plants were harvested as described in Section 2.3.1.

#### 2.3.3. Arseante uptake and aresnite efflux

According to the above results, 14 wheat cultivars were used to examine the As(V) uptake and As(III) efflux from roots of wheat

**Table 1**Cultivar name of winter wheat and their associated details

No.	Cultivar name	Abbreviation	Year of release	Origin
1	Mianmai45	MM45	2007	Sichuan
2	Nannong0686	NN0686	2010	Jiangsu
3	Fanmai8	FM8	2008	Henan
4	Jinmai85	JM85	2008	Shanxi
5	Yangmai11	YM11	2001	Jiangsu
6	Zhoumai16	ZM16	2003	Henan
7	Ningmai14	NM14	2006	Jiangsu
8	Ligao6	LG6	2006	Shannxi
9	Bainong160	BN160	2007	Henan
10	Wennong17	WN17	2011	Shandong
11	Nannong9918	NN9918	2002	Jiangsu
12	Kenong199	KN199	2006	Beijing
13	Mianmai37	MM37	2004	Sichuan
14	Zhoumai22	ZM22	2007	Henan
15	Jimai6	JM6	2008	Henan
16	Neimai988	NM988	2009	Henan
17	Yangmai15	YM15	2005	Jiangsu
18	lining16	IN16	2003	Shandong
19	Linkang11	LK11	2004	Shanxi
20	0			
	Yunhan22-33	YH22-33	2005	Shanxi
21	Jining12	JN12	2005	Shandong
22	Ji5265	J5265	2007	Hebei
23	Mianmai367	MM367	2010	Sichuan
24	Xinong979	XN979	2005	Shannxi
25	Mianmai48	MM48	2009	Sichuan
26	HedongTX006	HDTX006	2005	Shanxi
27	Yumai49	YM49	2000	Henan
28	Aikang58	AK58	2005	Henan
29	Yangmai158	YM158	1993	Jiangsu
30	Shannong22	SN22	2011	Shandong
31	Zhefeng2	ZF2	2001	Zhejiang
32	Wenmai19	WEM19	2004	Henan
33	Zhenmai366	ZM366	2005	Henan
34	Linfeng3	LF3	2004	Shanxi
35	Xiaoyan22	XY22	1998	Shannxi
36	Yannong19	YN19	2001	Shandong
37	Luyuan502	LY502	2011	Shandong
38	Yumai57	YM57	2003	Henan
39	Tainong19	TN19	2011	Shandong
40	Tainong18	TN18	2008	Shandong
41	Wennong14	WN14	2010	Shandong
42	Hengguan35	HG35	2006	Hebei
43	Zhenmai6	ZM6	2005	Jiangsu
44	Yannong21	YN21	2002	Shandong
45	Pingan6	PN6	2006	Henan
46	Shunmai1718	SM1718	2011	Shanxi
47	Iimai22	JM22	2006	Shandong
48	Huaimai29	HM29	2009	Jiangsu
49	Shannong15	SN15	2006	Shandong
50	Wanmai19	WAM19	1999	Anhui
51	Jinmai88	IM88	2009	Shanxi
52	Yannong9901	YN9901	2011	Shandong
53	Zhenmai5	ZM5	2004	Jiangsu
54	Ningmai13	NM13	2004	Jiangsu Jiangsu
55 55	Shixin539	SX539	2003	Hebei
56			2003	
56 57	Qin gfeng1	QF1	2008	Zhejiang
3/	Liangxing99	LX99	2004	Shandong

seedlings. After growing in 1.1-L 0.5 mmol  $L^{-1}$  CaCl $_2$  solution (containing 2 mmol  $L^{-1}$  MOPS-buffer) for 1-d, plants were cultured and treated under the same conditions as described in Section 2.3.1. Each treatment was replicated four times. After 72-h exposure, an aliquot of culture solution (1.0-ml) was collected from each pot. As speciation in the culture solutions were analyzed within 24-h after collection. Plants were harvested as described in Section 2.3.1.

#### 2.4. Analysis of total arsenic and arsenic speciation

Wheat root and shoot samples were digested with  $HNO_3 - H_2O_2$  (1:1, v/v) at 125 °C [23]. After cooling, solution was diluted to 10-ml and filtered. Total As concentration was determined by an inductively coupled plasma-optical emission spectrometer

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