MOLECULAR CHARACTERIZATION OF JASMINE SPECIES BY RAPD MOLECULAR MARKERS

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ABSTRACT

In the following study genomic DNA was extracted from eight Jasmine species. DNA fingerprinting was performed by the random amplified polymorphic DNA (RAPD)-PCR. RAPD has been one of the most commonly used molecular techniques to develop DNA markers since it does not require prior knowledge of a DNA sequence. RAPD-PCR produced a spectrum of amplification products which are characteristics of the selected Jasmine DNA. From the DNA fingerprinting, Dendrogram was constructed and genetic similarity matrix were estimated which revealed variations between selected species of Jasmine. A total of 15 random primers were used for conducting the RAPD analysis. The primers were OPZ5, OPZ6, OPZ7, OPZ8, OPZ9, OPZ10, OPZ11, OPZ12, OPZ13, OPZ14, OPZ15, OPZ18, OPZ19, OPZ20, OPZ21 and OPZ22. Of these selected random primers OPZ8, OPZ9, OPZ10 produced clear banding patterns which were used for constructing Dendrogram. OPZ8 produced a total 58 bands ranging from 7-11, OPZ9 produced a total 91 bands ranging from 10-13 and OPZ 10 produced a total 49 bands ranging from 5-8 for eight jasmine samples. Most of the bands were monomorphic with some polymorphic bands which can be used for marker development for these jasmine species. The described approach holds great promise for genetic diversity polymorphism, cultivar characterization and genetic population conservation of Jasmine species.

KEYWORDS: Jasmine, DNA fingerprinting, RAPD, Genetic Diversity.

INTRODUCTION

Plants of Jasmine species are also called as Chameli, Chambeli and Mallige belonging to the family Oleaceae. It is a plant with fragrant flowers generally found in all over India,
especially in the temperate regions and on the temperate Himalayas. The Chameli leaves contain resin, salicylic acid and jasminine an alkaloid. Herbal plants are pioneer for new drug discovery and development, not only for plant constituents used directly as therapeutic agents, but also as starting materials for synthesis of pharmacologically active compounds \(^1\). The use of plants for prevention and treatment of various health ailments has been in practice from time immemorial and it is estimated that about 25% of drugs prescribed are derived from plants, moreover, WHO's essential medicine list contains 252 drugs out of which 11% is exclusively of plant origin \(^2\). In the present scenario, pharmaceutical companies are involved in research on plant materials for their potential medicinal value as the demand for herbal products is growing exponentially due to its fewer side effects as compare to other system of medicines \(^3\). Jasminum genus with about 200 species belonging to family Oleaceae are of three types: shrub or bush form, vines and trees, native to tropical and warm temperate regions. Many jasminum plants prominently feature white, yellow or pink flowers with sweet fragrance and others are unscented \(^4\). Jasminum species is used to treat many conditions such as amenorrhoea, ringworm, leprosy, skin diseases and also as an analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorants, diuretics and among others \(^6\). The leaves of Jasminum grandiflorum is used in folk medicine for treating ulcerative stomatitis, toothache, skin diseases, ulcers, wounds, corms and also as gargles. It has been reported to possess antioxidant, anti-lipid peroxidation and spasmylytic activity \(^5\). Jasmine oil blends well with every floral scent imparting smoothness and elegance to the perfume composition. It is useful in treating diseases of the mouth and teeth, especially for toothache \(^8\). The essential oil in flowers is extracted through enfleurage which is widely used for production of Jasmine attars in India \(^12\). Jasmine oil has a wide range of medicinal applications and can be used in perfumery, soaps, flavorings and the cosmetic industry \(^9\). Therapeutically, jasmine oil is used as an antidepressant, antiseptic, antispasmodic, sedative and uterine tonic \(^10\). Essential oil of J. sambac is used as fragrance for skin care products as it tones the skin as well as reduces skin inflammation \(^11\). Essential oil and methanol extract from Jasminum sambac have in vitro antimicrobial and antioxidant activities which could support the use of the plant by traditional healers to treat various infective diseases \(^7\). In India, Jasmines are cultivated throughout the country. However, the largest area under Jasmine flower production is in Tamil Nadu followed by Karnataka \(^13\). The annual production of jasmine concrete is more than 15 tones in India, the largest producer being Egypt, followed by Morocco, India, Italy, France and China \(^14\).
MATERIALS & METHODS

