

Comparative genetic and epigenetic of the *Wedelia trilobata* (L.) from different regions

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Abstract

Background

Wedelia trilobata (L.) Hitchc., is a common and widespread invasive plant in South China. In order to further explore the molecular mechanisms of its rapid adaptation, we selected DNA methylation-sensitive amplified polymorphism (MSAP) and simple sequence repeats (SSR) to analyze 60 *W. trilobata* individuals from Fuzhou (FZ), Haikou (HK), Jinghong (JH) and Guangzhou (GZ).

Results

In this study, The Shannon diversity index (*H'*) of SSR and MSAP Nei are 0.236, 0.184; the average methylation rate of *W. trilobata* from the four regions is 18.25% – 32.92%, indicating that *W. trilobata* is highly methylated. In the unweighted pair group method with an arithmetic mean (UPGMA) phylogenetic tree, all populations in the MSAP are clustered together. The geographical distance between HK and JH is the closest, and GZ is the farthest from the other three populations. Epigenetic distance is more susceptible to geographical distance, as the correlation between epigenetic distance and geographical distance is significantly higher than that between genetic distance and geographical distance by Mantel analysis.

Conclusions

The result reveal that *W. trilobata* populations in different regions have high genetic and epigenetic diversity, as well as high methylation levels, in which epigenetic mechanisms play a stronger role.

1 Background

Wedelia trilobata (L.) Hitchc., belongs to the Asteraceae, a perennial herb native to tropical South America (Zhang et al., 2020). It had been listed as one of the 100 most invasive alien species in the world (IUCN, 2001). It was used widely in landscape greening because of its rapid reproduction, strong survival adaptability, and easy coverage. Introduced to China as a ground cover plant in the 1970s, *W. trilobata* quickly fled to the wild after settlement, and then developed and spread rapidly in southern China (Song et al., 2010). Previous studies found that *W. trilobata* can inhibit the growth and development of surrounding species, significantly threaten the local biodiversity, and cause the imbalance of local ecological diversity, due to its rapid coverage of occupied open spaces through allelopathic therapy and asexual reproduction (Qi et al., 2014; Dai et al., 2016). Since its stem can directly develop into a complete plant, it can also invade other remote areas through water flow or human activities (Sun et al., 2015). At present, its distribution is recorded in Guangdong, Guangxi, Fujian, Hainan, Yunnan and other provinces, mostly in parks, scenic spots, riversides, roadsides, fields, etc.

The key decisive factors of the establishment and spread of invasive species may be genetic and evolutionary processes, because invasive species must adapt to their new environments to survive and reproduce (Sakai et al., 2001), and genetic variation plays a major role in the process of plant adaptation (Henderson and Salt., 2017). Genetic diversities of high level are found in many invasive species and are associated with their successful invasion (Muller-Scharer et al., 2004). For example, the South American weed *Bidens pilosa* is introduced from Tianjin and spread rapidly in northern China. Its sexual reproduction and multiple introductions maintain high genetic diversity and promote its local adaptation and invasion (Ma et al., 2011). Due to the extremely low survival rate of its seed in the wild, it is assumed that the spread of *W. trilobata* mainly depends on its clonal reproduction. As a result, its clonal reproduction may reduce genetic biodiversity and cannot maintain high genetic diversity. However, epigenetics can provide clonal plants with more adaptive strategies. Epigenetics is one of the best example for cloning plants to replace the slow adaptation mechanism of natural selection. Epigenetics depends to varying degrees on asexual reproduction and preserves or reverses gene ability to buffer plants against current challenges and future rapid environmental changes (Duhovnikoff et al, 2015). In addition, epigenetic modification is also found to be genetically stable across generations (Gáspár et al., 2019), thereby transferring more favorable phenotypic variations to future generations. Epigenetics itself is a phenotypic trait, and its evolution may be driven by natural selection, indicating that in natural populations, epigenetics and genetic variation are two intertwined rather than independent evolutionary factors (Herrera et al., 2014; Bossdorf et al., 2008; Fujimoto et al., 2012). DNA methylation, RNA interference, and histone modification are considered to be the important components of epigenetic regulation. Among them, cytosine methylation is the most important epigenetic regulation strategy and plays a central role in the epigenetic control of gene expression (Angers et al., 2010; Law et al., 2010).

