

Application of molecular markers in medicinal plant studies

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Abstract. The World Health Organization has estimated that more than 80% of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs. Approximately two thirds of the 50 000 different medicinal plant species in use are collected from the wild and only 10% of medicinal species used commercially are cultivated. DNA-based molecular markers have utility in the fields like taxonomy, physiology, embryology, genetics, etc. DNA-based techniques have been widely used for authentication of plant species of medicinal importance. The geographical conditions affect the active constituents of the medicinal plant and hence their activity profiles. Many researchers have studied geographical variation at the genetic level. Estimates of genetic diversity are also important in designing crop improvement programmes for the management of germplasm and evolving conservation strategies. The DNA-based molecular marker helps in the improvement of medicinal plant species. DNA markers are more reliable because the genetic information is unique for each species and is independent of age, physiological conditions and environmental factors.

Keywords: polymorphism, authentication, identification, genetic variation

Introduction

With the cultivation of medicinal plants, traditional breeding techniques and biotechnology can be used to identify the genetic improvement of the growth of the plants [7]. Selections based on molecular markers have been used

widely in crop improvement. This method is desirable, genotypes detected in the early stages of the selection process will be expedited. This means that certain DNA sequences or alleles identify genes that are directly associated with the trait. Identifying functional genes and associating sequences with them is a costly process, but technical progress and results of whole-genome sequencing of model plants, such as *Oryza sativa* and *Arabidopsis thaliana*, is facilitated. There is high degree of similarity between the functional genes in the DNA sequences. Thus, DNA probes from one species can be used for the sequencing of related species. Availability of complete genome sequences for rice and *Arabidopsis* and the rapid increase in genomic resources for several other models such as *Medicago*, *Lycopersicon* and *Populus* has provided new opportunity for medicinal plant breeding through comparative genomics [15, 25].

A genetic marker is a gene or sequence with specific place on a chromosome which is associated with a particular trait [38]. The efficiency of molecular markers depends on the detected polymorphism [22, 31, 32].

Purpose

The purpose of this study is the evaluation of different DNA markers in classification, identification and breeding of medicinal plants.

DNA markers in medicinal plants

RFLP¹

Restriction fragment length of polymorphisms (RFLPs) were one of the first markers that were used to detect variation in a DNA sequence with restriction digestion by enzymes. One of the newest methods for the identification of individual diversity in nucleotides of DNA can be conceived directly through single-nucleotide polymorphism. Jana and Eva [19] performed RFLP analysis of the *Hypericum perforatum* L. They expressed that similar RFLP patterns in some somaclonal plants of R0 are related to the apomictic method of reproduction. RFLP patterns compared with parental plants showed that some offsprings have been established through sexual reproduction. Phylogenetic relationships among species of *Anthemideae* and *Asteraceae* have been studied using RAPDIS, SSR and RFLP markers in Egypt. In this study, twenty-six RFLP bands were identified after cutting with *EcoRI* and *BamHI*, and the

¹Restriction Fragment Length Polymorphism

similarity mean was between 0 and 0.5. Digested DNA products of *Achillea fragrantissima*, *Artemisia arborescens*, *Artemisia Judaica*, *Matricaria aurea*, *Glebionis Coronaria*, *Cotula barbata* and *Cotula cinerea* were 6, 4, 5, 4, 3, 2 and 2, respectively, while *Achillea santolina*, *Anacyclus monanthos* and *Matricaria recutita* had no band [1].

The taxonomy and physiology of India *Citrus* have been studied using PCR-RFLP with trnD-trnT and rbcL-ORF regions and sequence analysis of intra-genic spacer region trnD-trnT gene by Satya Narayan et al. [36]. In this experiment, 50 samples of genotype collected plant material of wild, semi-wild and domesticated. PCR-RFLP analysis generated of phylogenetic tree with moderate to high bootstrap values while trees based on trnL-trnF sequence showed only moderate to low bootstrap, which reflects the unknown origin of some *Citrus* genotypes. Nevertheless, the PCR-RFLP and trnL-trnF data distinguished *Citrus maxima*, *C. medica* and *C. Citrus* from each other.

