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Application of SRAP and CDDP Markers in Genetic Diversity Analysis of *Morinda officinalis* How.

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Abstract The analysis of the main germ plasm resources of *Morinda officinalis* How. in Zhaoqing area and the definition of the relationship among the varieties will provide scientific basis for the cultivation, protection and identification of *Morinda officinalis* How.. SRAP and CDDP molecular markers were used to analyze the genetic diversity of 9 *Morinda officinalis* How. materials in Zhaoqing. The genetic similarity coefficients among samples were calculated by NTSYS-pc2.1 software, cluster analysis was carried out according to UPGMA method, and the results of two kinds of molecular markers were compared and analyzed. 28 pairs of SRAP primers and 16 CDDP primers were used to amplify 140 and 83 bands respectively, of which the number of polymorphic bands was 68 and 63 respectively, the polymorphism ratio (PPB) was 48.6% and 75.9% respectively, the genetic similarity coefficient varied from 0.69 to 0.96 and 0.53 to 0.95, and the average genetic similarity coefficient was 0.83 and 0.69 respectively. When the genetic similarity coefficient of SRAP was 0.86 and that of CDDP was 0.71, the four varieties of Guangning special type, Guangning special grafting type, local large leaf type and local lobular type were grouped into one group. SRAP and CDDP molecular markers can be used for genetic diversity analysis of *Morinda officinalis* How.. There are some differences between SRAP and CDDP, so the combined analysis of the two kinds of markers can more accurately reveal the genetic relationship between *Morinda officinalis* How. germplasm. The results of CDDP clustering were more consistent with those of combined analysis, indicating that CDDP was more suitable for genetic diversity analysis of *Morinda officinalis* How. than SRAP.

Keywords *Morinda officinalis* How.; SRAP; CDDP; Genetic diversity; Cluster analysis

Morinda officinalis How. is a woody vine medicinal plant of *Morinda* in Rubiaceae, which is used as medicine with its fleshy dry root because it's sweet in taste and mild in nature. *Morinda officinalis* How. not only has the traditional curative effects of invigorating kidney yang, nourishing yin, benefiting qi, dispelling wind and eliminating dampness, but also has modern pharmacological effects of anti-cancer, anti-aging, anti-depression, anti-fatigue, improving body's immunity, strengthening muscles and bones and so on, which is known as one of China's famous 'Four Southern Medicines' (He et al., 2018; Zhang et al., 2018; Song et al., 2018; Yun, 2019; Zhan et al., 2019). *Morinda officinalis* How. is mainly distributed in Guangdong, Guangxi, Fujian, Hainan and other provinces to the south of the Yangtze River in China. Deqing County in Guangdong Province is currently recognized as the traditional land of *Morinda officinalis* How. (Wang et al., 2016; Rao et al., 2018).

Molecular markers are genetic markers based on the DNA level, which are not affected by environmental factors and restricted by the growth cycle. And different molecular markers have their unique functions (Liu, 2013). Sequence-related amplified polymorphism (SRAP) is a new type of molecular markers based on PCR (Sun et al., 2010). SRAP use double primers to amplify open reading frames (ORFs) and detect their polymorphisms, which has the advantages of universal primers, simple operation, high codominance, and no need to predict the sequence information of the material (Zhang et al., 2011; Yang et al., 2016). Conserved DNA-derived polymorphism (CDDP) is a new marker technique that uses single primer to amplify target molecules for conserved sequences of plant DNA (Zhai et al., 2019). CDDP marker has the advantages of simple operation, high polymorphism and low

cost. Besides, its PCR products can be separated by agarose gel (Fang et al., 2016; Zhou et al., 2019). Studies have shown that CDDP has a strong ability to distinguish closely related varieties and can be widely used in the study of plant genetic diversity (Xing et al., 2017).

The germplasm resources of *Morinda officinalis* How. have been differentiated through long-term natural selection, artificial grafting and the influence of ecological environment in different cultivation areas. At present, only Ding et al. (2008) used RAPD molecular markers, Liu et al. (2011) used ISSR molecular markers and Wei (2017) used DNA barcode sequence to identify *Morinda officinalis* How.. Compared with other medicinal plants, the study on the molecular markers of *Morinda officinalis* How. is relatively backward. So far, there are no reports on SRAP and CDDP molecular markers of *Morinda officinalis* How..

