浙江大学学报(农业与生命科学版) 33(5):473～478,2007
Journal of Zhejiang University (Agric. & Life Sci.)

Article ID: 1008-9209(2007)05-0473-06

Genotypic difference in growth and As accumulation in *Pteris vittata*

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Abstract: Some *Pteris vittata* genotypes, collected from different locations of China, were planted in pots to evaluate their ability in As tolerance and accumulation. Results showed that there were dramatic difference in plant height, biomass and frond number. The plant height ranged from 29.6 to 68.2 cm, frond number from 18.0 to 60.0 per plant, shoot fresh weight from 150 to 540 g per plant and root fresh weight from 20.3 to 94.9 g per plant. The distinct difference among the genotypes was also found in shoot and root As accumulation, ranging from 643.10 to 3009.03 mg·kg^{-1} and from 26.34 to 112.38 mg·kg^{-1}, respectively. It was found that accession C108 had the highest As accumulation in shoot, being significantly higher than all other accessions. There was a significant difference among the accessions of *Pteris vittata* in both transporting factor (TF) and biological factor (BF). There was a positive association between As accumulation and some growth characters, including plant height, frond and bud numbers, and shoot fresh weight.

Key words: *Pteris vittata* L.; arsenic; contamination; genotype; phytoremediation
CLC number: Q948; S564.4 **Document code:** A

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蜈蚣草生长特性和富集砷的基因型差异研究(英文).浙江大学学报(农业与生命科学版),2007,33(5):473-478

摘　要:利用从我国蜈蚣草主要分布地区收集到的不同蜈蚣草基因型进行田间试验,评价他们耐砷和富集砷的能力.试验结果表明,蜈蚣草基因型在株高、生物量、羽叶数上都表现出显著的不同.株高变化范围为29.6～68.2 cm;每株羽叶数变化范围为18.0～60.0个;每株地上部鲜重变化为150～540 g;每株根鲜重变化范围为20.3～94.9 g.蜈蚣草基因型地上部和根部的砷浓度也表现出显著差异.地上部砷积累浓度变化范围为643.10～3009.03 mg·kg^{-1},根部砷积累浓度变化范围为26.34～112.38 mg·kg^{-1}.基因型C108地上部积累砷最多,显著大于其它蜈蚣草基因型;研究还发现,蜈蚣草基因型间的转运系数和生物富集系数也存在显著差异.相关分析显示,蜈蚣草富集砷的量与株高、羽叶数、芽苞数等生长特性呈正相关.

Received date: 2005-09-15

Foundation item: Project Supported by the National Hi-Tech Research and Development Program (863) of China (2001AA640501); Knowledge Innovation Program from Chinese Academy of Sciences (KZCX-401-2); National Natural Science Foundation of China (40071075); National Basic Research Program (973) of China (G1999011808).

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关 键 词: 蜈蚣草; 砷; 污染; 基因型; 植物修复

Phytoextraction, defined as the use of plants to remove pollutants from the environment or to render them harmless[1-2], is being considered as a highly promising technology for the remediation of polluted sites due to its cost-effectiveness and environmental friendliness[3]. Plant cultivation and harvesting are inexpensive processes and less secondary waste compared with traditional engineering approaches. Furthermore, this technology creates minimal environmental disturbance[4-5]. Phytoextraction has generated increasing interest worldwide and the great effort has been done to improve its efficiency, so as to extend its application[6-9]. However, the ability to accumulate heavy metals varies significantly among plant species and among cultivars within a species. There are three strategies at present to improve the efficiency of phytoremediation, *i.e.* (1) to identify the hyper-accumulators with high ability to accumulate contaminants; (2) to enhance the capacity of the plant to uptake and accumulate contaminants by adding special reagents; and (3) to increase the biomass or contaminant-accumulating ability of hyper-accumulator by genetic modification. The first approach is the most practical one compared to others because it will not cause additional pollution.

Pteris vittata is widely grown in China, especially in southern China. Many researches show that there is a markedly genotypic difference in plant growth traits. For example plant height ranges from 0.2 to 2 meters, and fresh biomass ranges from 5 to 36 $t \cdot hm^{-2}$[10]. However, there has been less information about the relationship between As accumulation and plant growth. The objective of the present study is (1) to develope a method for selecting As hyper-accumulator in the fields; (2) to determine genotypic difference in As accumulation in *Pteris vittata* shoot; (3) to find relationship between shoot As content and growth character in fields.

1 Materials and Methods

The experiment was conducted in Chenzhou experimental station, Chenzhou City, Hunan Province, China, where soil was heavily contaminated with As. Ten *Pteris Vittata* genotypes (accessions) were collected from Guangxi, Hunan, Chongqing, Guangdong and Fujian provinces respectively. Pores were sowed on compost bed for germination and seedling growth. When seedlings grew into the seven leaves (180-day old), they were transplanted into a field. A completely randomized block design was used with three replications for each treatment. The plot consisted of 4 lines with 4 m of length and 0.4 m between lines. Phosphorus as $CaH_2PO_4 \cdot 2H_2O$, potassium as KCl and nitrogen as urea were supplied at a rate of 30, 60 and 160 $kg \cdot hm^{-2}$, respectively. All P and K fertilizers were applied before transplanting, while N fertilizer applied in four equal splits, the first one being applied before transplantation and three others at 1, 5 and 7 months after transplanting.