AFLP²

AFLP markers have been successfully used for linkage mapping with high saturation in *Lolium perenne* L. AFLP markers are done in *Lolium* genomic mapping as a prelude in the determination of agronomic traits QTLs and marker-assisted selection [6]. AFLP analysis is used in genus *Ocimum* species by Labra et al. [21]. *Ocimum* genus is over 150 species. They concluded that the combined analysis of morphological characteristics, composition of volatile oils and molecular markers is a robust method for the identification of classification and its correlation with agronomic traits. In this study, the genetic distances were calculated with Nei and Li index [6]. Genetic relationships among *Rosa damascena* plants were studied by AFLP markers in Turkey [5]. There was no polymorphism between plants and marker patterns derived from different plants were similar.

The collected data showed that all *R. damascene* plants derived from the same original genotype vegetative reproduction.

RAPD³

RAPD technique is used by Salim khan et al. for the authentication of *Glycyrrhiza glabra* L., differentiating it from *Abrus precatorius* L. [35]. Fifty-two primers were screened for identifying original and unfathered samples. Six-

²Amplified Fragment Length Polymorphism

³Rapid Amplification of Polymorphic DNA

teen primers generated reproducible and specific amplification product. The PCR amplification products were clearly distinguished original and counterfeit samples with similar morphology. So, RAPD can be used as a complementary tool in quality control [34].

In a study by Mehrnia et al. [24], fresh leaves of 20 plants belonging to Iranian *Astragalus microcephalus* were gathered and studied with RAPD markers. The results showed that RAPD markers could be used to study the systematic relationship between related species. In a similar work by Sultan et al. [39], *Podophyllum hexandrum* was collected from high-latitude regions of the north-western Himalayas. The RAPD analysis showed a high degree of genetic diversity among the 12 collected samples, which can be attributed to geographical and climatic conditions. This study demonstrated that RAPD markers are very useful tools for comparing the genetic relationship and diversity patterns between medicinal plants.

SSR⁴

Genetic characterization of *Rhodiola rosea* L. was performed using SSR markers. Genetic relationships of 30 samples of *Rhodiola rosea* were studied using 10 markers. SSR analysis produced 12 polymorphic locus with an average of 1.8 polymorphic band per primer. Genetic differentiation was significantly low which indicates a high level of gene flow and a strong influence on the genetic structure. Results showed a significant gene flow between the populations of *R. rosea* [37].

SNP⁵

Coles et al. [12] used tagged, sequenced and expressed libraries to identify SNP in *Chenopodium quinoa* willd. SNP markers identified in this study had particular value in the gene expression and regulation associated with seed, while distinguished SNPs had immediate application in genetic mapping experiments, germplasm introduction and evolutionary relationships within the genus *Chenopodium*.

⁴Simple Sequence Repeats

⁵Single-nucleotide polymorphisms

Authentication of medicinal herbs using molecular markers

Techniques based on DNA were widely used for the authentication of important medicinal plants. Molecular biology provides a set of techniques that can be useful for the authentication of medicinal plants. This fact is useful particularly in plants that are indistinguishable from other species or fake varieties in terms of morphology or phytochemistry [28].

Some researchers determined AFLP technique potential to analyse the genetic distances between varieties of *Ocimum basilicum* L. and the correlation between genetic distance, the patterns of essential oils and morphological characteristics [21]. RAPD technique is used for determining the components of the medicinal plants *Astragalus membranaceus*, *Ledebouriella seseloides* and *Atractylodes macrocephala*. Components in pharmaceutical formulations have been identified using single RAPD primer [2].

In the study by Sultan et al. [39], three RAPD primers successfully differentiated the *Podophyllum* species from the northwest of Himalayas. In another study, three random primers were used for the detection of genetic variation in *Astragalus* medicines sold on Taiwan markets [2]. SSCP analysis of the PCR products was performed to separate the two species of *Astragalus* [24]. RAPD analysis was used for the authentication of *Glycyrrhiza glabra* L., differentiating it from *Abrus precatorius* L. Fifty-two oligonucleotide primers screened for the detection of original from fake samples. The specified PCR products clearly distinguish the original and the fake samples having similar morphology. Thus, RAPD can be used as a complementary tool for quality control [35].

The dried samples of fruit of *Lycium barbarum* were distinguished by RAPD markers [45]. RAPD technique has been used to determine the components of Chinese drug “yu-ping-feng san”. In this study, the presence of three medicinal plants was evaluated in formulation using a single primer RAPD [10]. In another study, three random primers detected genetic variation *Astragalus* on Taiwan markets. SSCP analysis of PCR products recognized two species of *Astragalus* [9]. RAPD primers identified successfully *Atractylodes* species from Chinese formulations purchased from local markets [8].

