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A COMPARISON OF GENOMIC METHYLATION LEVEL BETWEEN TWO POPULATIONS OF *IVA XANTHIFOLIA* (NUTT.)

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Abstract

Methylation levels of the two populations of *Iva xanthifolia* Nutt. from Harbin and Daqing areas were determined using methylation-sensitive-amplification-polymorphism (MSAP) method. The results showed that there was no significant difference of the overall level of DNA "CCGG" sites with and without methylation Harbin and Daqing population. The level of fully methylation sites was higher than that of hemi-methylation sites in both the groups of *I. xanthifolia*, specially the level of fully methylation of Daqing population was about three times more than that of level of hemi-methylation.

Introduction

Iva xanthifolia Nutt. (Asteraceae) a weed was originated in North America (Huszár 2011). In China, it was first found in the region of Dayingzi commune of Liaoning Province in 1981 (Xu *et al*. 2012), and then it was also found in other areas such as Shenyang in 1982. At present, it has spread in most parts of Liaoning Province and Heilongjiang Province, and became one of the alien invasive species in China. *I. xanthifolia* has strong adaptability, growth, competitiveness and reproductive capacity. No matter, in barren soils along roads or beside fertile dunghill, *I. xanthifolia* can grow (Hodi and Torma 2002, Huszár 2011). It can destroy the environment as well as can create hazardous effect on human health. During flowering, the plant spreads large amount of pollens in the air leading Hay fever among patients. People with sensitive skin also suffer from dermatitis when they come in contact to the *I. xanthifolia* leaves. Existing studies show that *I. xanthifolia* is more harmful than ragweed (Juhasz and Juhasz 2006, Milanova *et al.* 2010). *I. xanthifolia* invades soybean (*Glycine max*), maize (*Zea mays*), sunflower (*Helianthus annuus*) and sugar beet (*Beta vulgaris*) farmlands as weed and cause economic loss by decreasing the production. Though the adaptive capability of *Iva xanthifolia* is extremely diverse (Jia 2007) yet its distribution is uneven. Wild populations follow contagious distribution. For example, in Harbin city of Heilongjiang Province, *I. xanthifolia* mostly distributes in Dianlan Street, Wanggang Town, Chenggaozi Town and both sides of the roads along the Sun Island. Every single population has its uneven size, distribution and density (Xu *et al.* 2012).

According to the survey samples, certain phenotypic differences in different population were found. This indicates the presence of phenotypic plasticity i.e., different phenotypes from the same genotype response to different environment (Bradshaw 1965, Pigliucci 2001). Baker (1974) suggested that phenotypic plasticity might be an important feature of some invasive species. Rejmanek (1996) constructed the general model of seed plants invasion ability, in which phenotypic plasticity was also included. According to him phenotypic plasticity could enhance the

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invasion ability directly or indirectly. Among the proposed hypotheses regarding the invasion mechanism of exotic plants, phenotypic plasticity and genetic differentiation are considered to be responsible for the adaptation to heterogeneous habitats. For those plants which are low genetic diversity but have the diverse habit at the same time, the positive correlation between phenotypic plasticity and invasion ability could be a universal law rather than an exception (Geng *et al.* 2004).

In order to explore the mechanism existing in phenotypic differences of different population of *I. xanthifolia*, two population one from Harbin City and another from Daqing City were selected as the study objects. The method of methylation sensitive amplification polymorphism (MSAP) was used to analyze the DNA methylation level in *I. xanthifolia* population.

Materials and Methods

Iva xanthifolia population from two different places namely, Shanxima village of Shuangyushu town in Daqing city and Wanggang town in Harbin city were used as test material for the present investigation. Young leaves from top of the branches of 10 randomly selected plants were collected separately from the above mentioned two localities. Samples from Shanxima village were collected on September 7, 2011 and those from Wanggang Town on September 11, 2011. Leaf samples collected from 10 different plants for each habitat were mixed together.

