An Interesting Phenomenon from Proline Accumulation in Miscanthus sinensis under Different Levels of Heavy Metal Pollution in Mining Area*

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Abstract: Proline content in plant was closely related with its endurance to enviroment stress. In present research, proline contents in Miscanthus sinensis populations growing in mining area, where the main pollutants were Zn, Cu, Pb and Cd, and in unpolluted plot were investigated to find a proof for its endurance changing. The result showed an interesting accumulation pattern of proline: ① in summer, proline content increased with soil heavy metal content, and significantly correlated with Cu accumulation in plant; ② in contrast to summer, proline content in the most heavily polluted plot decreased to the lowest in winter, while, in the slight and unpolluted plots, proline content increased most in winter; ③ population experienced moderate pollution showed a similar proline content and proline change pattern as unpolluted population between seasons. What the special accumulation pattern of proline in present study means, increasing or decreasing the stress endurance, needs further research.

Key words: Miscanthus sinensis; proline accumulation; different seasons; heavy metals stress
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Proline is an important compound that accumulates in plants exposed to environmental stresses such as drought[1], high salinity[2-5], freezing[6], UV radiation[7] and heavy metals[8-11]. Many studies focused on the roles of proline in defense mechanisms against impairments caused by osmotic stress[12]. The dominant results indicate that proline functions as osmoprotectants as a mediator of osmotic adjustment under osmotic stress conditions[13], as a stabilizer of subcellular structures[14], as a scavenger of free radicals[15-16], as a sink for energy[17], as a major constituent of cell wall structural proteins in plant[12] and even as a stress-related signal[18]. Plant species, variety or ecotype with higher proline content exist higher endurance[19] or higher proline can improve the tolerance to environment pressure[5]. Proline, therefore, was suggested to be used as a marker to test the level of heavy metal pollution[8] and to evaluate tolerance of plant species, varieties or ecotype.

Miscanthus sinensis, a dominant endemic perennial grass, is ubiquitously distributed in south China and was found to have wide adaptability to different environmental regimes, such as high salinity, polluted soil and severe dry land. M. sinensis was pro to evolve into different ecotypes under stresses due to its wide heterogeneity and the regulation of isoenzyme polymorphism[20]. The aim of the present work is to investigate the proline accumulation in natural M. sinensis populations growing in a heavy metal pollution gradient in a mining area to find a proof for its endurance changing.

1 Materials and methods

1.1 Plant material and field condition

M. sinensis leaves were sampled in Dabaoshan, an area with mining history of 30 a in south China, at E 111°55′~114°45′ and N 23°50′~25°31′. In this area, Cu, Cd, Pb and Zn were the dominant pollutants. Due to the intensive toxicity of the heavy metals and

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acid soil, few plant species can grow in this region except M. sinensis, the pioneer species, forming single species community. Based on extensive investigation to the soil condition, 4 plots with different heavy metal pollution levels in the mining area and one unpolluted plot outside the mining area with approximate pH, which formed a heavy metal pollution gradient, were selected. The detailed information about the corresponding populations and the soil condition was listed in table 1. Heavy metal content increased with the plot number from I to V, and plot V had highest contents of Pb, Zn and Cd, namely the population in plot V was under the highest pollution pressure.

In each population 5 individuals were selected. 2 ~3 leaves of every individual were sampled for proline analysis in November 2002 and July 2003, respectively. Three whole plants were harvested, including shoot and root, for heavy metal determination.

1.2 Determination of proline and metals

The proline content was determined by using the method of Hou[23]. Lyophilized leaf samples of 0.5 g was grinded in 3% (w/v) sulfosalicylic acid and transferred into centrifugal tube supplemented to 5 mL with 3% (w/v) sulfosalicylic. After 10 min incubation at 100 ℃, the tubes were cooled and centrifuged at 3000 r. 2 mL of extract was mixed with 2 mL distilled water and 6 mL of a mixture of glacial acetic acid, orthophosphoric acid (3∶2, v/v) and 25 mg minhydrin. After 1 h incubation at 100 ℃, the tubes were cooled and 4 mL toluene were added. The absorbance of the upper phase was spectrophotometrically determined at 520 nm. Then the proline concentration was calculated by using a standard curve.