Due to the advantages of abundant multiple alleles, high polymorphism and dominance, simple sequence repeat (SSR) is a powerful tool for studying genetic diversity (Powell et al., 1995). DNA methylation is one of the most common epigenetic modifications. It often occurs at the CG site in the promoter region of DNA sequence (Richards et al., 2017). DNA methylation-sensitive amplified polymorphism (MSAP) can be directly used to assess the methylation status of randomly distributed genomic cytosines. Since it does not require sequencing of the reference genome, this method is the most widely used analytical epigenetics for detecting cytosine methylation changes in the ecological environment (Schrey et al., 2013). The previous researches related to *W. trilobata*, more focus on the its physiology under different environmental factors, and there are few explorations based on genetics and epigenetics (Huang et al., 2022). This study aims to explore the genetic and epigenetic structures and differences among populations of *W. trilobata* under different habitats by using molecular markers SSR and MSAP.

2 Materials And Methods

2.1 Collection of Materials

The current distribution records of *W. trilobata* were collected, Maxent software as well as Arcgis was used to predict contemporary suitable areas, and samples were collected in high suitable areas. Environmental factor data were collected from China Meteorological Administration (<http://www.cma.gov.cn/>). In this study, a total of 120 samples from 4 different regions were collected in the highly suitable areas alone. 30 samples were collected from each region (Table 1). The detailed sampling method we applied was as follows: 1) three quadrats were randomly selected from each region and ensure each quadrat is more than 2 km apart; 2) ten *W. trilobata* plants were randomly selected from each quadrat and ensure each plant is more than 50 m apart; 3) After the mature leaves of the third pair were taken from each selected plant; 4) 30 samples of the mature leaves were collected and dried in the numbered silica gel from each region. DNA of each sample was extracted and stored at -20 °C. DNA extraction were extracted using plant genome DNA extraction kit (Tiangen).

Table1 The information and environmental factors of sampling sites of *Wedelia trilobata* from different regions

Population	Longitude	Latitude	Altitude (m)	Average annual temperature (°C)	Highest temperature (°C)	Lowest temperature (°C)	Average annual sunshine hours (h)	Average annual precipitation (mm)	Maximum daily precipitation (mm)	Average annual relative humidity (%)
FZ	119°18'	26°4'	84.00	19.68	38.21	1.32	1654.43	1374.36	103.81	74.58
JH	101°14'	21°55'	574.29	18.15	32.95	2.03	1994.19	1442.25	82.29	77.80
HK	101°15'	19°57'	46.68	37.31	7.67	24.13	83.50	1689.70	1982.64	167.64
GZ	113°27'	23°13'	18.00	22.06	36.78	2.63	1686.15	1766.05	126.53	77.42

Note: The data is the average from 1952 to 2016

2.2 SSR Method

Based on the transcriptome data, six pairs of SSR primers were used for capillary electrophoresis. The volume of Polymerase Chain Reaction (PCR) system was 10 µL, including 1 µL of DNA template, 5 µL of 2 × Taq PCR Master Mix, 0.5 µL of upstream primers (the 5' end of the upstream primer was labeled with phosphorescent dye during fluorescence PCR), 0.5 µL of downstream primers and 3 µL of ddH₂O. The PCR conditions were set as follows: 95°C, 3 min; 34 cycles of (95°C, 30 s; The annealing temperature is the annealing temperature of each primer, 30 s; 72°C, 60 s); and a final extension at 72 °C for 5 min, and finally stored at -20 °C. In the end, PCR products were separated by 6% non-denaturing polyacrylamide gel electrophoresis (Liu et al., 2016).

2.3 MSAP method

Using EcoRI and two restriction sites 5'-CCGG-3', restriction endonucleases MspI and HpaII with different sensitivity to methylation were cut at 37 °C for 12 h. DNA digested products were connected and amplified by PCR. After 2% agarose gel detection, 8 pairs of primers were selected for PCR selective amplification. The selective PCR products were detected with 6% polypropylene gel, and then the color was observed by silver staining. There were four cytosine methylation patterns in DNA (Table 2). Type I: sites with the presence of bands in both EcoRI/HpaII and EcoRI/MspI, namely (1.1), it represents CCGG site was unmethylated. Type II: bands present in EcoRI/HpaII, but absent in EcoRI/MspI, namely (1.0), indicating that the external cytosine site of only one DNA chain was methylated, which was also known as the semi-methylation of CCGG site. Type III: bands present in EcoRI/HpaII, but absent in EcoRI/MspI, namely (0.1), stands for the internal cytosine of the double DNA chain was methylated, which was also known as the complete methylation of CCGG site. Type IV: bands has not present in EcoRI/HpaII and EcoRI/MspI, namely (0.0). The markers of type IV mode (0.0) were treated as hypermethylated sites in this study followed Gupta et al. (2012) and Schulz et al. (2013).