At the end of the experiment, fronds, buds and fresh weight per plant were counted, and the plants were harvested as the sample for dry weight determination and As accumulation analysis. The plants were washed thoroughly with tap water to remove adhesive soils and dust, and then rinsed with deionized water three times, separated into shoots and roots and dried in an oven for 48 h at 85 ℃, and then the dry weight was recorded. The soils were then air-dried, ground to pass a 100-mesh screen. Both soil and plant samples were digested with nitric acid and perchloric acid (4/1, *V/V*), and arsenic content was determined by an Atomic Fluorescence

Spectrometer (Haiguang AFS-2202).

2 Results and analysis

2.1 Genotypic difference in growth traits

There was a significantly genotypic difference in plant height, frond number and fresh weight when the plants were grown in the soil with As accumulation of 59 mg•kg^{-1} (Table 1). Frond number per plant ranged from 18.0 for C109 to 60.0 for C105, plant height ranged from 29.6 cm for C109 to 68.2 cm for C103, shoot fresh weight ranged from 150 g•plant^{-1} for C109 to 540 g•plant^{-1} for C106, and root fresh weight ranged from 20.3 g•plant^{-1} for C109 to 94.9 g•plant^{-1} for C106. The plant height of C109 was the lowest, being 29.6 cm and 45% of the control (accession C101), while C103 had the highest plant height, being 68.2 cm and 103% of the control. Shoot and root fresh weight in C109 was also the smallest, and being only 62% and 39% of those in the control. C106 had the greatest shoot and root fresh weight, being 540 and 94.9 g•plant^{-1}, *i.e.* 222% and 182% of the control. Similarly, the remarkable difference in frond number per plant could be found among these accessions, in C109 being the smallest (18.0 per plant) and C105 the greatest (60.0 per plant), although the two accessions had the basically same plant height. There was also a dramatic difference in frond number per plant between C107 and C104 or C102, but no distinct difference was found in both shoot and root fresh weight. In general, plant height and frond number per plant were closely related to shoot biomass of *Pteris vittata*, thus it may be deduced that the accession of *Pteris vittata* with higher plant height and more fronds per plant is favorable for more As accumulation in above-ground parts.

Table 1 Genotypic difference in plant height, frond number and fresh weight per plant

Accession	Originated location	Plant height		Fronds/plant^{-1}		Fresh weight/(g•plant^{-1})		Fresh weight/(g•plant^{-1})	
		cm	%*	Frond	%	Shoot	%*	Root	%*
C102	Guangxi	44.2b	67	24.0a	180	273.3 abc	112	46.7abc	89
C103	Guangxi	68.2e	103	22.7a	171	306.7abc	126	63.0bcde	121
C104	Hunan	44.4b	67	27.7a	208	230.0 ab	95	33.4 ab	64
C105	Hunan	59.8cde	91	60.0c	451	493.3d	203	88.7 de	170
C106	Guangxi	54.2bcd	82	58.7c	441	540.0d	222	94.9 e	182
C107	Hunan	44.7b	68	30.0 b	226	260.0 ab	107	33.7 ab	65
C108	Chongqing	51.9bc	79	57.7 c	434	466.7 cd	192	83.5cde	160
C109	Guangdong	29.6a	45	18.0a	135	150.0 a	62	20.30a	39
C110	Fujian	53.3bcd	88	28.0a	211	429.0bcd	176	86.2cde	165
	Mean	50.0	78	36.3	273	349.9	144	61.2	117

Notes: The same letter after data within a column represents no significant difference at 95% probability. Asterisk shows that calculation is based on accession C101.

2.2 Genotypic difference in As concentration and accumulating capacity

All parameters measured in the present study differed significantly among the accessions of *Pteris vittata*. As concentration ranged from 643.10 to 3009.03 mg•kg^{-1} in shoots, with a mean of 1283.1 mg•kg^{-1}, and 26.34 to 112.38 mg•kg^{-1} in roots, with a mean of 61.86 mg•kg^{-1} (Table 2). C106 was the accession with the lowest As concentration in both shoots and roots, and C109 had the highest As concentration in shoots, followed by C108 which had the highest root As concentration.

Concerning As accumulation, which is the function of As concentration and corresponding plant part, C108 was the highest in both shoots and roots, being significantly higher than all other accessions. C104 accumulated the lowest amout of As in both roots and shoots, although it showed no significant difference with accessions, except in C109. In phytoremediation, the accession with more As accumulation in shoot is favorable, because the higher efficiency of remediation may be reached.

Transporting factor (TF) is defined as the ratio of shoot to root As concentration, and reflects As-transporting ability of *Pteris vittata* from root to shoot. Biological factor (BF) is defined as the ratio of shoot to soil As concentration, and reflects As uptake ability of *Pteris vittata* from soil. It may be seen from the Table 2 that there was a significant difference among the accessions of *Pteris vittata* in both TF and BF, with C109 (50.37) and C106 (10.77) having the highest and lowest BF, respectively, and C110 (81.90) and C108 (17.22) having the highest and lowest TF.