Heavy metals in plant material (the mixture of leaf, shoot and root) were determined by digestion of plant material in concentrated HNO_3-HClO_4 (3∶1), and then measured by atomic absorption spectrophotometry.

Tab.1 Heavy metal contents in soil and the polluted time of the populations1)

		Sample plots				
		I	II	III	IV	V
Polluted time (a)		0	15	25	15	20
Heavy metals (mg/kg)	Cu	13.67 a	283.23 b	498.67 c	756.67 d	709.16 d
	Zn	181.33 a	252.67 a	820.00 b	769.67 b	1326.33 c
	Pb	42.83 a	347.10 b	616.67 c	874.00 d	2845.36 e
	Cd	0.163 a	0.298 a	0.47 b	0.69 c	1.03 d

1) Numbers followed with different superscript letters between different sample plots in same heavy metal line indicate significantly different at P =0.05 (t test)

2 Result

2.1 Proline content in two seasons

Proline content in M. sinensis leaves in plots with different heavy metal pollution levels was detected in both seasons (Fig.1). In summer, population in higher heavy metal polluted plots showed higher proline accumulation except for plot III, in which proline content was similar to that in unpolluted plot (I) and in light polluted plot (II). In winter, however, a reversed pattern was observed: proline decreased with increasing pollution.

For the single plot, proline content in plot V in winter was lower than that in summer, while proline contents in other plots in winter were higher than those in summer. Especially for the unpolluted plot, 1.5 times higher proline content was detected in winter than in summer. Thus proline contents in the heaviest polluted plot were relatively stable between seasons comparing with other lighter polluted plots. It was different from the results in controlled experiments[9-11].

2.2 Proline accumulation and heavy metals

No significant correlation between proline content and single heavy metal content in soil was found. But from the results of proline accumulation and total heavy metal in heavily polluted plots (V), slight polluted plot (II) and unpolluted plot (I), it can be concluded that severe heavy metal pollution has affected proline synthesis or decomposition in the plant.

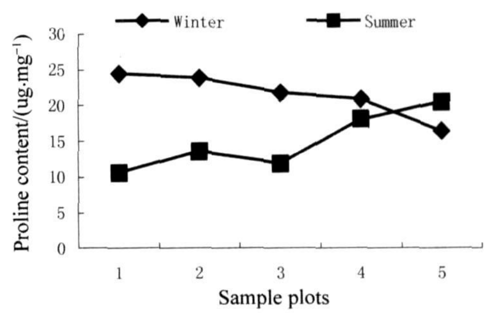

Fig.1 Proline content of M. sinensis populations in elevated polluted stands in two seasons. Serial number from I to V indicates an increasing extent of pollution

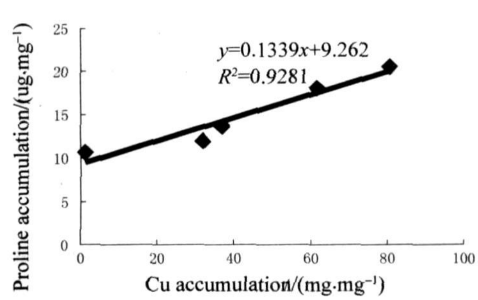

Fig.2 Correlation between proline content and Cu accumulation in M. sinensis

Average heavy metal accumulation in M. sinensis was listed in table 2. Regression analysis between proline content and heavy metal accumulation was conducted. Proline content was only positively correlated to Cu content ($P < 0.01$, $R^2 = 0.9281$) (Fig. 2). Thus, in this experiment site Cu might be the main inducer for proline accumulation except in plot V, in which the high concentrations of Pb, Zn and Cd worked together.