Molecular markers in herbal drug technology

RAPD markers are useful in distinguishing different samples of *Codonopsis pilosula* [14], *Allium schoenoprasum* L. [13] and *Andrographis paniculata* [29] collected from different geographical regions. Similarly, various samples of *Arabidopsis thaliana* [4] are differentiated with ISSR markers.

Inter and intra species diversity have been studied using molecular markers. Interspecies diversity using RFLP and RAPD markers has been studied in general such as *Glycerrhiza* [44], *Echinacea* [20] and *Arabidopsis* [23]. RAPD and RFLP markers for the characterization of species *Epimedium* [26] have been used on genetic level. Members of three different species of *Scutellaria* [18] and three sub-species of *Melissa officinalis* [43] were detected with RAPD. Varietal identification and genetic purity of hemp was performed using RAPD and morphological data [11].

RFLP technique was used to assess the genetic diversity intraspecies in the genus *Capsicum* and the DNA fingerprinting of pepper varieties [33]. RAPD marker was used as a tool to explore the diversity of *Simmondsia chinensis* L. Schneider [3], *Vitis vinifera* L. [40] and *Camellia sinesis* [42]. Genetic diversity within and between relationships of *Withania* [27] and the genetic relationships between wild relatives of *Caricaceae* was revealed using AFLP markers [41]. RAPD markers are used for genetic linkage mapping *Grandis Eucalyptus* and *Eucalyptus urophylla* [17]. RAPD markers are used for the genetic mapping of *Taxus braviifolia* Nutt. [16]. AFLP physical map of *Arabidopsis* genome is provided by AFLP marker [30].

Conclusion

Markers are different in their ability to differentiate, their mechanism of polymorphism and their genomic coverage. So, they can complement each other depending on the available techniques. Molecular technology provides an independent way to describe the medicinal plant materials.

References

- [1] Abd El-Twab MH, Zahran F. A. (2010), RAPD, ISSR and RFLP analysis of phylogenetic relationships among congeneric species (*Anthemideae*, *Asteraceae*) in Egypt. *International Journal of Botany* 6: 1–10.

- [2] Adiguzel A., Agar G., Baris O., Medine G., Fikrettin S., Meryem S. (2006), RAPD and FAME analyses of *Astragalus* species growing in eastern Anatolia region of Turkey. *Biochemical Systematics and Ecology* 34: 424–432.
- [3] Amarger V., Mercier L. (1995), Molecular analysis of RAPD DNA based markers: Their potential use for the detection of genetic variability in jojoba (*Simmondsia chinensis* L. Schneider). *Biochimie* 72: 931–936.
- [4] Barth S., Melchinger A. E., Lubberstedt T. (2002), Genetic diversity in *Arabidopsis thaliana* L. Heynh. investigated by cleaved amplified polymorphic sequence (CAPS) and inter-simple sequence repeat (ISSR) markers. *Molecular Ecology* 11: 495–505.
- [5] Bayder N. G., Bayder H., Debener T. (2004), Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. *Journal of Biotechnology* 111: 263–269.
- [6] Bert P. F., Charmet G., Sourdille P., Hayward M. D., Balfourier F. (1999), A highdensity molecular map for ryegrass (*Lolium perenne*) using AFLP markers. *Theoretical and Applied Genetics* 99: 445–452.
- [7] Braga R. A., Dal Fabbro I. M., Borem F. M., Rabelo G., Arizaga R., Rabal H. J., Trivi M. (2003), Assessment of seed viability by laser speckle technology. *Biosystems Engineering* 86: 287–294.
- [8] Chen K. T., Su Y. C., Lin J. G., Hsin L. H., Su Y. P., Su C. H., Li S. Y., Cheng J. H., Mao S. J. T. (2001), Identification of *Atractylodes* plants in Chinese herbs and formulations by random amplified polymorphic DNA. *Acta Pharmacologica Sinica* 22: 493–497.
- [9] Cheng K. T., Su B., Chen C. T., Lin C. C.)2000, RAPD analysis of *Astragalus* medicines marketed in Taiwan. *The American Journal of Chinese Medicine* 28: 273–278.
- [10] Cheng K. T., Tsay H. S., Chen C. F., Chou T. W. (1998), Determination of the components in a Chinese prescription, Yu-Ping-Feng San, by RAPD analysis. *Planta Medica* 64: 563–565.
- [11] Cheng Z., Lu B. R., Baldwin B. S., Sameshima K., Chen J. K. (2002). Comparative studies of genetic diversity in kenaf (*Hibiscus cannabinus* L.) varieties based on analysis of agronomic and RAPD data. *Hereditas* 136: 231–239.