2.4 Data Analysis

SSR bands of *W. trilobata* were counted by numbers, where "1" standing for a band and "0" standing for no band. MSAP bands of *W. trilobata* were counted by numbers, where "1" indicating DNA methylation, and "0" indicating non-methylation. The genetic diversity was calculated using Popgene software, and the phylogenetic trees of SSR with MSAP were constructed using MEGA7. NTSYS software was used to conduct evolutionary tree analysis; Pcoa analysis was performed on the results. Furthermore, GenAlex software was used for mantel test analysis and AMOVA analysis. The R package (4.1.2) and Python (3.10.5) were used to data analysis and figure drawing.

Table 2 DNA methylation band types

The type of bands		DNA methylation status	DNA methylation patterns
H	M		
1	1	Non-methylation	
1	0	Hemi-methylation	
0	1	Full-methylation	
0	0	Hypermethylation	

Note: H=EcoR +Hpa M=EcoR +Msp.

3 Result And Analysis

3.1 Genetic Diversity and Epigenetic Genetic Diversity Analysis

The numbers of alleles (Na) observed by SSR ranged from 1.656 to 1.750, while the number of effective alleles (Ne) range from 1.319 to 1.530. The average values of Na and Ne were 1.703 and 1.406, respectively. In MSAP, Na ranges from 1.689 to 2.000, while the Ne ranges from 1.148 to 1.344, with the average values of 1.798 and 1.275, respectively. The average values of gene diversity (H) and the Shannon diversity index (I) of SSR and MSAP Nei were 0.236, 0.184 and 0.354, 0.303, respectively, with little variance (Table 3). In summary, the genetic diversity of population in HK was the highest compared with other populations, key fact to remember its epigenetic diversity was significantly lower than that of the other three populations. The genetic diversity of JH is significantly lower than that of the other three regions. In the genetic distances (Table 4), HK and FZ were the closest, while GZ and JH were the farthest. The epigenetics of JH and FZ were the most recent, while JH and HK were the furthest ones.

Table 3 Genetic and epigenetic diversity analysis on the four *Wedelia trilobata* populations from different invasive areas

Population		PIC	Na	Ne	H	I
FZ	genetic	75.00%	1.750	1.375	0.228	0.349
	epigenetic	83.47%	1.835	1.344	0.224	0.357
HK	genetic	65.62%	1.656	1.319	0.193	0.297
	epigenetic	60.33%	1.603	1.148	0.101	0.174
JH	genetic	68.75%	1.688	1.399	0.232	0.348
	epigenetic	75.21%	1.752	1.327	0.207	0.326
GZ	genetic	71.88%	1.719	1.530	0.291	0.421
	epigenetic	100.00%	2.000	1.279	0.206	0.353
SSR Mean		70.30%	1.703	1.406	0.236	0.354
SSR Species level			1.969	1.528	0.313	0.472
MSAP Mean		79.75%	1.798	1.275	0.184	0.303
MSAP Species level			2.000	1.457	0.281	0.440

Table 4 The distance of genetic and the distance of epigenetic genetic within populations

genetic distance					epigenetic distance				
Population	FZ	HK	JH	GZ	Population	FZ	HK	JH	GZ
FZ	***	0.875	0.870	0.134	FZ	***	0.906	0.962	0.837
HK	0.134	***	0.788	0.142	HK	0.099	***	0.018	0.647
JH	0.139	0.238	***	0.078	JH	0.039	0.983	***	0.722
GZ	0.874	0.868	0.925	***	GZ	0.178	0.436	0.325	***

Note: Nei's genetic/epigenetic distance (left bottom) and genetic/epigenetic identity (top right).

3.2 Phylogenetic Tree

It was depicted in the Fig.1 that most samples from HK and GZ regions were obviously clustered into independent branches, and samples from JH and FZ regions were partially intertwined. The cluster of MSAP molecular markers was more independent compared with SSR in each region. Presumably, this may be due to the fact that MSAP amplifies more specific sites than SSR.

3.3 PCoA and AMOVA Analysis

The main coordinate (PCoA) results showed that the JH and GZ populations were close based on SSR , but they were separated from the FZ population (Fig.2a). The PCoA were consistent with the results from UPGMAclustering. Based on MSAP, FZ, HK and JH populations were close, the second axis separated the three populations from GZ (Fig.2b).

3.4 Mantel Test

Mantel analysis of relationship between geographical distance and genetic or epigenetic distance showed that the R values were 0.0093 and 0.475, respectively, both with the 0.01 P values (Fig. 3a, 3b). The Mantel analysis of relationship between genetic and epigenetic distances showed that the R value were 0.2 (Fig. 3c), suggesting that a stronger correlation as a contrast to genetic distance.

