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Iso-peroxidase profile as taxonomic criteria in the morphologically related species of *Jasminum* sps. (Oleaceae)

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ABSTRACT

Six species of *Jasminum* viz., J*asminum sambac* Ait, *Jasminum officinale* Linn, *Jasminum calophyllum* Wall, *Jasminum flexile* Vahl., *Jasminum grandiflorum* Linn. and *Jasminum pubescens* Willd. was characterized using iso-peroxidase isoenzyme as a identification marker. Each and every species expressed their similarities and variation by the presence and absence of their banding profiles in the gel system. A total of twenty three bands with fifteen different positions with four active zones were observed in this enzyme system. Isoperoxidase profiles distinguished the six species of Jasmine by their unique banding expression viz., *Jasminum sambac* ($PRX3^2$ and $PRX3^4$), *Jasminum officinale* ($PRX1^1$ and $PRX1^6$), *Jasminum calophyllum* ($PRX1^4$), *Jasminum flexile* ($PRX1^7$), *Jasminum grandiflorum* ($PRX1^2$ and $PRX2^2$) and *Jasminum pubescens* ($PRX1^{5,}$ $PRX1^9$ and $PRX3^1$). The cladogram of Isoperoxidase illustrated the genetical similarity and variation between the selected six species of *Jasminum*.

KEYWORDS

Chemotaxonomy;
Isoenzyme;
Peroxidase;
Cladogram.

ABBREVIATION

PRX – Peroxidase,

INTRODUCTION

Electrophoresis is a versatile biochemical technique to detect genetic variation within and between the species or populations[1]. In the middle of the nineteenth century, the basis of electrophoretic analysis of isozyme is employed to understand the gene action in differentiation and development of the species[2]. The genetic similarity co efficiencies provide to summarization of isoenzyme data for inter sample comparative studies and characterization[3]. Nowadays, molecular diagnostic techniques are applied to estimate the extent of genetic diversity within and between populations[4]. Unlike, morphological markers, biochemical markers are not prove to environmental influences and port ray the genetic relationships between plant groups. Isozymes are adaptable tool for the species diversity analysis within and between the species of the plants and animals[5-8]. In addition, most of population DNA based marker studies provides the same type of in-

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formation as isozymes[9]. Isozyme provides relatively simple and inexpensive method of attaining genetic information. Most of the population, conservation and rescue projects hold the role 'the cheaper and better' because cost can be crucial point. In addition to developmental studies, isoenzymes are employed to reveal the variation between and within the plant populations as taxonomical criteria[10], genetic uniformity confirmation[11,12], Somoclonal variation studies[13-15] and crop development, differentiation, speciation programme[16-19]. *Jasmine* is grown commercially in various part of the world especially in India. The genus *Jasminum* comprised about 300 species, of which 72 species are present in India. The Jasmine oil extracted from *Jasminum* sps has high value; the amount of oil was varied from species to species. In addition to perfume industry, they are used for various pharmaceutical industries. Based on this background, six *Jasminum* species were selected for present investigation. In this study, we investigated the genetic control of the isozyme of the enzyme peroxidase, in order to illustrate the use of enzymatic variation for the identification of selected six species. The results obtained in the present investigation are expected to be useful for researchers dealing with plant systematists, perfume industries, pharmaceuticals and for plant breeders.

MATERIAL AND METHOD

The present study was included six species of *Jasminum* viz., *Jasminum sambac* Ait (Lane 1), *Jasminum calophyllum* Wall (Lane 2), *Jasminum officinale* Linn (Lane 3), *Jasminum flexile* Vahl (Lane 4), *Jasminum grandiflorum* Linn (Lane 5) and *Jasminum pubescens* Willd (Lane 6). The six species were collected from the wild and confirmed the identification with flora. For peroxidase (EC 1.11.1.7), 500 to 1000 mg of freshly harvested young leaves were taken and homogenized with 3.5 ml of ice-cold 0.1M phosphate buffer (pH 7.0) in a pre-chilled pestle and mortar and centrifuged at 12,000 rpm for 10 min and the supernatant was collected used for iso enzyme (peroxidase) analysis[7]. The Poly acrylamide gel electrophoresis was performed by Anbalagan[20] method. The staining and fixation of the enzyme was performed by the Sadasivam and Manickam[21] method.

