

Analysis on DNA Methylation in *Chromolaena Odorata* from Different Area and at Different Nitrogen Level by MSAP

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ABSTRACT: Two species of *Chromolaena odorata*, with one indigenous in Mexico and the other introduced to Sipsongpanna, were cultivated by water culture method, and treated by high, normal and low nitrogen respectively, and the technology of methylation sensitive amplification polymorphism (MSAP) was used to explore the changes of DNA methylation polymorphism of the *C. odorata* with different sources and the influence of nitrogen nutrition levels on their DNA methylation polymorphisms. The study showed that DNA methylation levels and modes of the two species that undergone high nitrogen and low nitrogen treatments were changed, DNA methylation level of *C. odorata* invaded in Sipsongpanna was higher than that in the place of origin, and the higher the nitrogen supply level, the higher the DNA methylation.

KEYWORD: *Chromolaena odorata*; Mexico; Sipsongpanna; DNA methylation; MSAP

1 INSTRUCTIONS

DNA methylation is a modification process of transforming cytosine of CpG 5' dinucleotide into 5' methylcytosine under the effect of methylation transferase (Zhang *et al.*, 2010; Vanyushin and Ashapkin, 2011), it has been involved in a lot of basic research and application fields of life science, and is closely related to the regulation of gene expression, genomic imprinting, sex chromosome inactivation, cell differentiation, embryonic development and other biological processes(Gehring and Henikoff, 2007; Zilberman *et al.*, 2007; Zilberman, 2008). DNA methylation has two main functions: a. defense, protecting genome being damaged by foreign insertion sequence and maintaining the stability of genome; b. regulator gene expression, playing an important regulatory role in cell development (Mandrioli, 2004; Meyer, 2011; Alba *et al.*, 2012; Chang and Smith, 2012; Drenovsky *et al.*, 2012).

Invasive plants refer to the species that are introduced by human activities, intentionally or unintentionally, from their native region to a new region, in environmental ecological system of which they form self-regeneration capacity, cause harm or impact on the surrounding ecosystems, industries, or human health, disrupting the ecological balance (Wan *et al.*, 2009; Navajas *et al.*, 2013; Parepa *et al.*, 2013). They often have a strong ability to reproduce and spread, and the so-caused huge damage has

become the global hot issue in the biological environment, safety and economy, and aroused great concern of all countries around the world. These species have caused serious economic losses to our country, with the most serious harm in the destruction of ecosystem and the loss of biodiversity, which will cause the loss of the rich, indigenous and peculiar biodiversity, and hard recovery (Drenovsky, *et al.*, 2012). *C. odorata* has been recognized in the world as a kind of perennial invasive weed, originated in Central-South America and invaded to Asia and Africa in the middle period of the 19th century and then China in 1934, it has widely distributed in southern and other parts of China, with a continuous expansion trend (Ding and Blossey, 2009; Tang *et al.*, 2011; Ramaswami and Sukumar, 2013; te Beest *et al.*, 2013).

Explanation about invasion manners of the exotic plants has been given in some invasion mechanism hypothesis, such as enemy release hypothesis (Funk and Throop, 2010), novel weapon hypothesis (Bais, 2003; Levine *et al.*, 2006), resource competition hypothesis (Eppstein and Molofsky, 2007), and nitrogen distribution evolutionary hypothesis (Feng *et al.*, 2009), etc. There has been no report about the role of DNA methylation in exotic plant invasion. In this test, *C. odorata*, a typical invasive plant, was taken as the experimental object, the MSAP technology was used to explore the influence of DNA methylation polymorphism changes and nitrogen nutrition levels of the *C. odorata* with

different sources on their DNA methylation polymorphisms, to reveal the correlation between DNA methylation and plant invasion and provide reference for enriching the plant invasion mechanism.

2 MATERIALS AND METHODS

Indoor seeding of the two species of *C. odorata* (respectively from Mexico and Sipsongpanna) was conducted in June 2011, illuminating for at 18 hours in 29°C illumination incubator and six hours in 25°C dark one. Hydroponics was adopted for the young plants and treated firstly by normal nitrogen treatment (nitrogen content of 210 mg L⁻²) with half the amount of Hoagland nutrient solution, and then by high nitrogen treatment (nitrogen content of 315 mg L⁻²) with nitrogen content being increased to two-thirds of the original dose, and lastly by low nitrogen treatment (nitrogen content of 105 mg L⁻²) with nitrogen content being reduced to half of the original dose. Total nutrient solution concentration was controlled by the regularly measured conductance value.

MSAP analysis: refer to the methods of Xiong et al (Xiong *et al.*, 1999)

Select clear stripes from PAGE gel, mark "with strip" by "1", "without stripe" by "0", and classify by

"with or without strip" into three types of A, B and C: *Hpa* II and *Msp* I with stripes, i.e. none methylation or interior cytosine methylation, marked as (1, 1); Type B: *Hpa* II with stripes, *Msp* I without stripes, i.e. exterior cytosine methylation, marked as (1, 0); C type: *Hpa* II without stripes, *Msp* I with stripes, i.e. interior cytosine methylation, marked as (0, 1) (Ashikawa 2001).

3 RESULTS

3.1 Comparison of DNA methylation levels between indigenous and invasive *C. odorata*

DNA methylation of the *C. odorata* from two places are shown in Table 1. At "CCGG" of *C. odorata* indigenous in Mexico, the overall DNA methylation level was 21.17%, among which the whole methylation point level was 9.49%, and semi methylation point level of 11.68%; while at "CCGG" of that introduced to Sipsongpanna, the overall DNA methylation level was 29.44%, among which the whole methylation point level was 14.12%, and semi methylation point level of 15.33%. No matter for whole methylation and semi methylation, with regard to point level and the overall level of DNA methylation, the indigenous *C. odorata* was higher than the invasive one.

