INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 2, p. 117-125, 2018

RESEARCH PAPER OPEN ACCESS

RAPD based genetic diversity analysis among wild and cultivated genotypes of *Sarcandra glabra*

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Key words: *Sarcandra glabra*,RAPD, Genetic diversity, Cluster analysis, Medicinal.

http://dx.doi.org/10.12692/ijb/12.2.117-125 Article published on February 10, 2018

Abstract

Sarcandra glabra(*S. glabra*) is famous traditional medicinal herb in China, but in past years the wildgenetic resources have strongly declined so thestudy of genetic diversity and relationship among the various relativesis necessary to protect the germplasm resources. In this study, the genetic diversity among 9 accessions of *S. glabra*from different regions was analyzed by using therandom-amplified polymorphic DNA (RAPD) markersand the genetic relationship was revealed by cluster analysis and correlation coefficient analysis. Polymerase chain reactions were performed for 22 RAPD primers.Total 117 loci were detected by 22 primers and the amplified bands ranged from 3 to 9 with an average of 5.32 bands per pair of primer. There were 87 polymorphic and 30 monomorphic sites accounted for all genotypes and 74.36% polymorphism was found. The clusters were greatly associated with the origins and the morphologic characters of the tested germplasm.The genetic similarity coefficients between provenances ranged from 0.513 to 0.889.Clusterdiagram divides 9 samples into two categoriesrevealed that many years of domestication between different provenances and geographical distance has no significant correlation between wild and cultivated species. TheGenetic difference between populations was normal, and the estimation of gene flow between populations was low. RAPD markers were effective for all populations and exhibited a high level of polymorphism. The genetic distance between resources gives useful information to guide parent selection for breeding program.

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Introduction

S. glabra (Thunb) Nakai (Chinese name: Caoshanhu), an evergreen herbwhich is distributed in the southern parts of China and also in other Asian countries. It is an important crude herb used in traditional Chinese medicine. It has been demonstrated that the dried *S. glabra* has extensive pharmacological actions and is effective in the treatment of pneumonia, cancer, rheumatism, appendicitis, gastritis, enteritis, and injuries due to fall and fracture (China Pharmacopeia 2010; Xu *et al.* 2011). Due to its useful pharmacologic effects, particularly the increasing recognition of the utilization of Chinese medication in cancer treatment, the wild resources of S. glabra dramatically reduced because of years of overharvesting and curtailment of habitat since the Eighties.(Xu *et al.* 2011).

Knowledge of genetic variation is essential for breeding programs because it provides information about the molecular structure of plant genetics, so it can be used as a basis for the selection of crops to be cultivated. For the efficient evaluation, conservation and utilization of germplasm the understanding of genetic basis of any population is very essential.

The idea of sequence-related amplified polymorphism (SRAP) method was developed by Li and Quiros (2001)to efficiently target the overlapping coding and non-coding regions of any organism's genome. SRAP is highly effective technique and widely used for tagetted genetic map construction, gene tagging and cloning, genealogical classification, genetic diversity,population structure and to study gene linkage in plants (Zheng *et al.* 2010; Lu *et al.* 2012; Alghamdi *et al.* 2012;Jiang and Liu 2011;Yuanyuan *et al.* 2007). Moreover, it is alsowidely used in medical health to study genetic diversity of human and animal parasites (Li *et al.* 2009; Song *et al.* 2011). However, the genetic variation in *S. glabra*population has only been reported by using ISSR markers (Ni *et al.* 2008). Improvementin the field of molecular DNA analysis these days is often used to characterize genetic variation and relationship in a genus, species, cultivars, or accessions. Information on genetic diversity is the basis for the breeding and population genetics experts in the development and improvement of plants, especially as a first step in the selection of plants.

This step is especially important to distinguish between individuals within a species as well as the precise identification of samples and the identification of genes that are potential carriers of a single character. To measure the genetic variation within differentgermplasm collections the use of RAPD for identification of cultivars through DNA profiling isvery efficient and reliable for future breeding. (Hernendez *et al.*, 2001;Williams *et al.*, 1990). For genetic linkage and gene mapping the RAPD markers are widely used and dominant than other molecular primers (Chalmers *et al.*, 2001).