Morinda officinalis How. has high requirements for planting environment and long growth cycle, so there is few artificial planting and it is in short supply on the market. In addition, the folk believe that wild *Morinda officinalis* How. has better medicinal effects, which has led to wild *Morinda officinalis* How. being excavated. In order to protect the germplasm resources of *Morinda officinalis* How., accurately identify its varieties, and cultivate excellent strains, SRAP and CDDP molecular markers were used to analyze the genetic diversity of 9 *Morinda officinalis* How. materials in Zhaoqing, which has important scientific significance.

1 Results and Analysis

1.1 Polymorphism analysis of *Morinda officinalis* How. germplasm

1.1.1 Polymorphism analysis of SRAP marker

From 80 pairs of SRAP primers, 28 primer combinations with better amplification effect were selected to amplify 9 *Morinda officinalis* How. germplasms. A total of 140 bands were detected, with an average of 5 bands per primer combination. The number of polymorphic bands was 68, and the percentage of polymorphic bands was 48.6%. Among them, the primer combination Me6Em2 could amplify 9 bands, while the primer combinations Me2Em3, Me2Em7, Me4Em3 and Me8Em2 could only amplify 2 bands. The primer combinations ME4EM10 and ME6EM7 could amplify 5 polymorphic bands, which is the most, while the primer combinations ME2EM2, ME2EM3, ME2EM7, ME3EM4, ME3EM5, ME3EM8 and ME4EM3 could only amplify 1 polymorphic band. The polymorphism ratio of the primer combinations ME3EM1, ME4EM5, ME4EM9, ME4EM10 and ME8EM2 is as high as 100%. While the percentage of polymorphic bands of the primer combinations ME2EM2, ME3EM4, ME3EM5 and ME3EM8 was only 16.7%, which was the lowest (Table 1). All amplified fragments were in the range of 100 ~ 2 000 bp. The primer combination ME1EM10 could amplify 4 bands from 9 *Morinda officinalis* How., including 3 polymorphic bands (Figure 1).

1.1.2 Polymorphism analysis of CDDP marker

From 17 primers, 16 primers with better amplification effect were selected to amplify 9 *Morinda officinalis* How. germplasms., and the effective rate of primer was 94.1%. Then a total of 83 bands were detected, with an average of 5.2 bands per primer. Among them, there were 63 polymorphic bands and the percentage of polymorphic bands was 75.9%. The primer ABP1-1 could amplify 9 bands, including 7 polymorphic bands, which is the most (Figure 2). The primer MADS-2 could only amplify 2 bands, which was the fewest. Both MADS-2 and KNOX-1 could only amplify 2 polymorphic bands, which was the fewest as well. The primers with 100% percentage of polymorphic bands were ABP1-3, MADS-1, MADS-2, MYB-2, WRKY-R1 and WRKY-R3 (Table 1).

1.1.3 Polymorphism comparison between SRAP and CDDP markers

From the bands covered by primers, the average number of bands covered by CDDP was 5.2, which was slightly higher than 5.0 of SRAP. And the percentage of polymorphic bands of CDDP was 75.9%, which was much higher than 48.6% of SRAP (Table 1). The results showed that the two molecular markers could amplify abundant polymorphic bands, but CDDP marker was better than SRAP marker in revealing the genetic diversity of *Morinda officinalis* How..

Table 1 Amplification results of SRAP primers and CDDP primers

SRAP primer combination	Total bands	Number of polymorphic bands	Percentage of polymorphic bands (%)	CDDP primer	Total bands	Number of polymorphic bands	Percentage of polymorphic bands (%)
ME1EM2	4	2	50.0	ABP1-1	9	7	77.8
ME1EM10	4	3	75.0	ABP1-3	4	4	100.0
ME2EM2	6	1	16.7	ERF-1	5	3	60.0
ME2EM3	2	1	50.0	ERF-2	6	3	50.0
ME2EM4	4	3	75.0	ERF-3	4	3	75.0
ME2EM5	4	2	50.0	KNOX-1	3	2	66.7
ME2EM7	2	1	50.0	KNOX-2	6	4	66.7
ME3EM1	4	4	100.0	MADS-1	3	3	100.0
ME3EM4	6	1	16.7	MADS-2	2	2	100.0
ME3EM5	6	1	16.7	MADS-4	4	3	75.0
ME3EM8	6	1	16.7	MYB-1	6	3	50.0
ME3EM9	6	2	33.3	MYB-2	5	5	100.0
ME4EM3	2	1	50.0	WRKY-F1	6	3	50.0
ME4EM5	3	3	100.0	WRKY-R1	8	8	100.0
ME4EM8	4	3	75.0	WRKY-R3	5	5	100.0
ME4EM9	3	3	100.0	WRKY-R3B	7	5	71.4
ME4EM10	5	5	100.0				
ME6EM1	8	4	50.0				
ME6EM2	9	3	33.3				
ME6EM5	4	3	75.0				
ME6EM7	7	5	71.4				
ME7EM2	7	2	28.6				
ME7EM4	6	2	33.3				
ME7EM5	8	4	50.0				
ME7EM6	8	2	25.0				
ME7EM9	6	2	33.3				
ME8EM2	2	2	100.0				
ME8EM3	4	2	50.0				
Total	140	68		Total	83	63	
Mean	5	2.4	48.6	Mean	5.2	3.9	75.9