The genome DNA was extracted using CTAB method and preserved at –20°C. Methylation sensitive amplification polymorphism (MSAP) analysis was done following Xiong (1999). The DNA samples were digested by two combinations of restriction endonuclease *Eco*R I*/Hpa* II and *Eco*R I/*Ms*p I, and then connected with adapter. Enzyme digestion system: 300 ng DNA, 10U *Eco*R I, 5U *Hpa* II (or 5U *Msp* I), digested 6 h at 37°C, using agarose gel electrophoresis inspecting the digesting effect. Connection system: the digestion products 10 µl, T4 DNA ligase 2 U, 1×T4 reaction buffer, 5 pmol *Eco*R I adapter, 50 pmol *Hpa* II/*Msp* I adapter, 10 mg/l BSA, added in a total volume of 20 µl. The ligation mixture was incubated overnight at 16°C, and was preserved at –4°C in a refrigerator. Adapters with 100°C modified 5 min and slow cooling to room temperature before use.

PCR pre-amplification system: 1 × PCR reaction buffer, 5 nmol dNTPs, each pre-amplification primer 5 pmol, 1U Taq DNA polymerase, digested ligation products 2 µl, added in a total volume of 20 µl. 29 cycles of thermal profile: 95°C for 45s, 56°C for 45s, 72°C for 60s. The pre-amplification DNA was diluted 1:20 in TE buffer and used 2 µl as template for selective amplification, each selective amplification primer 5 pmol, other components were the same with pre-amplification system. The system was amplified with the thermal cycle profile: the 16 cycles of 94°C for 45s, 65°C (decreasing by 0.7°C) for 45 s and 72°C for 80 s, followed by 26 cycles of 94°C for 45s, 55°C for 45s and 72°C for 80s. Selective amplification primer were respectively eight *Eco*R I primers and eight *Hpa* II/*Msp* I primers, a total of 64 primer combinations. The bands of the final eight for primer combinations were clear, good repeatability, for analysis. Selective amplification products were separated on a 6% polyacrylamide gel and detected by silver stain.

The restriction enzymes *Eco*R I, *Hpa* II and *Msp* I were bought from New England Biolabs (NEB), the DNA ligase and polymerase were bought from TaKaRa Company (Dalian City), the primers and adapters were synthesized by Sangon Company (Shanghai City).

The clear bands from polyacrylamide gel carried on the statistics, having band is marked as 1, no bands are marked as 0. The bands were classified on the basis of reaction to the restriction enzumes and are divided into three types namely, A-type: *Hpa* II and *Msp* I have bands, no methylation or medial cytosine hemi-methylation, marked as (1,1); B-type: *Hpa* II has bands, *Msp* I has no bands, lateral cytosine hemi-methylation, marked as (1, 0); C-type: *Hpa* II has no band, *Msp* I has bands, medial cytosine methylation, marked as(0, 1) (Ashikawa 2001).

Results and Discussion

Based on data of all primers selective amplification was summarized in Table 1. According to Table 1, on the "CCGG" sites of the Hrb1 plant DNA, the overall level of non-methylation is 73.83%; the overall level of methylation is 23.05%, in which the level of hemi-methylation sites is 12.01% and the level of fully methylation sites is 11.04%. On the "CCGG" sites of the Hrb2 plant DNA, the overall level of non-methylation is 58.33%; the overall level of methylation is 35.26%, in which the level of hemi-methylation sites is 14.72% and the level of fully methylation sites is 20.54%. On the "CCGG" sites of the Hrb3 plant DNA, the overall level of non-methylation is 64.34%; the overall level of methylation is 27.12%, in which the level of hemi-methylation site is 13.95% and the level of fully methylation sites is 13.17%. On the "CCGG" sites of the Hrb4 plant DNA, the overall level of non-methylation is 61.24%; the overall level of methylation is 32.16%, in which the level of hemi-methylation site is 8.52% and the level of fully methylation site is 23.64%. On the DNA "CCGG" sites of those four plants (Hebs), the overall level of non-methylation is 64.38%; the overall level of methylation is 29.40%, in which the level of hemi-methylation site is 12.30% and the level of fully methylation site is 12.30%.