RAPD primers also used for the targeted and effective identification of loci linked with different characters (Sun*et al.*, 2003) although the problems associated with the process such as reproducibility. A RAPD marker has been widely used for diversity analysis and genotype identification in several and different crop plants (Abdellatif and Khidr 2010; Malviya and Yadav 2010;Jubera*et al.*, 2009; Plaschke *et al.*, 1995; Skaria *et al.*, 2011;Wu *et al.*, 2006).

Previous study revealed that *S. glabra*is genetically diverse and has good immunologic response including natural killer (NK) cell activity and its antioxidative capacity in splenocytes(He *et al.* 2009; Ni *et al.* 2008). Keeping in view the medicinal importanceand its current ecological status in focus we have identifiedthe genetic diversity among nine genotypes of *S. glabra*with RAPD marker. Furthermore, the genetic polymorphism among the nine *S. glabra* populationswas investigated at the molecular levels using RAPD analyses.

The obtained data was used to construct the phylogenetic tree by cluster analysis. In order to understand the genetic diversity of *S. glabra* among different regions, this research will be a foundation for further cultivation and development fine breeding

program for *S. glabra*.According to our studies, this is the first assessment of DNA sequence variation using RAPD analyses in *S. glabra*.

Material and methods

Plant material

Ninegenotypes of *S. glabra* were used in this experiment. Among of themsix genotypes were obtained from Guangxi province and three from Fujian provinceto build the experimental materials for this study. The experimental material was planted in Guangxi University, China. All material used in this experiment is listed in Table 1.

DNA extraction

DNA extraction was performed with the established CTAB method (Lee *et al.* 1988; Wu *et al.* 2001).

Genomic DNA extraction was performed from fresh leaves of *S. glabra* genotypes by CTAB method. RNA was removed from the DNA preparation by adding 10 µl of RNAase (10 mg/ml) and incubation at 37 °Cfor 30 min and then was purified using phenol and chloroform isoamyl alcohol (24:1) solution. DNA quantification of concentrations was done with few modifications by the procedure of Zachleder (1984). DNA sample concentration was adjusted at 25 ng/µl and stored at −20 °C until used in PCR.

Primer design and PCR amplification

RAPD-PCR was performed according to the modified procedure protocol of Williams *et al.* (1990). The RAPD primers used in this experiment were synthesized by Shanghai Bioengineering Company enlisted in the table 2. There were twenty two primers used in this experiment.

The amplification reactionwas performed in 20 µL volume containing 2.0 µL of 10 × Buffer, 1 µL of template DNA (25 ng / µL), 1.2 µL of $MgCl_2$ (25 mmol / µL), 0.25 µL of DNTPs), 0.2 µL of Taq DNA polymerase (5 U / µL).

The amplification program was carried out with denaturation at 94 ℃ for 2mins, denaturation at 94 ℃ for 30s, annealing at 37 ℃ for 30s, extension at 72 ℃ for 80s, 40 cycles, extension at 72 ℃ for 8mins.

When the program was finished, the 0.2 ml thin walled PCR tubes were removed and stored at 4 °C.

Electrophoresis detection

To clearly detect the RAPD-PCR product 1.5% agarose gel was used in 1× TAE buffer (pH 8.0, 242 g Tris Base, 57.1 ml glacial acetic acid, 0.5 M EDTA per litre of the buffer) and run in the same buffer for95 minutes with the voltage at 85V. Gels were stained with 0.5 µg/ml of ethidium bromide for 20seconds and washed in distilled water for 15minutes. DNA banding profiles were visualized on a UV trans illuminator and pictures taken gel documentation system.

Data processing and analysis

Data were subjected to correlation and cluster analysis for clear results. Only the most intense and reproducible DNA bands were considered for analysis. The bands of amplified fragments were coded as random binary characters (0 and 1), corresponding to the absence or presence of bands, respectively.

The cluster analysis and distance matrix of the complete data set was performed by usingNTSYS-PC Version 2.10e. Genetic similarities between different genotypes were measured by the Jaccard's similarity coefficient (Jaccard, 1908) for pair-wise comparisons based on the proportion of shared bands produced by the primers by using the SIMQUAL module. Similarity coefficients were used to construct a dendrogram.