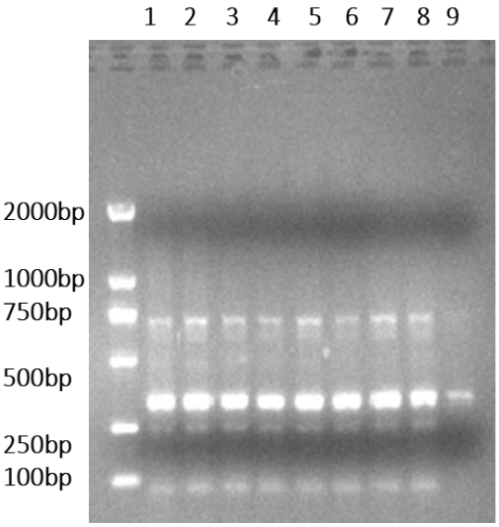

Figure 1 Amplification results of total DNA based on SRAP primer (ME1EM10) from 9 types of *Morinda officinalis* How.

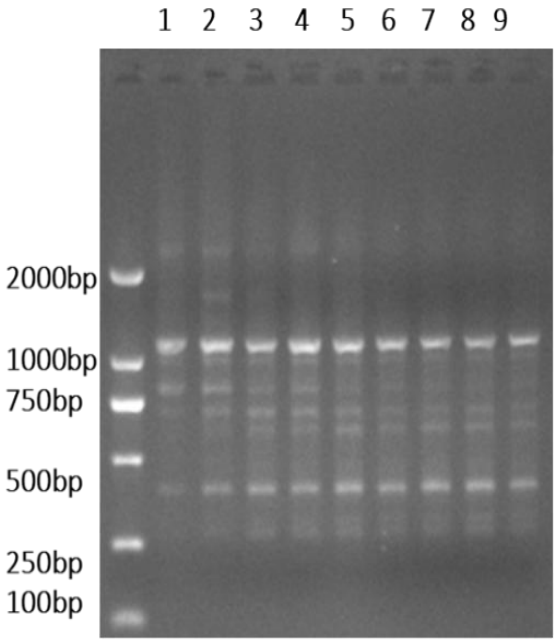

Figure 2 Amplification results of total DNA based on CDDP primer (ABP1-1) from 9 types of *Morinda officinalis* How.

1.2 Genetic similarity analysis of *Morinda officinalis* How. with these two marker methods

NTSYS-pc 2.1 software was used to calculate the genetic similarity coefficients of 9 *Morinda officinalis* How. based on SRAP molecular marker. The results showed that the genetic similarity coefficient of 9 samples varied from 0.69 to 0.96, the genetic similarity (GS) was 0.83, and the genetic distance (GD) was 0.17. The genetic similarity coefficient of local lobular type and local large leaf type was the highest, which was 0.96, indicating that they had the closest genetic relationship and the highest genetic similarity. The genetic similarity coefficient of lobular improvement and willow leaf type was 0.69, indicating that the genetic relationship between them was the farthest (Table 2).