Table 2 Genotypic difference in As concentration and accumulating capacity among accessions of *Pteris vittata*

Accession	Originated location	As concentration/ (mg·kg^{-1})		As content/ (mg·plant^{-1})		TF	BF
		Shoot	Root	Shoot	Root		
C101	Guangxi	1236.86ab	66.67abc	49.97a	0.85a	17.72a	20.70ab
C102	Guangxi	1159.69ab	44.91a	62.61a	0.49a	26.59b	19.41ab
C103	Guangxi	1070.32ab	59.35ab	76.97a	1.14a	18.28a	17.92ab
C104	Hunan	845.13ab	44.44a	50.36a	0.48a	20.01a	14.15ab
C105	Hunan	743.38a	31.61a	94.38a	0.97a	23.98ab	12.44a
C106	Guangxi	643.10a	26.34a	83.31a	0.79a	26.44b	10.77a
C107	Hunan	1465.74ab	75.05abc	91.04a	0.80a	19.73a	24.54ab
C108	Chongqing	1621.53b	112.38c	174.66b	2.84b	17.22a	27.14b
C109	Guangdong	3009.03c	110.68bc	82.60a	0.55a	27.88b	50.37c
C110	Fujian	1036.13ab	47.13a	91.86a	1.07a	81.90c	17.34ab
	Mean	1283.09	61.86	85.78	1.00	27.97	21.48

Notes: The same letter after data within a column represents no significant difference at 95% probability; TF: Transporting factor; BF: biological factor.

2.3 Relationship between shoot As concentration, accumulation and plant growth characters

Relationships between shoot As concentration, accumulation and plant growth characters were shown in Table 3. There were significantly negative correlations between shoot concentration and plant height or fresh weight, indicating that the accessions with larger plants

Table 3 Relationship between shoot As concentration, accumulation and plant growth characters

Character	Line regression	Significance
Plant height	$y_{\text{As concentration}} = 2707.62 - 27.81x$	$r = -0.48^{*}$
	$y_{\text{As accumulation}} = 73.12 + 0.25x$	$r = 0.07$
Frond number per plant	$y_{\text{As concentration}} = 1665.62 - 11.25x$	$r = -0.28$
	$y_{\text{As accumulation}} = 42.14 + 1.28x$	$r = 0.57^{*}$
Bud number per plant	$y_{\text{As concentration}} = 1460.54 - 6.72x$	$r = -0.19$
	$y_{\text{As accumulation}} = 61.08 + 0.94x$	$r = 0.48^{*}$
Shoot fresh weight	$y_{\text{As concentration}} = 2197.22 - 2.77x$	$r = -0.54^{*}$
	$y_{\text{As accumulation}} = 36.34 + 0.15x$	$r = 0.52^{*}$

Notes: $n = 12$; Asterisk shows significance at 95% probability.

tend to contain lower As concentration. On the other hand, the significant positive correlations were found between shoot As accumulation and fronds per plant, buds per plant or fresh weight. However there was no significant correlation between shoot As accumulation and plant height.

3 Discussion

Arsenic contamination has emerged as a major environmental issue worldwide. Health of millions of people has been damaged in Bangladesh and Indian because of arsenic poisoing[11-21]. In China, arsenic pollution has also caused the great damage to human health. Therefore, the remediation of arsenic contamination has been paid special attention.

Successful phytoextraction requires those plants which are capable of producing high biomass while accumulating large amount of contaminants through uptaking from soil. *Pteris vittata* has been proved to be capable of accumulate high As and maintaining a large biomass, thus it is a promising candidate for As phytoremediation[10,22-25]. Moreover, it was shown that its bio-accumulating factor (BF) of As ranged from 7 to 80[10]. In addition, unlike most other As-tolerant plants, which have lesser accumulation coefficient in shoot, *Pteris vittata* shows quite high accumulation coefficient, transferring approximately 90 % of As taken up to the above-ground parts[10,22]. However, there are few reports on the differences among the various accessions (genotypes) of the species in As accumulation and growth response to As toxicity. The present results showed that there is a large difference in plant height, shoot and root biomass among the accessions with different origin.

The plants used as extractant for contaminants should have the following traits: (1) to be tolerant to high levels of metalloid; (2) to accumulate a large amount of metalloid in its harvestable parts; (3) to be fast in growth rate exposed to the contamination; (4) to be great in biomass production; (5) to have a developed roots[26-28]. Unfortunately, most hyper-accumulators available now are relatively small in plant size and slow in growth rate[4,27]. Data from the current experiment illuminated that it is possible to screen out the accessions of *Pteris vittata* with high biomass and As accumulation in above-ground parts. However, more research should be done in the field condition to identify the accession or genotype suitable for use in the practice of remediation of As contaminated soils. In addition, the experiment showed that the frond and bud number are positively correlated with As accumulation ability, suggesting that these growth parameters may be used as the indicator of As accumulation ability of *Pteris vittata*.

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