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- [12] Coles N. D., Coleman C. E., Christensen S. A., Jellen E. N., Stevens M. R., Bonifacio A., Rojas-Beltran J. A., Fairbanks D. J., Maughan P. J. (2005), Development and use of an expressed sequenced tag library in quinoa (*Chenopodium quinoa* Willd.) for the discovery of single nucleotide polymorphisms. *Plant Science* 168: 439–447.
- [13] Friesen N., Blattner, F. R. (1999), RAPD analysis reveals geographic differentiations within *Allium schoenoprasum* L. (Alliaceae). *Planta Meica* 65: 157–160.
- [14] Fu R. Z., Wang J., Zhang Y. B., Wang Z. T., But P. P., Li N., Shaw P. C. (1999), Differentiation of medicinal *Codonopsis* species from adulterants by polymerase chain reaction-restriction fragment length polymorphism. *Planta Meica* 65: 648–650.
- [15] Gilmore S., Peakall R. (2003), Isolation of microsatellite markers in *Cannabis sativa* L. (Marijuana) in fibre crop varieties. *Molecular Ecology Notes* 3: 105–107.
- [16] Gocmen B., Jermstad K. D., Neale D. B., Kaya Z. (1996), Development of random amplified polymorphic DNA markers for genetic mapping in Pacific yew (*Taxus brevifolia*). *Canadian Journal of Forest Research* 26: 497–503.
- [17] Grattapaglia D., Sederoff R. (1994), Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics* 137: 1121–1137.
- [18] Hosokawa K., Minami M., Kawahara K., Nakamura I., Shibata T. (2000), The sequences of the spacer region between the atpF and atpA genes in the plastid genome allows discrimination among three varieties of medicinal *Angelica*. *Planta Medica* 66: 270–272.
- [19] Jana H., Eva C., (1997), RFLP analysis of *Hypericum perforatum* L. somaclones and their progenies. *Euphytica* 95: 229–235.
- [20] Kapteyn J., Goldsbrough B., Simon E. (2002), Genetic relationships and diversity of commercially relevant *Echinacea* species. *Theoretical and Applied Genetics* 105: 369–376.
- [21] Labra M., Mariangela M., Bernardetta L., Fabrizio G., Mauro M., Francesco S., (2004), Morphological characterization, essential oil com-

- position and DNA genotyping of *Ocimum basilicum* L. cultivars. *Plant Science* 167: 725–731.
- [22] Leela T., Suhas P. W., Seetha K., Naresh B., Thakur K. S., David A. H., Prathibha Devi, Rajeev K. V. (2009), AFLP-based molecular characterization of an elite germplasm collection of *Jatropha Curcas* L., a biofuel plant. *Plant Science* 176: 505–513.
- [23] Lind-Hallden C., Hallden C., Sall T. (2002), Genetic variation in *Arabidopsis suecica* and its parental species *A. arenosa* and *A. thaliana*. *Hereditas* 136: 45–50.
- [24] Mehrnia M., Shahin Z. and Ashrafoldin S. S. (2005), Intra- and inter-specific relationships within the *Astragalus microcephalus* complex (Fabaceae) using RAPD. *Biochemical Systematics and Ecology*. 33: 149–158.
- [25] Miller Coyle H., Palmbach T., Juliano N., Ladd C., Lee H. C. (2003), An overview of DNA methods for the identification and individualization of marijuana. *Croatian Medical Journal* 44: 315–321.
- [26] Nakai R., Shoyama Y., Shiraishi S. (1996), Genetic characterization of *Epimedium* species using random amplified polymorphic DNA (RAPD) and PCR-restriction fragment length polymorphism (RFLP) diagnosis. *Biological and Pharmaceutical Bulletin* 19: 67–70.
- [27] Negi M. S., Singh A., Laksmikumaran M. (2000), Genetic variation and relationship among and within *Withania* species as revealed by AFLP markers. *Genome* 43: 975–80.
- [28] Ngan F., Shaw P., But P., and Wang J. (1999), Molecular authentication of *Panax* species. *Phytochemistry* 50: 787–791.
- [29] Padmesh P., Sabu K. K., Seeni S., Pushpangadan P. (1999), The use of RAPD in assessing genetic variability in *Andrographis paniculata* Nees, a hepatoprotective drug. *Current Science* 76: 833–835.
- [30] Peters J. L., Constandt H., Neyt P., Cnops G., Zethof J., Zabeau M., Gerats T. (2001), A physical amplified fragment-length polymorphism map of *Arabidopsis*. *Plant Physiology* 127: 1579–1589.