Results and discussion

Primers amplification and polymorphism

Based on 22 RAPD primers, a total of 117 bands were detected, 87 of which were polymorphic (Table 4). Variation between 2 and 7 polymorphic loci was observed with a mean of 3.95 bands per primer. The genetic polymorphism was 74.36% among all population.

Table 1. List of *S. glabra* sample and characteristics from different places

Sr. No.	Origin	Cultivar Type	Name	Sr. No.	Origin	Cultivar Type	Name
1	Guangxi Lingchuan	Wild	YGX1	6	Guangxi Lingchuan	Wild	YGX6
2	Fujian	Cultivated	YGX2	7	Guangxi Heng County	Wild	YGX7
3	Fujian	Cultivated	YGX3	8	Guangxi Rongan	Cultivated	YGX8
4	Guangxi Heng County	Cultivated	YGX4	9	Fujian	Cultivated	YGX9
5	Guangxi Heng County	Cultivated	YGX5				

Table 2. Selected DNA (RAPD) primers with their respective sequences.

Sr. No.	Primer	Sequence (5′–3′)	Sr. No.	Primer	Sequence (5′–3′)
1	S2	TGATCCCTGG	12	LS1102	ACTTGACGGG
2	LS206	CAAGGGAGA	13	LS2139	GGTCTCGCTC
3	LS1101	TCACGTACGG	14	LS1084	ACAACGGTCC
4	LS1120	ACCAACCAGG	15	LS2144	ACCTGCCAAC
5	LS2127	CAGAGGTTCC	16	LS1132	AACGGCGGTC
6	LS2141	CCGACTCTGG	17	LS2089	CCCGCTTTCC
7	LS1087	CCGTCCATCC	18	LS2134	GACCAGCCCA
8	LS2146	TCGTGGCACA	19	LS1066	TCACGTCCCT
9	LS1137	TCAGCACAGG	20	CP714	GGGTGGGTGT
10	LS1083	CCCACCCTTG	21	LS2156	CTGCGGGTTC
11	LS2155	GAGAACGCTG	22	CP733	GGGAAGGGAG

The high polymorphism between genotypes was clearly demonstrated by the RAPD band patterns obtained using the primer LS1137(Table 4).The lowest numbers of polymorphic bands (2) were obtained with primer LS206, LS1087 and LS2089.The size of the amplification product ranged from 100bp to 2000bp. The percentage of polymorphism ranged from 50% to 83.33%. Primer LS1083, LS1102 and CP714, revealed the highest polymorphism (83.33%) while primer LS2089 exhibited the lowest polymorphism (50%).

Table 3. The similarity coefficient by for 9 sample of *S. glabra*.

	YGX1	YGX2	YGX3	YGX4	YGX5	YGX6	YGX7	YGX8	YGX9
YGX1	1.000								
YGX2	0.598	1.000							
YGX3	0.556	0.667	1.000						
YGX4	0.513	0.624	0.838	1.000					
YGX5	0.598	0.658	0.821	0.829	1.000				
YGX6	0.581	0.641	0.803	0.726	0.709	1.000			
YGX7	0.650	0.572	0.769	0.761	0.778	0.795	1.000		
YGX8	0.641	0.564	0.795	0.769	0.752	0.752	0.889	1.000	
YGX9	0.590	0.632	0.778	0.718	0.752	0.735	0.786	0.812	1.000

The primer LS1132 showed 3 monomorphic bands were in the range of 100bp to 1000bp (Fig. 1). The primer LS206, LS1101, LS2127, LS2141, LS1087, LS1083, LS2155, LS1102, LS2139, LS2144, LS2089, LS2134 and LS1066showed minimum (9) monomorphic bands. The primer LS1137 generated maximum(9)amplified bands of size 1000bp while 1 band was absent in YGX1 and present in the other samples. The primer LS206, LS1087 and LS2089 generated lowest (3) bands. The primer LS1137 showed 2 monomorphic bands and 9 polymorphic bands were in the range of 250bp to 1300bp. All the 9 samples reveal monomorphic and polymorphic bands ranged from ~200bp to 1400bp.