Table 2 Genetic similarity coefficient table of *Morinda officinalis* How. based on SRAP molecular marker

Number	1	2	3	4	5	6	7	8	9
1	1.00								
2	0.84	1.00							
3	0.76	0.78	1.00						
4	0.77	0.85	0.80	1.00					
5	0.81	0.86	0.85	0.86	1.00				
6	0.82	0.86	0.84	0.86	0.94	1.00			
7	0.85	0.84	0.81	0.82	0.87	0.90	1.00		
8	0.85	0.87	0.84	0.85	0.91	0.94	0.96	1.00	
9	0.75	0.77	0.69	0.74	0.77	0.79	0.80	0.84	1.00

NTSYS-pc 2.1 software was used to calculate the genetic similarity coefficients of 9 *Morinda officinalis* How. based on CDDP molecular marker. The results showed that the genetic similarity coefficient of 9 samples varied from 0.53 to 0.95, the genetic similarity (GS) was 0.69, and the genetic distance (GD) was 0.31. The genetic similarity coefficient of local lobular type and black spike was the lowest, which was 0.53, indicating that the genetic relationship between them was the farthest; The genetic similarity coefficient of local lobular type and local large leaf type was the highest, which was 0.95, indicating that the genetic relationship between them was the closest (Table 3).

Table 3 Genetic similarity coefficient table of *Morinda officinalis* How. based on CDDP molecular marker

Number	1	2	3	4	5	6	7	8	9
1	1.00								
2	0.83	1.00							
3	0.61	0.71	1.00						
4	0.58	0.65	0.84	1.00					
5	0.58	0.70	0.67	0.64	1.00				
6	0.55	0.67	0.67	0.59	0.90	1.00			
7	0.58	0.70	0.80	0.66	0.81	0.83	1.00		
8	0.53	0.65	0.75	0.61	0.76	0.81	0.95	1.00	
9	0.66	0.66	0.71	0.67	0.63	0.60	0.67	0.67	1.00

The genetic similarity coefficient of SRAP varied from 0.69 to 0.96, and the average genetic similarity coefficient was 0.83. While the genetic similarity coefficient of CDDP varied from 0.53 to 0.95, and the average genetic similarity coefficient was 0.69. It can be seen that the variation range of genetic distance detected by CDDP is larger than that of SRAP.

1.3 Cluster analysis of *Morinda officinalis* How.

1.3.1 Cluster analysis of *Morinda officinalis* How. based on SRAP marker

According to the GS matrix, the UPGMA method was used for cluster analysis, and 9 *Morinda officinalis* How. were clustered to construct phylogenetic tree of *Morinda officinalis* How. varieties. When the genetic similarity coefficient of SRAP was 0.86, black spike, black spike grafting type, willow leaf grafting type, willow leaf type and lobular improvement were clustered separately. While the four varieties of Guangning special type, Guangning special grafting type, local large leaf type and local lobular type were closely clustered into one group (Figure 3), All of which have the characteristics of low yield and high effective medicinal ingredients (Table 4).