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- [31] Pourmohammad A., Moghaddam Vahed M., Khosrowchahli M., Mohammadi S. A., Yousefi A. (2010a), Genetic variation of hulless barley with agronomic traits and RAPD markers. 11th Iranian crop science congress. Iran, Tehran, Environmental Science Research Institute, Shahid Beheshti University, 24–26 July 2010. (In Farsi).
- [32] Pourmohammad A., Moghaddam Vahed M., Khosrowchahli M., Mohammadi S. A., Yousefi A. (2010b), Study of genetic diversity by RAPD markers and identification of informative markers for grain yield and its components in hulless barley genotypes. Seed and Plant Improvement Journal 253–267. (In Farsi).
- [33] Prince J. P., Lackney V. K., Angels C., Blauth, J. R., Kyle M. M. (1995), A survey of DNA polymorphism within the genus *Capsicum* and the fingerprinting of pepper cultivars. Genome 38: 224–231.
- [34] Salim Khan, Khanda J. M., Malik Z. A. (2010), Development of RAPD markers for authentication of medicinal plant *Cuscuta reflexa*. Eurasian Journal of Biosciences 4: 1–7.
- [35] SalimKhan, Khanda J. M., Md Tayaab, Malik Z. A. (2009), RAPD profile for authentication of Medicinal plant *Glycyrrhiza glabra* L. Internet Journal of Food safety 11: 24–28.
- [36] Satya Narayan J., Susheel Kumar and Narayanan K. Nair (2009), Molecular phylogeny in Indian *Citrus* L. (Rutaceae) inferred through PCR-RFLP and trnL-trnF sequence data of chloroplast DNA. Scientia Horticulturae. 119(4): 403–416.
- [37] Soni K., Shruti R., Ankit G., Karma Y., Saurabh P., Pradeep K. N. and Harvinder Singh (2010), Genetic characterisation of *Rhodiola rosea* using gene specific SSR and CAPS molecular markers. Genetic Engineering & Biotechnology Journal 11: 1–10.
- [38] Srivatsava S., Nidhi M. (2009), Genetic Markers - A cutting edge technology in herbal Drug research. Journal of Chemical and Pharmaceutical Research 1: 1–18.
- [39] Sultan P., Shawl A. S., Rehman S., Fayaz Ahmed S., Ramteke P. W. (2010), Molecular characterization and marker based chemotaxonomic studies of *Podophyllum hexandrum* Royle. Fitoterapia 81: 243–247.

- [40] Tessier C., David J., This P., Boursiquot J. M., Charrier A. (1999), Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. *Theoretical and Applied Genetics* 98: 171–177.
- [41] Van D. B., Breyne P., Goetghebeur P., Romijn-Peeters E., Kyndt T., Gheysen G. (2002), AFLP analysis of genetic relationships among papaya and its wild relatives (*Caricaceae*) from Ecuador. *Theoretical and Applied Genetics* 105: 289–297.
- [42] Wachira F. N., Waugh R., Hackett C. A. Powell W. (1995), Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* 38: 201–210.
- [43] Wolf H. T., Berg T. V. D., Czygan F. C., Mosandl A., Winckler T., Zundorf I., Dingermann T. (1999), Identification of *Melissa officinalis* subspecies by DNA fingerprinting. *Planta Medica* 65: 83–85.
- [44] Yamazaki M., Sato A., Shimomura K., Saito K., Murakoshi I. (1994), Genetic relationships among *Glycyrrhiza* plants determined by RAPD AND RFLP analyses. *Biological and Pharmaceutical Bulletin* 17: 1529–1531.
- [45] Zhang K. Y., Leung H. W., Yeung H. W., Wong R. N. (2001), Differentiation of *Lycium barbarum* from its related *Lycium* species using random amplified polymorphic DNA. *Planta Medica* 67: 379–381.