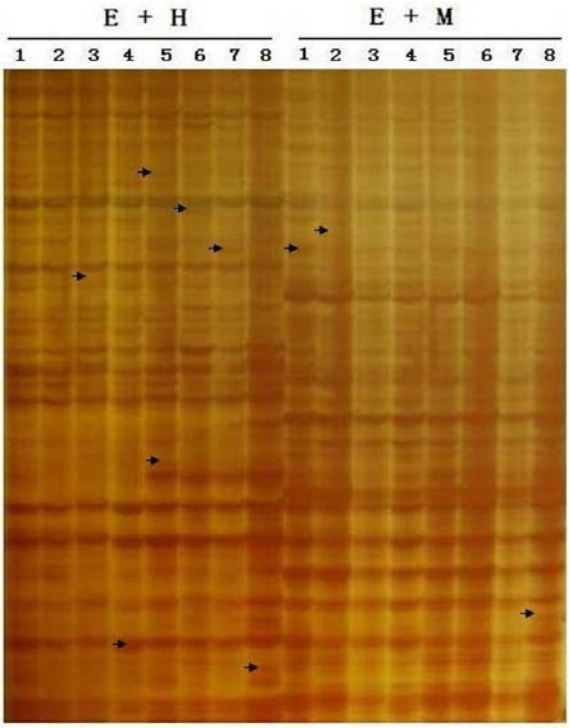

Fig. 1. The result of selective amplification with primer combination of E_G and H/M_8. 'E+H' is the samples digested with *Eco*RI/*Hpa*, 'E+M' is the samples digested with *Eco*RI/*Msp*. The bands in channel 1~4 were amplified from the samples of Harbin City, marked as Hrb1, Hrb2, Hrb3 and Hrb4; the bands in channel 1~4 were amplified from the samples of Daqing city, marked as Dq1, Dq2, Dq3 and Dq4.

On the "CCGG" sites of the Dq1 plant DNA, the overall level of non-methylation is 64.34%, overall level of methylation is 27.89%, in which the level of hemi-methylation sites is 8.13% and the level of full methylation sites is 19.76%. On the "CCGG" sites of the Dq2 plant DNA, the overall level of non-methylation is 64.72%, overall level of methylation is 29.45%, in which the

level of hemi-methylation sites is 9.30% and the level of full methylation sites is 20.15%. On the "CCGG" sites of the Dq3 plant DNA, the overall level of non-methylation is 60.85%, overall level of methylation is 30.99%, in which the level of hemi-methylation sites is 8.13% and the level of full methylation sites is 22.86%.On the "CCGG" sites of the Dq4 plant DNA, the overall level of non-methylation is 70.93%, overall level of methylation is 23.25%, in which the level of hemi-methylation sites is 4.65% and the level of full methylation sites is 18.60%. On the DNA "CCGG" sites of those four plants (Dqs), the overall level of non-methylation is 65.21%; the overall level of methylation is 27.90%, in which the level of hemi-methylation site is 7.55% and the level of fully methylation site is 20.34% (Figs 2, 3).

Table 1. Methylation level of CCGG site detected by MSAP.

Plant No.	Total No. of bands	No methylated CCGG sites	Methylated CCGG sites		
			Hemi-methylated sites	Full-methylated sites	Total
Heb1	516	381	62	57	119
Heb2	516	300	76	106	182
Heb3	516	332	72	68	140
Heb4	516	316	44	122	166
Total	2064	1329	254	353	607
Dq1	516	332	42	102	144
Dq2	516	334	48	104	152
Dq3	516	314	42	118	160
Dq4	516	366	24	96	120
Total	2064	1346	156	420	576

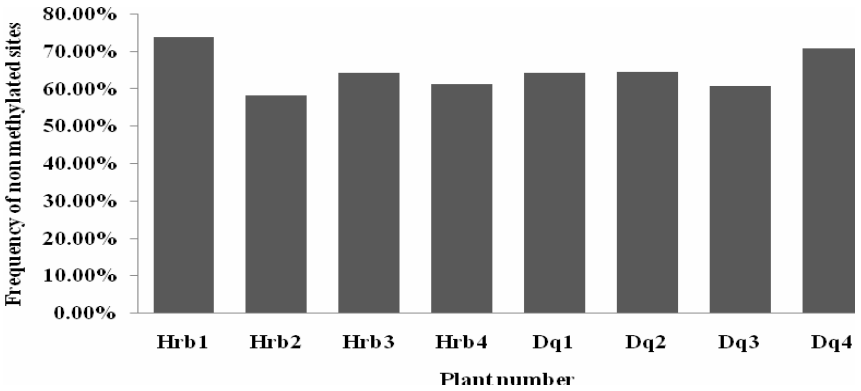

Fig. 2. The frequency of non methylated sites in all plants.