Genetic similarity

Genetic similarity among various sources lies between 0.513~0.889 as shown in table 3, most of which are

above 0.6, indicating that there are significant differences among different provenances of *S. glabra,* but the genetic relationship between same region provenances is relatively close. The results clearly show that the genetic similarity between the YGX1 and YGX4is the smallest with the similarity coefficient value of 0.513, and the genetic distance amongYGX7 andYGX8 is the largest with the similarity coefficient value of 0.889.

Table 4. Details of amplification obtained with different RAPD primers.

Sr. No.	Name of primer	Nucleotide Sequence (5'-3')	Total bands	No. of Fragments		Polymorphism %age
				Polymorphic	Monomorphic	
1	S2	TGATCCCTGG	7	5	2	71.43
2	LS206	CAAGGGAGA	3	2	1	66.67
3	LS1101	TCACGTACGG	4	3	1	75.00
4	LS1120	ACCAACCAGG	8	6	2	75.00
5	LS2127	CAGAGGTTCC	4	3	1	75.00
6	LS2141	CCGACTCTGG	5	4	1	80.00
7	LS1087	CCGTCCATCC	3	2	1	66.67
8	LS2146	TCGTGGCACA	5	4	1	80.00
9	LS1137	TCAGCACAGG	9	7	2	77.78
10	LS1083	CCCACCCTTG	6	5	1	83.33
11	LS2155	GAGAACGCTG	5	4	1	80.00
12	LS1102	ACTTGACGGG	6	5	1	83.33
13	LS2139	GGTCTCGCTC	4	3	1	75.00
14	LS1084	ACAACGGTCC	7	5	2	71.43
15	LS2144	ACCTGCCAAC	5	4	1	80.00
16	LS1132	AACGGCGGTC	6	3	3	50.00
17	LS2089	CCCGCTTTCC	3	2	1	66.67
18	LS2134	GACCAGCCCA	5	4	1	80.00
19	LS1066	TCACGTCCCT	4	3	1	75.00
20	CP714	GGGTGGGTGT	6	5	1	83.33
21	LS2156	CTGCGGGTTC	5	3	2	60.00
22	CP733	GGGAAGGGAG	7	5	2	71.43
Total			117	87	30	74.36
Average			5.32	3.95	1.32	3.38

Cluster analysis

A dendrogram (Fig. 2) based on NTSYS 2.10e, building genetic distance clustering map of provenances.

The analysis grouped the 9 samples into 2 main groups, with similarity coefficient ranging from 0.51 to 0.88. The dendrogram obtained 2 main groups (A and B) with 1 and 8 samples respectively. The main group A has one cluster (I). Cluster I has YGX1. The main group B has two clusters (I, II and III).