3.5 AMOVA analysis

AMOVA analysis (Table 5) revealed that the epigenetic fixation index (Fst) of *W. trilobata* in different regions was 0.475, which was higher than genetic fixation index (0.277). It was suggested that the differentiation among populations was mainly attributed to epigenetic. Genetic and epigenetic gene flow (Nm) were 0.652 and 0.276, respectively, which were less than 1, indicating that gene flow among *W. trilobata* populations in different regions was limited by geographical distance.

Table 5 AMOVA analysis of Carex accessions

Source		df	SS	MS	Est. Var.	%	Fst	Nm	P value
SSR	Among Pops	3	141.233	47.078	1.529	18%	0.277	0.652	0.001
	Among Indiv	56	67.800	1.211	0.000	0%			
	Within Indiv	60	406.000	6.767	6.767	82%			
	Total	119	615.033		8.296	100%			
MSAP	Among Pops	3	3806.350	1268.783	40.222	47%	0.475	0.276	0.001
	Among Indiv	56	3478.133	62.110	17.646	21%			
	Within Indiv	60	1609.000	26.817	26.817	32%			
	Total	119	8893.483		84.686	100%			

3.6 Mode Structure of Epigenetic Regions

The total methylated rates in the four populations (Fig. 4) were 23.82 %, 18.25 %, 32.92 % and 27.55 %, respectively; the fully methylated rates were 16.25 %, 11.51 %, 25.75 % and 24.43 %, respectively; the hemi-methylation rates were 7.57 %, 6.75 %, 7.16 % and 3.12 %, respectively; moreover, the non-methylated rates are 76.18 %, 81.75 %, 67.08 % and 72.45 %, respectively.

4 Discussion

4.1 Genetic and Epigenetic Diversity

In this study, the Shannon Diversity Indexes (I) of genetic diversity and epigenetic diversity in *W. trilobata* were 0.472 and 0.440, respectively. By contrast, the indexes in *Thlaspi arvense* (field pennycress) were only 0.12 and 0.11 (Guan et al, 2020), whereas in barley the indexes were 0.637 and 0.639 (Li et al, 2021). These results showed that *W. trilobata* has relatively higher genetic and epigenetic diversity.

The Shannon Diversity Index of genetic and epigenetic diversity in HK was low. The reason could be HK population was located in Hainan Province, an independent island, resulting in less frequent gene exchange between HK population and the other populations. As the earliest region in China to introduce *W. trilobata*, Guangzhou has the longest time for the formation of its escape zone and natural community, which may be the reason for its highest genetic diversity. In the process of clonal reproduction, plants evolve complex adaptation with defense mechanisms through potential epigenetic traits due to the stress they feel from the environment, and are genetically mediated to produce phenotypic changes called phenotypic plasticity. Studies had shown that invasive species tend to had higher phenotypic plasticity than non-invasive or native species, even that populations of invasive species in invasive regions were able to evolve greater plasticity than native populations (Davidson et al., 2011). The FZ population had the highest epigenetic diversity, it may be due to the unique distribution of samples in FZ area. Farther distribution between different populations and denser the distribution

within the population, would bring on greater epigenetic differentiation between different populations. However, the distribution of samples in other areas was more uniform.

As for the UPGMA phylogenetic tree, all populations were clustered together based on MSAP. The geographical distance between HK and JH population was the closest, while GZ population was the farthest from the three others. Since Guangzhou was the first region in China to introduce cultivation, that is, its population had undergone a long stage of adaptive evolution, its epigenetic degree was quite different from other regions that were later introduced for cultivation.

4.2 Comparison of the different populations in genetic distances and epigenetic distance with geographical

Although GZ and HK populations were relatively independent in SSR, they had the closest geographical distance and close genetic distance. Mantel's analysis showed that the correlation between epigenetic distance and geographical distance was significantly higher than that between genetic distance and geographical distance, which signified that epigenetic distance was more susceptible to geographical distance. In addition, the Fst results suggested that epigenetics had a higher differentiation than genetic. Herrer et al (2017) found that the differences in functional traits of *Helleborus foetidus* were related to environment and epigenetic distance. Pagel et al (2020) observed a strong correlation between genetic distance and epigenetic distance in *Linum catharticum* from dry and wet grasslands. The results proved the environmental influences played an important role at the genetic and epigenetic levels. In this study, the genetic and epigenetic distances in *W. trilobata* just showed significantly weakly correlated, where the habitats of our four regions were not extreme opposition. Besides, Ne, H, I and altitude were significantly positively correlated in genetic, as well as PIC, Na, Ne, H, and I in epigenetic were all positively correlated with the highest temperature. As a consequence, environmental factors had a certain degree of influence on genetic and epigenetic level.