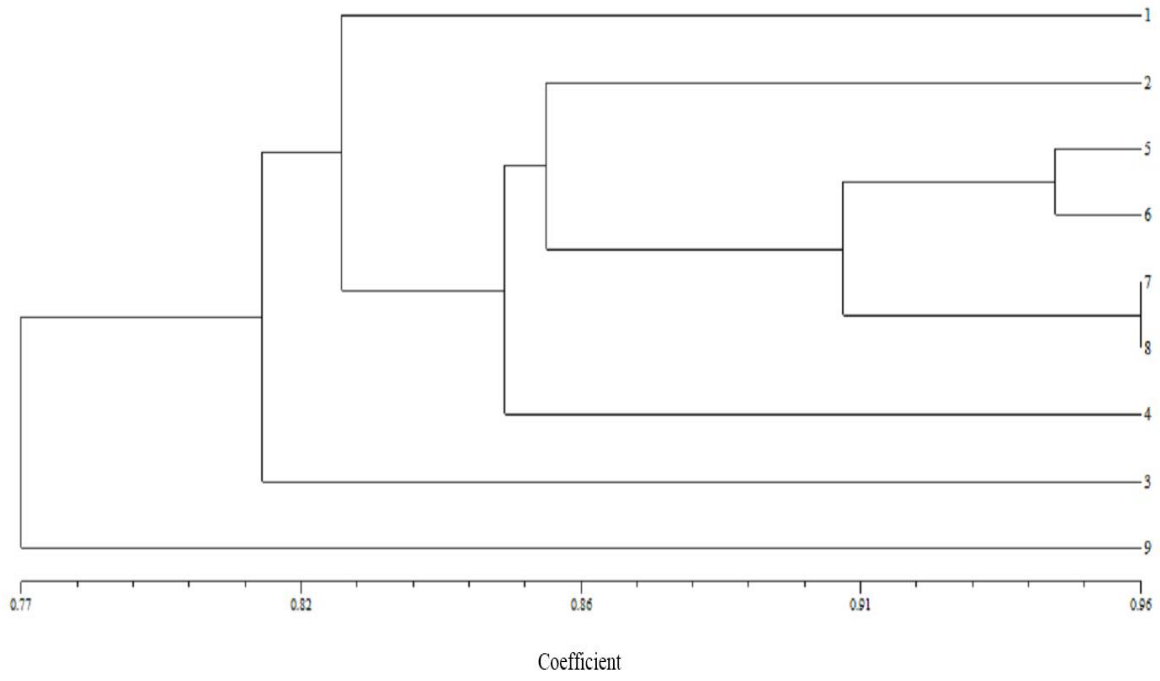

Figure 3 Phylogenetic tree of *Morinda officinalis* How. based on SRAP marker

Table 4 Information of germplasm of *Morinda officinalis* How.

Number	Variety	Characteristics
1	Black spike	Precocious, high yield, high effective medicinal ingredients
2	Black spike grafting type	Precocious, high yield (Higher than black spike), high effective medicinal ingredients
3	Willow leaf type	Late ripening, low yield, high effective medicinal ingredients
4	Willow leaf grafting type	Late ripening, low yield, high effective medicinal ingredients (Higher than willow leaf
5	Guangning special type	Late ripening, low yield, high effective medicinal ingredients
6	Guangning special grafting	Late ripening, low yield, high effective medicinal ingredients
7	Local large leaf type	Ancient variety of Deqing, low yield, high effective medicinal ingredients
8	Local lobular type	Ancient variety of Deqing, low yield, high effective medicinal ingredients
9	Lobular improvement	Late ripening, high yield, high effective medicinal ingredients

1.3.2 Cluster analysis of *Morinda officinalis* How. based on CDDP marker

According to the GS matrix, the UPGMA method was used for cluster analysis, and 9 *Morinda officinalis* How. were clustered to construct phylogenetic tree of *Morinda officinalis* How. varieties. When the genetic similarity coefficient of CDDP was 0.71, the 9 *Morinda officinalis* How. germplasms were clustered into 4 groups. Both black spike and black spike grafting type belong to group I, which have the characteristics of precocious, high yield, and high effective medicinal ingredients. Both willow leaf type and willow leaf grafting type of group II have the characteristics of late ripening, low yield and high effective medicinal ingredients. Lobular improvement belongs to group III alone. Among the 9 *Morinda officinalis* How. germplasms, only the lobular improvement has the characteristics of late ripening, high yield and high effective medicinal ingredients. Guangning special type, Guangning special grafting type, local large leaf type, and local lobular type belong to group IV. All four varieties have the characteristics of low yield and high effective medicinal ingredients (Figure 4). When the genetic similarity coefficient of SRAP was 0.86, these four varieties were also clustered into one group (Figure 3).