Tab. 2 Heavy metal accumulation in different M. sinensis populations (Cu, Zn, Pb: mg/kg; Cd: g/kg)

Heavy metals	Average Accumulation				
	I	II	III	IV	V
Cd	30.6[a]	80.06[ab]	88.64[ab]	134.56[b]	318.50[c]
Pb	5.70[a]	66.79[bc]	46.72[b]	90.37[c]	200.70[d]
Cu	1.09[a]	37.10[b]	32.07[b]	61.57[bc]	80.49[c]
Zn	42.73[a]	143.17[a]	220.96[ab]	364.10[b]	345.07[b]

Numbers followed with different superscript letters between different sample plots in same accumulated heavy metal indicate significantly different at $P = 0.05$ (t test).

3 Discussion

Most heavy metal toxicity studies indicated that heavy metals, to some extent, could induce rapid proline accumulation[9-11,19]. And the proline accumulation apex occurred at lower heavy metal concentrations, such as 5 ppm for Lemna minor[9], then decreased rapidly with heavy metal concentration increased. In this study higher proline content was detected in summer in the populations growing in severe polluted plots. But the changing pattern of decrease was not found even in the most heavily polluted plot, in which the contents of Cu, Zn, Pb and Cd were high up to 709.16, 1326.33, 2845.36 and 1.03 mg/kg respectively. Two possible reasons for such a result are: ① M. sinensis had strong stress resistance, and heavy metal stress intensity in this study was within its tolerance; ② Materials in this study were sampled in natural populations that experienced longer time stress, therefore, the plants had basically adapted to the polluted environment compared to other controlled toxicity experiments[9-11].

Proline contens in plot III, the moderate polluted level, was similar to those in the unpolluted and light polluted populations (I and II) in both summer and winter. Longer stressed time maybe the main reason to this result. The study on the dynamic changes of proteins and nucleic acid metabolism in Datrua stramonium, exposed to different heavy metal pollution duration, got similar result: heavy metal pollution reduced the nucleic acid and protein contents in D. stramonium, which had been growing in polluted areas for short time, and with pollution time prolonging, the changing rules began to recover to the initial (unpolluted population)[22-23]. Parameters of quantitative characters (POCs) of Vicia faba treated with low dose of heavy metal ions also recovered to the unpolluted population in the proceeding generations[24].

In winter a reversed proline accumulation pattern was found comparing to that in summer: the proline content decreased with the increasing heavy metal content. This result, together with the accumulation pattern in summer, suggests that the population experienced heavy metal stress got a changed proline metabolism. Proline has been proved to be beneficial to plant resistance against environmental stress[5,26-27] via osmoregulate[13], as a stabilizer of subcellular structures[14], and as a scavenger of free radicals[15-16]. If the changed proline metabolism population in this study is helpful to its stress endurance, still needs further research.

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金属矿区芒草脯安酸积累的一个有趣现象

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摘　要：植物的脯安酸含量与其对环境的耐受性有很大的关系。通过采集金属矿区（主要污染物为 Zn，Cu，Pb，Cd）不同污染梯度、不同季节的芒草种群材料，测定其脯安酸含量，从脯安酸角度探讨受不同污染强度胁迫的种群的耐性变化。结果发现：①夏季芒草种群的脯安酸含量随土壤重金属含量的升高而升高，与体内 Cu 积累量呈显著的正相关关系；②冬季脯安酸含量呈现相反的趋势，即重度污染土壤的种群脯安酸含量降到最低，而受轻度污染样地的种群与清洁种群一样，脯安酸含量升到最高；③受中度污染种群的脯安酸含量变化与清洁种群相似。这一结果说明重度的重金属污染已引起芒草种群在脯安酸水平发生代谢变化，意味着这一芒草种群可能已获得较强的环境耐性，但这一猜测还有待于进一步研究。

关键词：芒草；脯安酸积累；季节；重金属胁迫
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