Table 1 DNA methylation levels in *C. odorata* from different area by MSAP analysis

Locality of growth	Total	None-methylated sites	Methylated sites		
			Fully methylated sites	Semi- methylated sites	Total
Mexico	411	324	39(9.49%)	48(11.68%)	87(21.17%)
Sipsongpanna	411	289	58(14.12%)	63(15.33%)	121(29.44%)

3.2 Effect of nitrogen nutrition level on DNA methylation of *C. odorata*

DNA methylations obtained after treatment with different nitrogen levels are shown in Table 3. DNA methylations obtained after treatment with different nitrogen levels are shown in Table 2. At DNA "CCGG" of *C. odorata*, DNA methylation of both species after low nitrogen treatment was 19.23% on

average, that after normal nitrogen treatment was 26.29%, and high nitrogen treatment was 33.34%. According to the data, the different nitrogen supply level will affect the degree of DNA methylation, for both the indigenous and invasive species, the higher the nitrogen supply level, the higher the DNA methylation.

Table 2 DNA methylation in *C. odorata* from two area at three nitrogen levels by MSAP

Nitrogen level	Methylated sites in <i>C. odorata</i> from Mexico			Methylated sites in <i>C. odorata</i> from Sipsongpanna		
	Fully methylated sites	Semi-methylated sites	Total	Fully methylated sites	Semi-methylated sites	Total
Low nitrogen	39(9.49%)	27(6.57%)	16.06%	49(11.93%)	43(10.47%)	22.4%
Normal nitrogen	40(9.74)	52(12.66%)	22.4%	58(14.12%)	66(16.06%)	30.18%
High nitrogen	61(14.85%)	58(14.12%)	28.96%	76(18.50%)	79(19.23%)	37.73%

3.3 Changing pattern of DNA methylation of *C. odorata*

The DNA methylation patterns of the *C. odorata*. Demethylation at DNA "CCGG" of *C. odorata* with high nitrogen treatment accounted for 22.51%, 35.89% lower than that with low nitrogen treatment; However, in terms of methylation enhancement, *C. odorata* with high nitrogen treatment was higher than that with low nitrogen treatment, 40.21% and 31.03%, respectively; and the ratio of methylation patterns remaining unchanged at DNA "CCGG" were basically similar, 33.08% and 37.28%, respectively. There have been the changes of DNA methylation pattern in the *C. odorata* with high or low nitrogen treatment and normal nitrogen treatment.

4 DISCUSSION

Until now, there is no research report about the relevant between plant invasion and DNA methylation. This study shows that the DNA methylation polymorphism are significantly differ among the two *C. odorata* from their origins respectively. The DNA methylation extent of *C. odorata* from Sipsongpanna is significantly higher than that from Mexico. Only from this point of view, gene expression of the *C. odorata* in the invaded area is suppressed by the increase of methylation. According to the analysis of influence of different nitrogen levels on DNA methylation of *C. odorata*, high nitrogen level led to higher level of methylation, and more genes could not be expressed; low nitrogen level led to lower methylation level, and more genes are expressed, which is called demethylation. Whether they were *C. odorata* from different origins or undergone treatment of different nitrogen supply levels, the circumstances of increased and decreased methylation have occurred. Previous studies showed that methylation happening within in the internal and adjacent regions of the genes could suppress the expression of these genes, and demethylation then could activate the expression (Zhang *et al.*, 2010; Vanyushin and Ashapkin, 2011; Hernandez and Singleton, 2012). The successful invasion of the *C. odorata*, enhanced adaptability to the invaded area is a very important cause (Riis *et al.*, 2010; Lankau, 2013; Monty *et al.*, 2013). Epigenetic research showed that biological enhanced adaptability tends to be phenotype change rather than genotype change (Brock *et al.*, 2005, Weinig *et al.*, 2007). Therefore, successful invasion of *C. odorata* may result from suppression of gene expression due to methylation, or expression of certain genes due to demethylation's suppression relief, or the joint action of both methylation and demethylation. In order to thoroughly reveal the role

of methylation in plant invasion needs to be further identified genes which were methylated or demethylated.

In Liu Bao task group's MSAP analysis of the induced rice DNA methylation variance under low nitrogen level, it was demonstrated that significant decrease of rice DNA methylation happened when treated with low level of nitrogen and zero nitrogen, and the mode of decrease was mainly the lowering of CNG methylation level(Kou *et al.*, 2011). Similar results were obtained in this study. In addition, the demethylation proportion of *C. odorata* treated with low nitrogen was higher than that treated with high nitrogen, but enhancement proportion of methylation was lower. Our speculation is that more nitrogen will mobilize more carbohydrates to synthetize protein after entering plants; however, the carbohydrates that enter into the secondary metabolism pathways to synthetize "new weapon" allelochemicals will be greatly reduced, thus more secondary metabolism-related genes are turned off, resulting in increase of DNA methylation and decrease of demethylation ratio. This inference will also be proved by another of our findings to be published (i.e. low nitrogen treatment makes the allelopathy effect of *C. odorata* more prominent).

5 CONCLUSION

DNA methylation levels and modes of the two species *Chromolaena odorata* that undergone high nitrogen and low nitrogen treatments were changed, DNA methylation level of *C. odorata* invaded in Sipsongpanna was higher than that in the place of origin, and the higher the nitrogen supply level, the higher the DNA methylation.

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