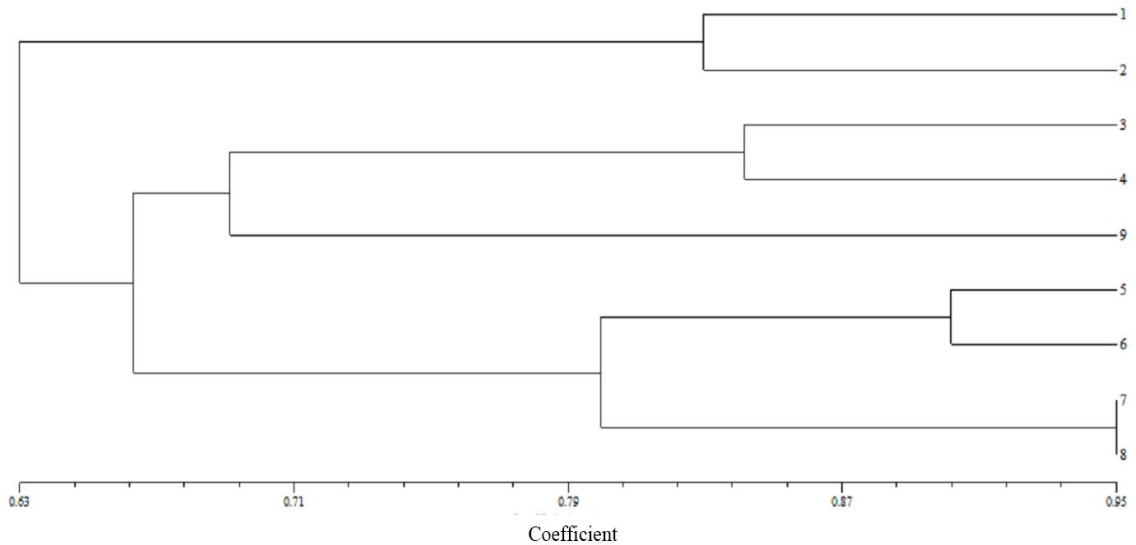

Figure 4 Phylogenetic tree of *Morinda officinalis* How. based on CDDP marker

1.3.3 Cluster analysis based on SRAP marker and CDDP marker

The results (Figure 5) based on the combined analysis of SRAP markers and CDDP markers were consistent with the results of CDDP clustering when the genetic similarity coefficient was 0.79 and that of CDDP was 0.71. Guangning special type, Guangning special grafting type, local large leaf type, and local lobular type were clustered into one group, which is consistent with the results of SRAP clustering and CDDP clustering. Although all these four varieties have the characteristics of low yield and high effective medicinal ingredients, local large leaf type and local lobular type are distinguished from the Guangning special type and the Guangning special grafting type because they are ancient varieties in Deqing.

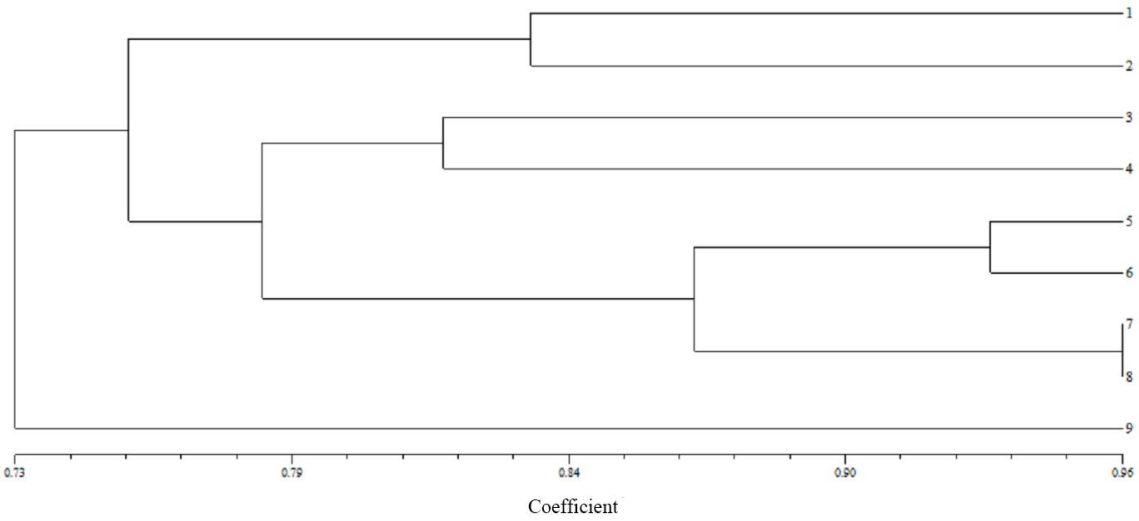

Figure 5 Phylogenetic tree of *Morinda officinalis* How. based on SRAP marker and CDDP marker

2 Discussion

The genetic background of *Morinda officinalis* How. is complicated, and traditional morphological studies can no longer accurately analyze the rich genetic diversity of *Morinda officinalis* How.. With the continuously further research on *Morinda officinalis* How., It is of great significance for the research on genetic breeding of *Morinda officinalis* How. to discover molecular markers which are easy to operate, rich in polymorphism and directly reflect genetic variation at the DNA level. In this study, SRAP and CDDP molecular markers were used to analyze genetic diversity and clustering of 9 *Morinda officinalis* How. germplasms. The results of the two molecular markers both showed that the genetic distance between local large leaf type and local lobular type of Deqing was the closest, and the Guangning special type and the Guangning special grafting type were closely clustered, indicating that the two molecular markers could mutually confirm the accuracy of the genetic diversity analysis of *Morinda officinalis* How. germplasm. The results of cluster analysis of *Morinda officinalis* How. germplasm showed that different varieties and their grafting varieties showed a certain clustering, which indicated that there was little genetic difference between grafting before and after. In this study, the results of SRAP clustering and those of combined analysis showed that lobular improvement and the other 8 *Morinda officinalis* How. varieties were clustered into 2 groups respectively, and lobular improvement was clustered into 1 group alone, which speculated that lobular improvement was the source of rich genetic diversity of *Morinda officinalis* How. germplasm.