4.3 Methylation Status

The methylation rate in plants usually ranges from 20 % to 40 % (Portis et al., 2004). In this study, the average methylation rate of *W. trilobata* from four regions was 18.25 % - 32.92 %. The results manifested that *W. trilobata* was significant differences in methylation among the four populations. The methylated DNA can make chromatin extremely compact (Fletcher et al., 2013), which may reduce genetic exchange, resulting in the reduction of polymorphic bands. Moreover, type IV (0 0) was an unintuitive amplification site, so the number of methylation sites detected by MSAP was lower than the actual number, in other word, the actual methylation level of *W. trilobata* may be higher. The total methylated rate (%) and fully methylated rate (%) were both the highest in HK population, whereas those in GZ population were the lowest. The hemi-methylated rate (%) was the highest in FZ population. The non-methylated rate was the highest in GZ population and the lowest in HK population. Overall, the methylation level of GZ population was significantly lower than other populations, indicating that there were some differences in methylation patterns between different populations. The reason may be related to local temperature and different habitats at sampling sites. In cloned plants, epigenetic modifications created new modules over time and accumulate them for adaptations (Verhoeven et al., 2014; Douhovnikoff., 2015). There was a lot of evidences that sexual reproduction and clonal reproduction can be effectively superimposed, with epigenetic regulation can participate in and regulate this clonal reproduction (Hand et al., 2014). For example, Wen et al (2018) found a large number of epigenetic differentiations in the clone population of *Alternanthera philoxeroides* in the same environment, which can be maintained until 10 generations before gradually disappearing, indicating that epigenetic variation was likely caused by environmental induction and spontaneous mutations. Guarino et al (2019) denoted on the invading clonal population *Arundo donax*, which showed that epigenetic diversity was higher than genomic diversity, suggesting its invasion under different environmental conditions was attributed to higher epigenetic variability.

5 Conclusion

As can be concluded from this study, *W. trilobata* populations in different regions have high genetic and epigenetic diversity, as well as high methylation levels. The correlation between epigenetic and geographical distance is significantly higher than that between genetic and geographical distance, suggesting epigenetic mechanism partly compensates for the restriction of gene exchange between populations due to its clonal reproduction. This paper further elucidates the molecular mechanism of rapid adaptation of *W. trilobata* in the invasion process.

Declarations

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Availability of data and materials

All relevant data can be found within the manuscript and its supporting materials.

Authors' contributions

L.S., Y.X. and X.C. designed the study. Y.X., X.C., Y.Y., J.Z., H.Y. collected the samples and performed the experiment. Y.X., X.C. performed data analysis. Y.X. drafted the manuscript. L.S. revised the manuscript and edited the language of the manuscript. All authors have read and agreed to the published version

of the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

All data analyzed during this study are included in the supplementary information fles.

Ethics approval and consent to participate

We promise that *Wedelia trilobata* (L.) Hitchc. material from all regions has been licensed for collection. The authors confirm that all methods comply with relevant institutional, national, and international guidelines and legislations, including the collection of plant materials for this study. The plant materials were collected from the regions of Fuzhou city, Fujian; Haikou city, Hainan; Jinghong city, Yunan; Guangzhou city, Guangdong by the authors (Y.X. and X.C.), and identified as *Wedelia trilobata* (L.) Hitchc. by Prof. Guowen Xie of GZHU. The voucher specimen (No# GZHU-SLY-2020-07-FZ; No# GZHU-SLY-2020-07-HK; No# GZHU-SLY-2020-07-JH; No# GZHU-SLY-2020-07-GZ) were deposited at the Herbarium of School of Life Sciences, GZHU. L.S. undertook the formal identification of the plant material used in our study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