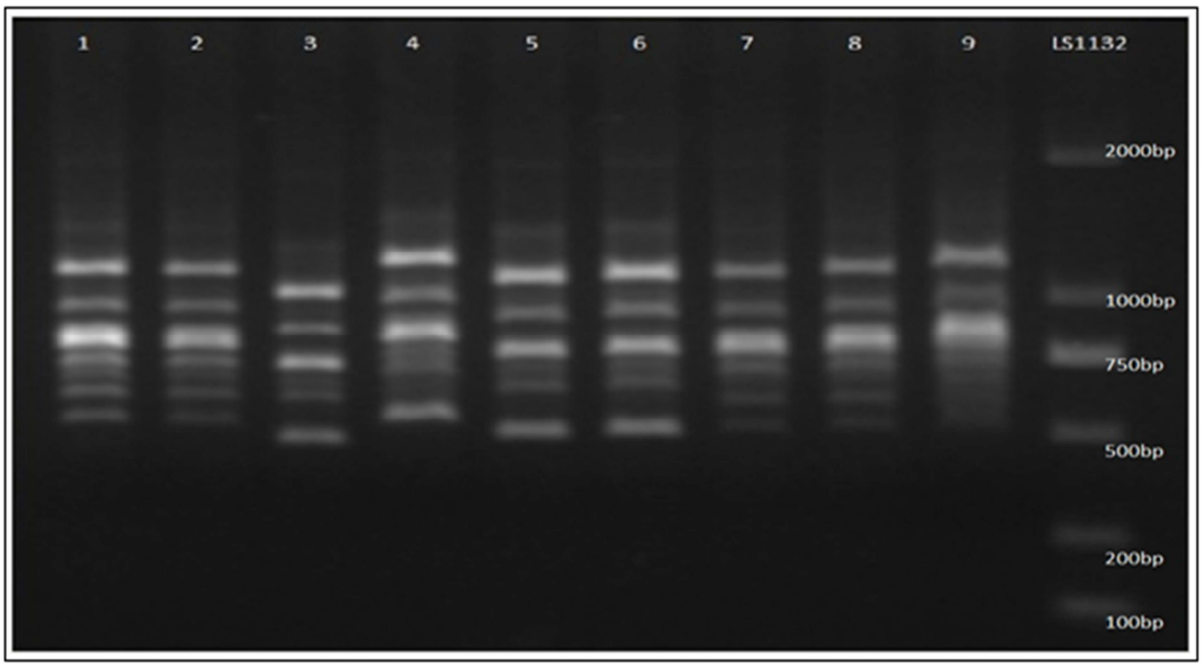

Fig. 1. The Electrophoretic profile obtained by DNA amplification of 9 plants of *S. glabra*with RAPD primer LS1132.

The main groups B with clusters I hasYGX2, and cluster II hasYGX3, YGX4 and YGX5. Clusters III from main group B containedYGX6, YGX7, YGX8, and YGX9.The results revealed that YGX1 is the first large group, the remaining eight YGX2, YGX3, YGX4, YGX5, YGX6, YGX7, YGX8, YGX9constitute the second largest group. The division of clusters shows that the there is a large genetic difference among provenance,such as YGX4, YGX5 and YGX7 have large genetic distance with YGX1 and YGX6.

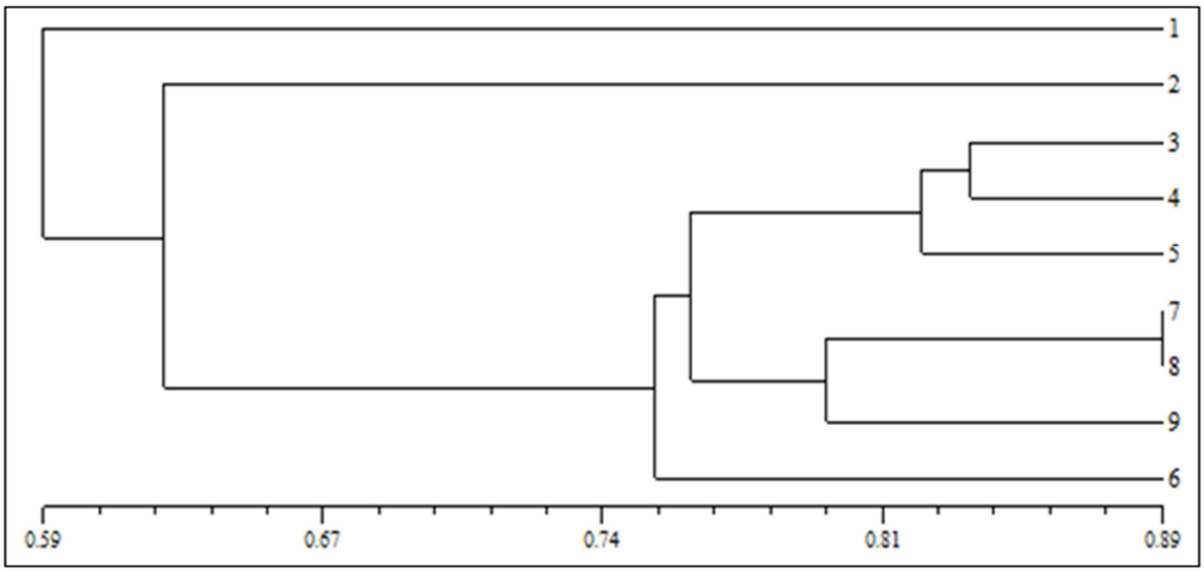

Fig. 2. Dendrogram constructed based on the genetic similarities between 9 plants of *S. glabra*.