In this study, a total of 83 bands were amplified by 16 CDDP primers, of which 63 were polymorphic bands, and the percentage of polymorphic bands was as high as 75.9%, which was higher than that of *Morinda officinalis* How. grown in Guangdong analyzed by RAPD molecular marker, the percentage of polymorphic bands of which was 50% (Ding, 2008). When the percentage of polymorphic bands is higher than 50%, it can be considered that the genetic diversity of the material is abundant (Wang, 2019). Therefore, it showed that CDDP marker could reflect the rich genetic diversity of *Morinda officinalis* How. and explore more genetic differences in *Morinda officinalis* How..

Both SRAP and CDDP molecular markers could reveal the genomic information of 9 *Morinda officinalis* How. germplasm, but from the overall effect, the CDDP molecular marker showed higher average number of primers and percentage of polymorphic bands than SRAP molecular marker. And from the phylogenetic tree, it could be seen that the results of CDDP clustering were more consistent with those of combined analysis than SRAP. Since CDDP marker is based on single primer amplification, compared with random amplification of SRAP marker, the CDDP marker is closely linked to a part or gene of the target gene, and has more advantages in the analysis of genetic diversity of *Morinda officinalis* How. (Zhou et al., 2019), so it was speculated that CDDP molecular markers could more accurately reveal the genetic relationship between *Morinda officinalis* How. germplasm.

SRAP uses unique double primers to amplify introns and promoters. Different introns and promoters have different intervals, resulting in polymorphisms (Guo et al., 2013). CDDP can produce functional molecular markers linked to target traits (Wu et al., 2017). Different molecular markers are not mutually exclusive and cannot replace each other. Using different molecular markers to complement each other can more effectively reveal the genetic diversity among germplasms. Since SRAP and CDDP molecular markers reveal different locus information in *Morinda officinalis* How. genome, combining these two molecular markers can more reasonably and accurately reflect the genetic differences among *Morinda officinalis* How. varieties (Guo et al., 2013). Therefore, combining the advantages of these two molecular markers, we can reduce the error and more accurately classify the genetic relationship of *Morinda officinalis* How., indicating that the results of the combined analysis of these two markers are more reliable and have more scientific value.

In this study, the genetic diversity of *Morinda officinalis* How. germplasm was analyzed based on SRAP marker, CDDP marker, and SRAP and CDDP combined markers, which provides a theoretical basis for molecular marker assisted selection of *Morinda officinalis* How., and plays an important role in shortening the breeding process and variety improvement of *Morinda officinalis* How..

3 Materials and Methods

3.1 Materials

The tested 9 *Morinda officinalis* How. germplasms were provided by teacher Zhang Weili, which were common *Morinda officinalis* How. varieties in Zhaoqing, Guangdong. The number, variety and characteristics of the tested *Morinda officinalis* How. (Table 4). The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd.. The sequence information of SRAP primers and CDDP primers (Table 5).

3.2 Extraction of genomic DNA from *Morinda officinalis* How.

The fresh healthy leaves of *Morinda officinalis* How. were extracted with the genomic DNA extraction kit of Tiangen Biotech (Beijing) Co., Ltd..

3.3 Reaction system of SRAP

The total reaction volume is 20 μL, including 1 μL DNA template, 0.1 μL 100 μmol/L positive primers, 0.1 μL 100 μmol/L negative primers, 1.5 μL 20 μmol/L Mg^{2+}, 1 μL 10 mmol/L dNTPs, 1 μL 2U/μL *Taq* DNA polymerase, and 15.3 μL ddH_2O.

SRAP-PCR was amplified by renaturation and temperature variation method. The amplification procedure referring to Li et al. (2001) was as follows: pre-denaturation at 94℃ for 5 min; denaturation at 94℃ for 1 min, renaturation at 35℃ for 1 min, extension at 72℃ for 1 min and cycle for 5 times; denaturation at 94℃ for 1 min, renaturation at 50℃ for 1 min, extension at 72℃ for 1 min and cycle for 35 times; finally, extension at 72℃ for 10 min.