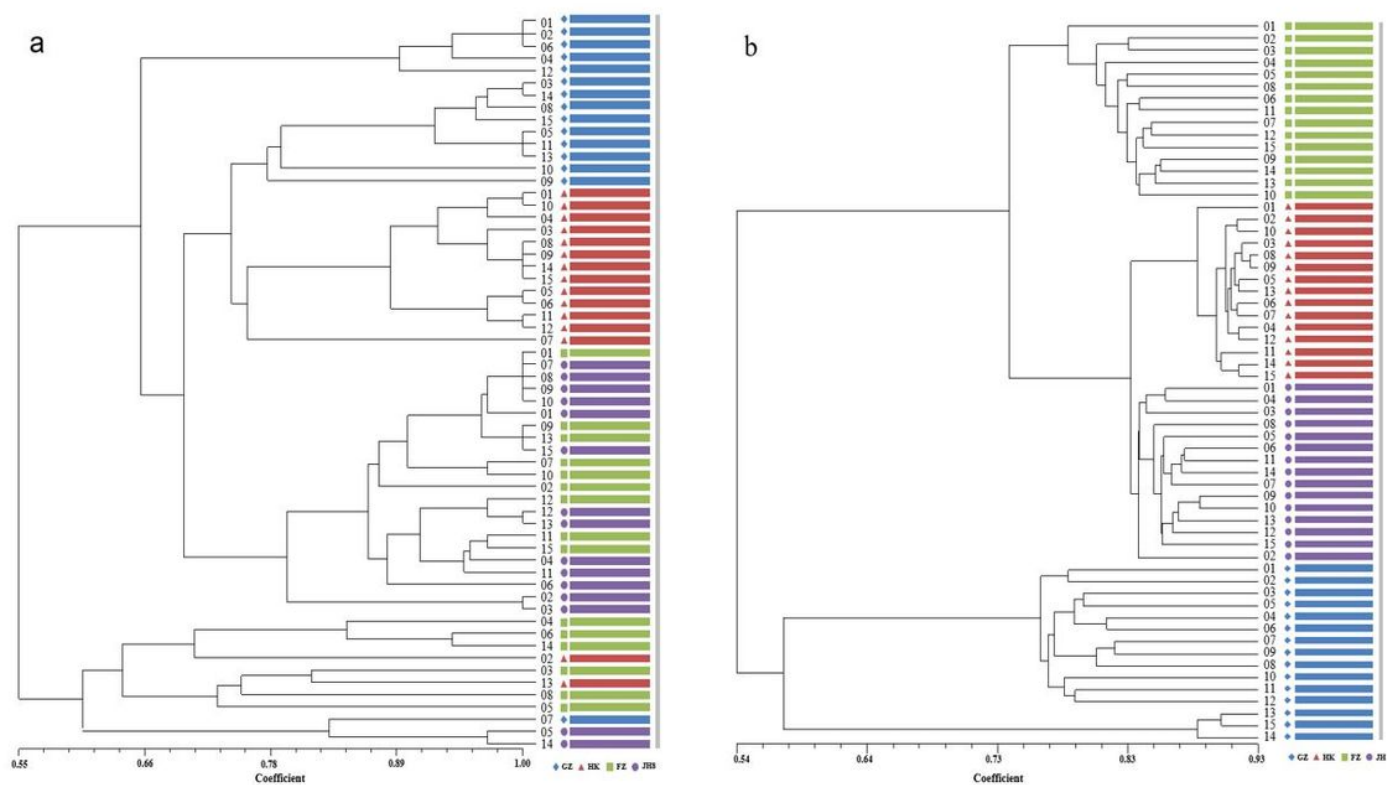


Figure 1

UPGMA clustering analysis of SSR(a) and MSAP(b)

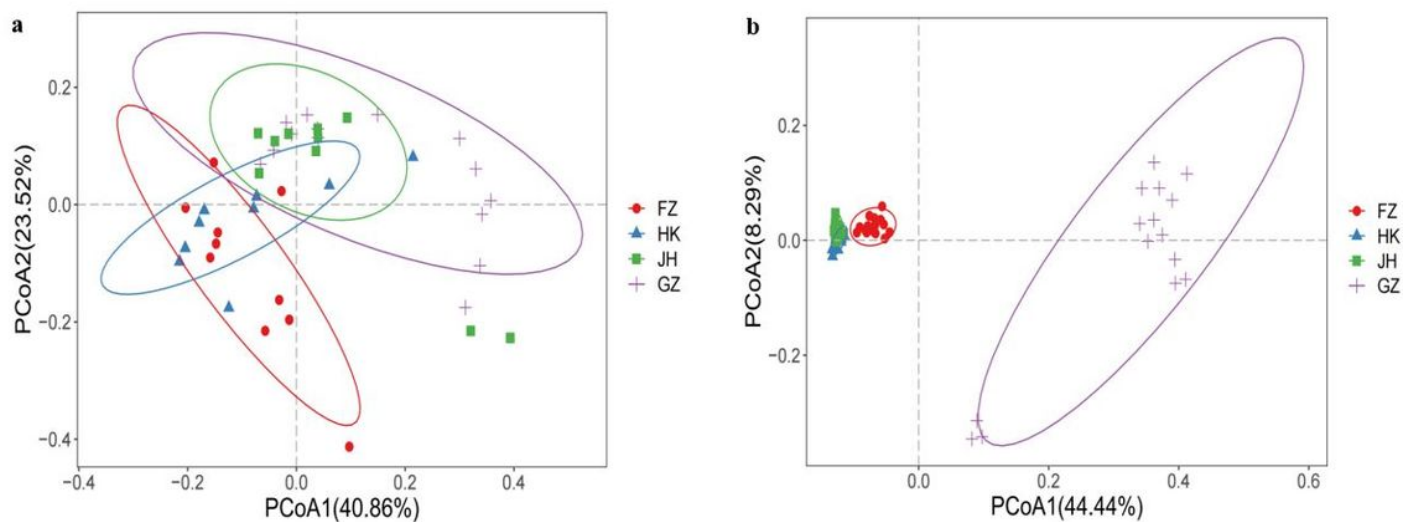


Figure 2

Cluster analysis showing the relationship among *Wedelia trilobata* based on SSR(a) and MSAP(b) data