The genetic distance between Guangxi accessions and Fujian accessions is large which shows that *S. glabra* in China has very large diversity among wild and cultivated relatives from different regions.

Conclusion

Based on the data obtained from the present study it is clear thatRAPDmolecular marker technique is of great significance to the study of genetic relationship

and diversity of *S. glabra*. It is also clear from the results that there is no obvious correlation between genetic distance and spatial distance between samples. This may be due to geographical isolation and different habitat conditions between provenances. Although YGX4andYGX5are very close geographically but theyhave no close genetic relationship, on the contraryYGX3 and YGX4 are from different regions and geographically distant provenance but their genetic distance is very close to each other. This may be due to a similar niche in the provenance, which is determined by the Tang*et al.*(2012).The results showed that the genetic distance between different provenances had no obvious correlation with the administrative division and geographical distance of *S. glabra*.

This indicated that both the accessions were genetically distant. It is clear from results that YGX1 is most different from the all other accessions. Results of RAPD marker analysis also illustrate that YGX1is genetically diverse from others. The samples taken from sameregion also showed the large diversity. *S. glabra*has medicinal and health care value andpresently its domestic and foreign demand is increasing so in order to ensure the sustainable use of *S. glabra*resources thetimely measures must be taken to effectively protect the existing germplasm.

Acknowledgements

The authors express a deep sense of gratitude the colleagues of State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Guangxi University, for all the support, assistance and constant encouragements to carry out this work. We are thankful toState Forestry Administration of China for their financial support.

References

Abdellatif KF, Khidr YA. 2010. Genetic diversity of new maize hybrids based on SSR markers as compared with other molecular and biochemical markers. Journal of Crop Science and Biotechnology, p. 139-145.
https://doi.org/10.1007/s12892-010-0068-x

Alghamdi SS, Al-Faifi SA, Migdadi HM, Khan MA, El-Harty EH, Ammar MH. 2012. Molecular Diversity Assessment Using Sequence Related Amplified Polymorphism (SRAP) Markers in Vicia faba L. International journal of molecular sciences, 16457-16471 p.
https://doi.org/10.3390/ijms131216457

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S, Harker N, Pallotta M, Cornish GB, Shariflou MR, Rampling LR. 2001. Construction of three linkage maps in bread wheat, Triticum aestivum. Australian Journal of Agricultural Research, 1089-1119 p.
https://doi.org/10.1071/ar01081

He RR, Yao XS, Li HY, Dai Y, Duan YH, Li YF. Kurihara H. 2009. The antistress effects of Sarcandra glabra extract on restraint-evoked immunocompromise. Biological and Pharmaceutical Bulletin, 247-252 p.
https://doi.org/10.1248/bpb.32.247

Hernández P, De La Rosa R, Rallo L, Dorado G, Martin A. 2001. Development of SCAR markers in olive (Olea europaea) by direct sequencing of RAPD products: applications in olive germplasm evaluation and mapping. Theoretical and Applied Genetics, 788-791 p.
https://doi.org/10.1007/s001220100603

Jaccard P. 1908. Nouvelles researches sur la distribution florale. Bulletin de la Société vaudoise des sciences naturelles, 223-270 p.

Jiang Y, Liu JP. 2011. Evaluation of genetic diversity in Piper spp using RAPD and SRAP markers. Genetics and Molecular Research, 2934-2943 p.
https://doi.org/10.4238/2011.november.29.4

Jubera MA, Janagoudar BS, Biradar DP, Ravikumar RL, Koti RV, Patil SJ. 2009. Genetic diversity analysis of elite Jatropha curcas L. genotypes using randomly amplified polymorphic

DNA markers. Karnataka Journal of Agricultural Sciences, 293-295 p.