3.4 Reaction system of CDDP

The total reaction volume is 20 μL, including 0.7 μL DNA template, 0.1 μL 100 μmol/L primer, 1.5 μL 10×buffer (including Mg^{2+}), 1.2 μL 10 mmol/L dNTPs, 1 μL 2 U/μL *Taq* DNA polymerase and 15.5 μL ddH_2O.

CDDP-PCR amplification procedure: pre-denaturation at 94℃ for 3 min; denaturation at 94℃ for 1 min, renaturation at 50℃ for 1 min, extension at 72℃ for 2 min and cycle 35 times; finally, extension at 72℃ for 5 min.

3.5 Separation of PCR products

The PCR products of SRAP and CDDP were separated by agarose gel electrophoresis at a concentration of 2.0% and 1.2% respectively, and the PCR products were observed and photographed with the Tanon4100 gel imaging system.

Table 5 Primer sequences of SRAP and CDDP

Primer type	Primer name	Sequence (5'- 3')
SRAP	SRAP-Me1	TGAGTCCAAACCGGATA
	SRAP-Me2	TGAGTCCAAACCGGAGC
	SRAP-Me3	TGAGTCCAAACCGGAAT
	SRAP-Me4	TGAGTCCAAACCGGACC
	SRAP-Me5	TGAGTCCAAACCGGAAG
	SRAP-Me6	GTAGCACAAGCCGGAGC
	SRAP-Me7	GTAGCACAAGCCGGACC
	SRAP-Me8	CGAATCTTAGCCGGATA
	SRAP-Em1	GACTGCGTACGAATTAAT
	SRAP-Em2	GACTGCGTACGAATTTGC
	SRAP-Em3	GACTGCGTACGAATTGAC
	SRAP-Em4	GACTGCGTACGAATTTGA
	SRAP-Em5	GACTGCGTACGAATTAAC
	SRAP-Em6	GACTGCGTACGAATTGCA
	SRAP-Em7	GACTGCGTACGAATTCAA
	SRAP-Em8	GACTGCGTACGAATTGAC
	SRAP-Em9	GACTGCGTACGAATTTGA
	SRAP-Em10	CGCACGTCCGTAATTAAC
CDDP	ABP1-1	ACSCCSATCCACCGC
	ABP1-3	CACGAGGACCTSCAGG
	ERF1	CACTACCGCGGSCTSCG
	ERF2	GCSGAGATCCGSGACCC
	ERF3	TGGCTSGGCACSTTCGA
	KNOX-1	AAGGGSAAGCTSCCSAAG
	KNOX-2	CACTGGTGGGAGCTSCAC
	KNOX-3	AAGCGSCACTGGAAGCC
	MADS-1	ATGGGCCGSGGCAAGGTGC
	MADS-2	ATGGGCCGSGGCAAGGTGG
	MADS-4	CTSTGCGACCGSGAGGTG
	Myb1	GGCAAGGGCTGCCGC
	Myb2	GGCAAGGGCTGCCGG
	WRKY-F1	TGGCGSAAGTACGGCCAG
	WRKY-R1	GTGGTTGTGCTTGCC
	WRKY-R3	GCASGTGTGCTCGCC
	WRKY-R3B	CCGCTCGTGTGSACG

3.6 Primer screening

28 pairs with clear bands and high percentage of polymorphic bands were selected from the electrophoretic map of 80 pairs of SRAP primer combinations; 16 primers with clear bands and high percentage of polymorphic bands were screened out from the electrophoretic map of 17 CDDP primers.

3.7 Data processing

In the electropherogram, the bands at the same migration position are homologous. According to this principle, the positions on the electropherogram were manually counted, and the clearly identifiable bands were recorded. If there were bands, we marked '1', while if there were no bands, we marked '0'. In this way, a binary data matrix was constructed.

NTSYS-pc 2.1 software was used to calculate the genetic similarity coefficient between samples, and cluster analysis was performed according to unweighted pair-group method with arithmetic mean.

Authors' contributions

LMX, as the executor of experimental research of this study, completed data compilation, wrote and revised the first draft of the manuscript. LFM participated in some experiments, completed data compilation and directed the writing of the first draft of the manuscript. ZWL, as the person in charge of the project, directed the experimental design, data statistics, paper writing and revision. CJT, LDH and CJY participated in the experiments. DZY directed the paper writing and revision. All authors read and approved the final manuscript.

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