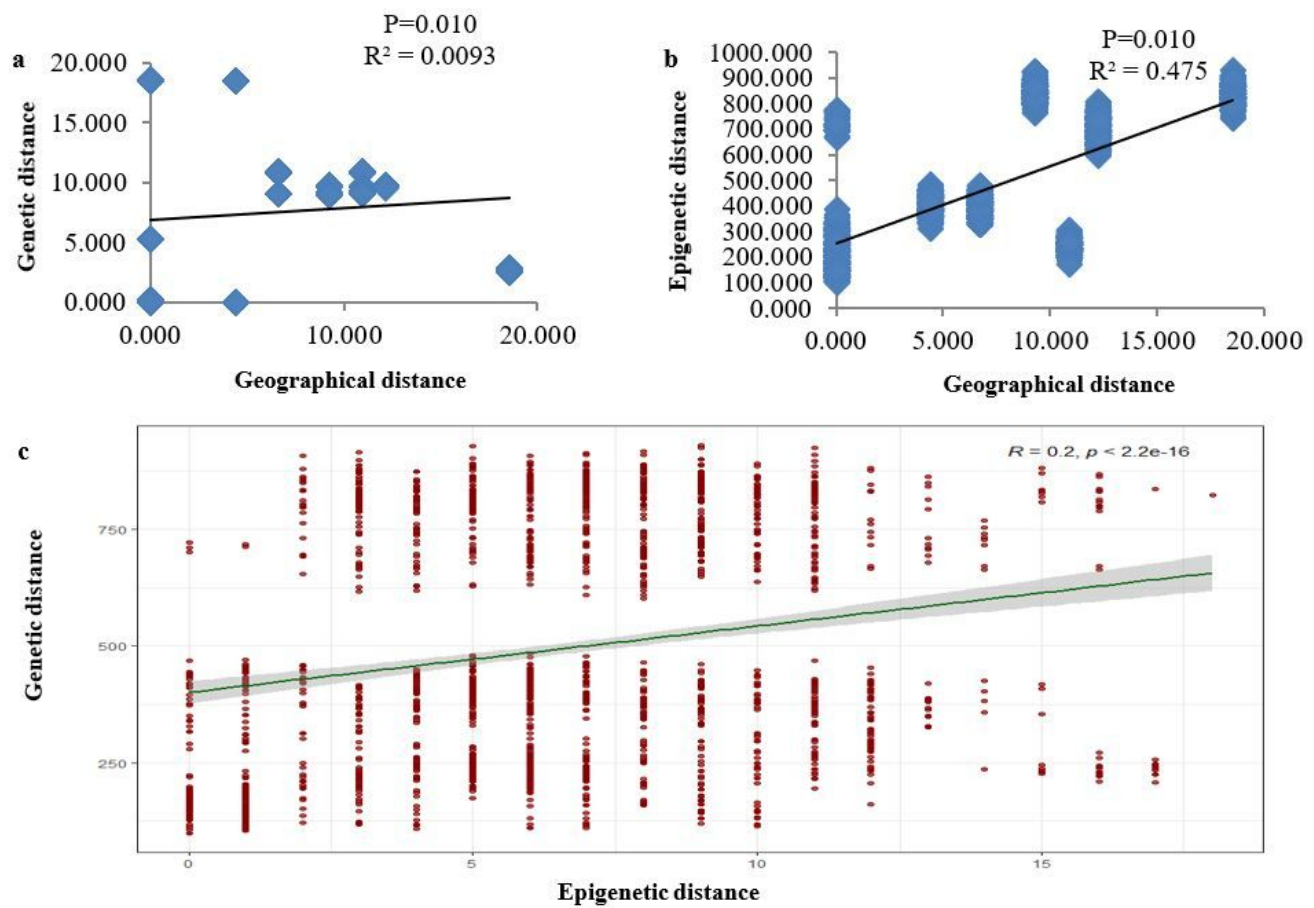


Figure 3

Genetic distance(a) and Epigenetic distance(b) of the all populations correlations to variation in Geographic,as well as calculate the correlation between Genetic and Epigenetic distances(c).

