Genetic Diversity Determination of Jasmine Species by DNA Fingerprinting Using Molecular Markers

Sushant Shekhar*, Sujatha Sriram¹, Prasad M.P¹

*Research Scholar, Department of Microbiology, Tumkur University, Tumkur
¹Sangenomics Research Labs, Bangalore- 560071.

Abstract

Plants are potent biochemical factories for bioactive components for medicinal use. Plant constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds etc. Jasmine plants are of great economic value as a field crop for the florist, landscape, medicinal and pharmaceutical industries. In this 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. OPX 6 primers produced most distinguished 75 bands with 8-11 bands per sample. 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.
1. Introduction

*Jasminum* commonly known as Jasmine is a well-known glabrous twining shrub widely grown in gardens throughout India. Its leaves are mostly ternate or pinnate; the flowers, usually white with a tubular, five- or eight-crested calyx, a cylindrical corolla-tube, with a spreading limb and two stamens enclosed in the corolla-tube. The flower is acrid, bitter with a sharp taste. Although more than 2,000 species are known, 40 species have been identified in India and 20 are cultivated in South India (Bhattacharjee, 1980). Jasmine plants are of great economic value as a field crop for the florist, landscape, medicinal and pharmaceutical industries (Green & Miller, 2009). Jasmine can be grown in a variety of climate and soils. Generally, it prefers mid-tropical climate for proper growth and flowering. Mostly, jasmine plants are grown in houses and gardens for ornamental purposes, and are sometimes also used for cut flowers to make garlands. However, there are a few species with fragrant flowers. Among these species, *J. grandiflorum*, *J. auriculatum* and *J. sambac* are commercially cultivated for oil extraction (Green & Miller, 2009).

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 (Kirtikar KR 1993). The essential oil in flowers is extracted through enfleurage which is widely used for production of Jasmine attars in India (Sharma et al., 1980). Jasmine oil has a wide range of medicinal applications and can be used in perfumery, soaps, flavorings and the cosmetic industry (Lawless, 1995). Therapeutically, jasmine oil is used as an antidepressant, antiseptic, antispasmodic, sedative and uterine tonic (Kang & Kim, 2002; Maxia et al., 2009). Essential oil of *J. sambac* is used as fragrance for skin care products as it tones the skin as well as reduces skin inflammation. 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 [Nayak & Mohan 2007]. In India, Jasmines are cultivated throughout the country. However, the largest area under Jasmine flower production is in Tamil Nadu followed by Karnataka. 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.

2. Materials and Methods

2.1 Plant material

The plant material of *Jasminum species* were obtained from University of agriculture, Bangalore India.

2.2 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$_2$ (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore)
Genetic Diversity Determination of Jasmine Species by DNA Fingerprinting

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.

2.3 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).

3. Results and Discussion
The genomic DNA from the jasmine samples was isolated by Phenol-chloroform extraction method. The qualitative estimation of the DNA on 0.8% agarose gel gave single, sharp and distinct bands devoid of any smear. Thus, genomic DNA of good quality without any degradation was successfully isolated from all the jasmine 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.

Figure 1: DNA fingerprint of Jasmine by OPX 6 primer.
DNA fingerprinting was performed for the eight jasmine samples using Random Amplified Polymorphic DNA (RAPD) technique. RAPD has some advantages such as efficiency to generate a large number of markers without any previous knowledge about the organism genetics, the requirement of small amount of DNA, the quickness and simplicity (Williams et al., 1990; Welsh and Mclelland, 1990). Ten random primers were used for DNA fingerprinting out of them two primers OPX6 and OPX9 produced clear banding patterns (figure 1 and 2). OPX6 produced a total 75 bands ranging from 8-11 and OPX9 produced a total 48 bands ranging from 3-8 for each sample (Table 1 and 2).

**Table 1:** Band scoring and matching for fingerprint of OPX 6.

**Table 2:** Band scoring and matching for fingerprint of OPX 9.
The amplification product generated by RAPD primer was scored as present or absent of specific bands. To estimate the similarity and genetics distance cluster analysis based on frequency similarity with unweighted pair-group with arithmetic average (UPGMA) was performed and dendrogram was constructed (figure 3). Sample 2 and 3 as well as sample 4 and 5 showed maximum similarity match of (90.91%). The results interpret that these sample share same morphological and physiological characteristics.

![Figure 3: Dendrogram generated for DNA fingerprint of OPX 6.](image)

4. Conclusion
RAPD DNA fingerprinting used in this study can used in variety of applications for jasmine such as identification of cultivars, differentiation of accessions; genetic diversity within breeding populations, and characterization of species for indistinct traits.

References