So far there is no such research report relates plant invasion to DNA methylation. Most studies focused on phenotypic plasticity, discussing how phenotypic plasticity and genetic differentiation enhanced the invasion capability of exotic plants. The AFLP analysis of *Ageratina*

adenophora's genetic diversity and genetic structure studied by Huang Wenkun indicated that with spreading eastward and northward, the genetic diversity of plants in new invasion area gradually reduced (Huang *et al.* 2007). Generally, speaking for invasion plants, going through bottle neck effect and genetic drift leads to the decrease of genetic diversity, which reduces the adaption ability to new environment (Lee 2002). For instance, it has been found that the phenomenon of genetic diversity decrease exists in the exotic invasion plant *Rubus alceifolius* (Amsellem *et al.* 2000).

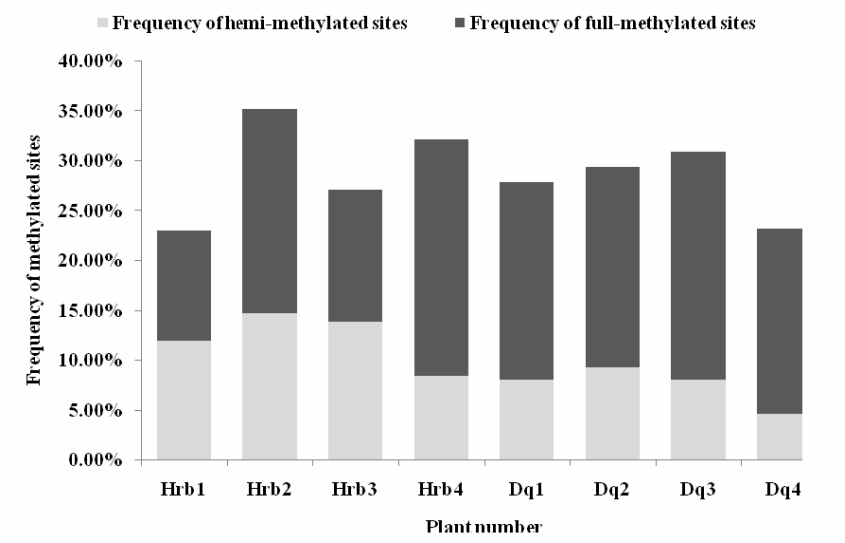

Fig. 3. The frequency of hemi-methylated sites and full-methylated sites in all plants.

Both groups of *Iva xanthifolia* have difference in DNA methylation polymorphisms. Comparing the DNA methylation level of *Iva xanthifolia* genome between two cities, Harbin and Daqing, the overall level of DNA "CCGG" sites without methylation or with methlation has no difference, while the level of hemi-methylation sites and fully methylation sites have considerable difference between the Harbin and the Daqing. Moreover, no matter area the level of fully methylation sites is higher than the level of hemi-methylation sites. However, in Harbin city, the rate of fully methylation to hemi-methylation in *Iva xanthifolia* is approximately 1, which means that fully methylation sites approximately equal to hemi-methylation sites. In Daqing, the rate of fully methylation to hemi-methylation in *Iva xanthifolia* is approximately 3, which means that fully methylation is as 2.7 times as hemi-methylation. The cause of the methylation difference may account for the difference of the soils. The soil in Shanxima village of Daqing is alkaline-saline, while the soil from Wanggang town in Harbin city is black soil, *Iva xanthifolia* may probably enhance the adaption to environment by changing the level of methylation.

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