Plant Material: The plant material of Jasminum species were obtained from University of agriculture, Bangalore India. The different species of Jasmine that were collected are Jasminum grandiflorum, J. sambac, J. humile, J. sambac wild, J. arborescens, J. angustifolium, J. auriculatum, J. officinale, J sambac L

DNA Isolation and PCR Amplification
DNA was isolated from fresh leaf tissues as per the procedure described previously. The polymerase chain reaction was carried out in final volume of 25 μl containing 100 ng DNA, 1 U of Taq DNA polymerase (Chromous Biotech, Bangalore), 2.5 mM MgCl (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 p mol of primers (GeNei, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94°C for 45 sec, 35°C for 1 min and 72°C for 1.5 min) followed by 30 cycles of amplification (94°C for 45 sec, 38°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5 min). All PCR products were separated on 1.5% (w/v) Agarose gel containing ethidium bromide (0.5 μg / ml). The gel was photographed with HP Alpha-imager.

Data Analysis
The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of frequency similarity. The matrix of genetic distance was used for grouping the lemongrass cultivars based on the Dendrogram constructed by UPGMA (unweighed pair group method with Arithmetic averages)

RESULTS
On the basis of the DNA markers generated by the primers, considerable genetic diversity was observed among the varieties. The different primers produced different number of bands in PCR. OPZ8 produced a total 58 bands ranging from 7-11, OPZ9 produced a total 91 bands ranging from 10-13 and OPZ 10 produced a total 49 bands ranging from 5-8 for eight jasmine samples. This variation in the number of bands may be due to the sequence of primer, availability of annealing sites in the genome or template quality. To estimate the similarity and genetics distance among different jasmine, cluster analysis based on frequency similarity with weighted pair-group with arithmetic average (UPGMA) was performed using the Alpha
Imager HP and dendrogram was constructed. The genetic similarity coefficients among genotypes estimated on the basis of Nei and Li (1979) varied from sample to sample in RAPD markers.

Figure 1: Nanodrop Quantification of DNA.

Figure 2: DNA fingerprint, Dendrogram and Matrix for OPZ 8.
Figure 3: DNA fingerprint, Dendrogram and Matrix for OPZ 9.
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Figure 4: DNA fingerprint, Dendrogram and Matrix for OPZ 10.
DISCUSSION
The present study deals with determining genetic diversity and phylogenetic relationship among eight *jasmine* species by RAPD markers. The isolated genomic DNA gave single, sharp and distinct bands devoid of any smear on 0.8% agarose gel. Thus, genomic DNA of good quality without any degradation was successfully isolated from all the eleven samples. Here, the quantitative estimation of genomic DNA was done by Thermo Scientific Nanodrop 1000 spectrophotometer. The genomic DNA were obtained in high concentration for all the samples and they showed a good 260/280 ratio (i.e. between 1.8 and 2.0) indicating absence of any protein or RNA contaminants. Fifteen random primers were used for conducting the RAPD analysis. The primers were OPZ5, OPZ6, OPZ7, OPZ8, OPZ9, OPZ10, OPZ11, OPZ12, OPZ13, OPZ14, OPZ15, OPZ18, OPZ19, OPZ20, OPZ21 AND OPZ22 of which three primers produced clear banding patterns and were used for dendrogram construction.

The amplification product generated by each RAPD primer were scored as “1” or “0” for presence or absence of specific band. DNA fingerprinting, a tool widely used in forensic science is also useful in a variety of applications with plants. It is used to identify cultivars; to positively identify and differentiate accessions; genetic diversity within breeding populations, and species that might be difficult to characterize due to similar morphological characteristics or indistinct traits. It’s also used to identify plants containing genes of interest such as the confirmation of transformation events. A number of molecular tools and procedures are being employed to establish DNA fingerprinting profiles and each of these procedures has its strengths and weaknesses.

REFERENCE