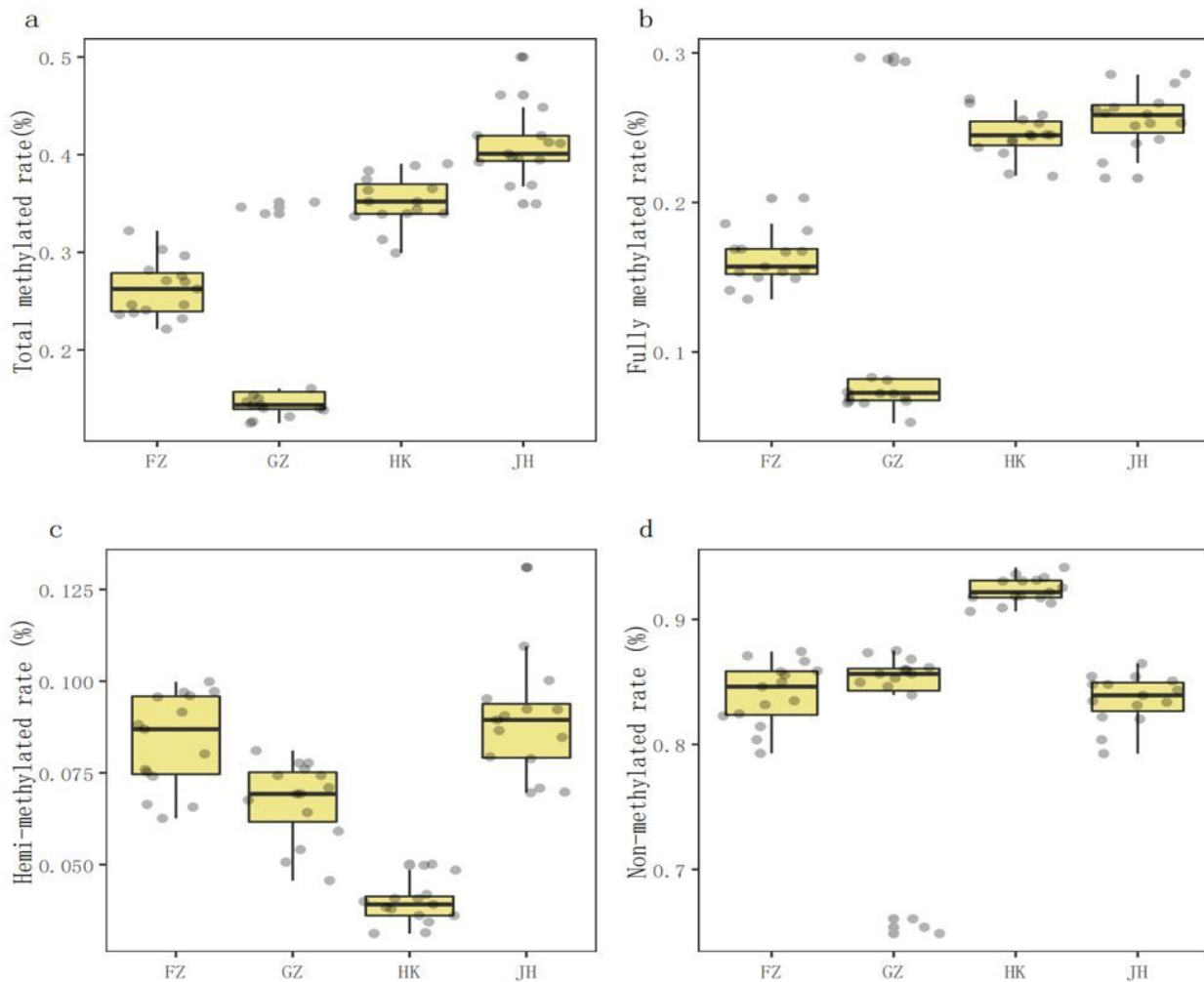


Figure 4

Comparison of *Wedelia trilobata* methylation status from different invasive areas

Note: Total methylated bands=I+II+III+IV; Full-methylated bands=III+IV;

Total methylated rate/% = $[(I + II + III + IV) / (I + II + III + IV)] \times 100\%$;

Fully methylated rate(%) = $[(III + IV) / (I + II + III + IV)] \times 100\%$;

Hemi-methylated rate (%) = $[(II) / (I + II + III + IV)] \times 100\%$;

Non-methylated rate (%) = $[(I) / (I + II + III + IV)] \times 100\%$.

Supplementary Files

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