Lee SB, Milgroom MG, Taylor JW. 1988. A rapid, high yield mini-prep method for isolation of total genomic DNA from fungi. Fungal Genetics Reports, 23 p.
https://doi.org/10.4148/1941-4765.1531

Li G, Quiros CF. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theoretical and applied genetics, p. 455-461.
https://doi.org/10.1007/s001220100570

Li QY, Dong SJ, Zhang WY, Lin RQ, Wang CR, Qian DX, Lun ZR, Song HQ, Zhu XQ. 2009. Sequence-related amplified polymorphism, an effective molecular approach for studying genetic variation in Fasciola spp. of human and animal health significance. Electrophoresis, 403-409 p.
https://doi.org/10.1002/elps.200800411

Lu JJ, Wang S, Zhao HY, Liu JJ, Wang HZ. 2012. Genetic linkage map of EST-SSR and SRAP markers in the endangered Chinese endemic herb Dendrobium (Orchidaceae). Genetics and Molecular Research, 4654-4667 p.
https://doi.org/10.4238/2012.december.21.1

Malviya N, Yadav D. 2010. RAPD analysis among pigeon pea [Cajanus cajan (L.) Mill sp.] cultivars for their genetic diversity. Genetic Engineering and Biotechnology Journal, 1-9 p.

Ni KC, Fang MN, Guo ED, Si JP, Zhang ZZ, Li HJ. 2008. Analysis on genetic diversity of Sarcandra glabra collected from eight provenance based on ISSR markers. Journal of Chinese Traditional and Herbal Drugs, 1392-1396 p.

Plaschke J, Ganal MW, Röder MS. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theoretical and Applied Genetics, 1001-1007 p.
https://doi.org/10.1007/bf00223912

Skaria R, Sen S, Muneer PM. 2011. Analysis of genetic variability in rice varieties (Oryza sativa L.) of Kerala using RAPD markers. Genetic Engineering and Biotechnology Journal, 1-24 p.

Song HQ, Mo XH, Zhao GH, Li J, Zou FC, Liu W, Wu XY, Lin RQ, Weng YB, Zhu XQ. 2011. Electrophoretic detection of genetic variability among Schistosoma japonicum isolates by sequence-related amplified polymorphism. Electrophoresis, 1364-1370 p.
https://doi.org/10.1002/elps.201000600

Sun G, Bond M, Nass H, Martin R, Dong Z. 2003. RAPD polymorphisms in spring wheat cultivars and lines with different level of Fusarium resistance. Theoretical and Applied Genetics, 1059-1067 p.
https://doi.org/10.1007/s00122-002-1163-2

Tang M, Wei R, Yao S, Lan Z, Ling Z. 2012. AFLP analysis on genetic diversity of Sarcandra glabra in Guangxi region. Journal of Chinese Traditional and Herbal Drugs, 1398-1402 p.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids research, 6531-6535 p.
https://doi.org/10.1093/nar/18.22.6531

Wu YJ, Chen Y, Wang J, Zhu CX, Xu BL. 2006. RAPD analysis of jasmine rice-specific genomic structure. Genome, 716-719 p.
https://doi.org/10.1139/g06-018

Wu ZH, Wang TH, Huang W, Qu YB. 2001. A simplified method for chromosome DNA preparation from filamentous fungi. Mycosystema, 575 p.

Xu YQ, Liu XL, Huang XF, Ge F. 2011. Status and prospect of research on important medicinal plant, Sarcandra glaba (Thunb.) Nakai. Journal of Chinese

Traditional and Herbal Drugs, 2552-2559 p.

Yuanyuan L, Jinxiong S, Tonghua W. 2007. Construction of a linkage map using SRAP, SSR and AFLP markers in Brassica napus L. Scientia Agricultura Sinica, 1118-1126 p.

Zachleder V. 1984. Optimization of nucleic acids assay in green and blue-green algae: extraction procedures and the light-activated diphenylamine reaction for DNA. Fundamental and Applied Limnology 313-328 p. https://doi.org/10.1127/algol_stud/67/1984/313

Zheng L, Qi J, Fang P, Su J, Xu J, Tao A. 2010. Genetic diversity and phylogenetic relationship of castor germplasm as revealed by SRAP analysis. Journal of Wuhan Botanical Research, 1-6 p.