Pairing affinity or similarity index was calculated by the method described by Sokal and Sneath[22]. Based on the results of the electrophoresis analysis of the peroxidase, the degree of pairing affinity (PA) was calculated by the following formula.

$$PA = \frac{\text{Bands common to species A \& B}}{\text{Total bands in A \& B}} \times 100$$

OBSERVATIONS AND DISCUSSION

Multiple zones of activity were obtained for this enzyme (peroxidase) system PRX1 to 4. Zone one (PRX1) contained nine bands with nine different (MW – Rf values) positions (Figure 1). $PRX1^1$ (0.028) showed its unique presence only in *J. officinale*. $PRX1^2$ (0.037) was restricted to *J. pubescens*. $PRX1^3$ (0.040) was shared by *J. sambac* and *J. flexile*. $PRX1^4$ (0.042) was restricted with *J. calophyllum*. $PRX1^5$ (0.045) was present only in *J. pubescens*. $PRX1^6$ (0.048) was expressed only in *J. officinale*. $PRX1^7$ (0.068) was restricted to *J. flexile*. $PRX1^8$ (0.074) showed its presence in J. calophyllum and J. grandiflorum. $PRX1^9$ (0.096) showed its unique presence in *J. pubescens*. Zone / Region two illustrated two bands in two different positions viz., $PRX2^1$ (0.119) and $PRX2^2$ (0.180). $PRX2^1$ (0.119) was shared by *J. sambac, J. flexile* and *J. grandiflorum*. $PRX2^2$ (0.180) was restricted its presence to *J. grandiflorum*. Zone / region three showed two bands $PRX3^1$ and $PRX3^2$ with two different MW – Rf values viz., 0.249 and 0.255. $PRX3^1$ showed its presence only in *J. pubescens* and $PRX3^2$ was restricted to *J. sambac*. Zone / Region four illustrated with two bands viz., $PRX4^1$ and $PRX4^2$ (0.331 and 0.371). $PRX4^1$ (0.331) was shared by *J. sambac, J. calophyllum, J. flexile, J. grandiflorum* and *J. pubescens*. $PRX4^2$ (0.371) was expressed its expression only in *J. sambac*.

A total of twenty three bands with fifteen rf values in four active zones / regions were observed in this enzyme system. The isoperoxidase banding profile system revealed the biochemical variation and similarity between the six selected species of Jasmine. The isozy-

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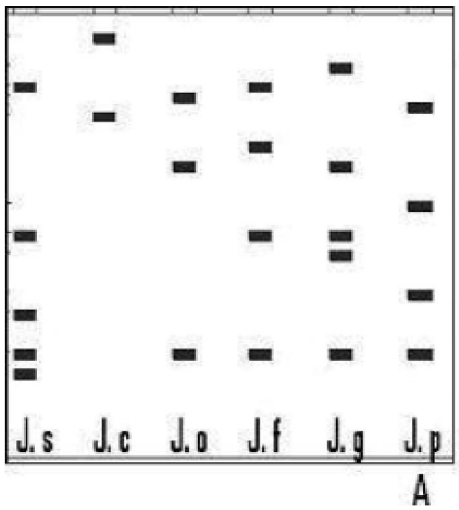

Figure 1A : Zymogram of the isoperoxidase profile of six selected species of Jasminum J.s.-J.sambac; J.c.-J.calophyllum; J.o.-J.officinale; J.f.-J.flexile; J.g.-J.grandiflorum; J.p.-J.pubescens

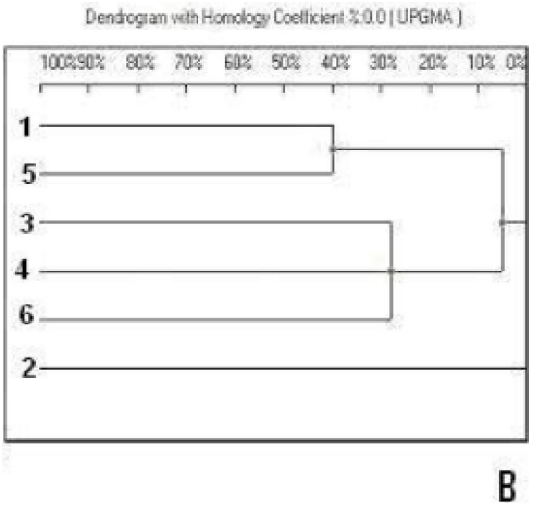

Figure 1B : Cladogram of the isoperoxidase profile of six selected species of Jasminum 1-J.sambac; 2-J.officinale; 3-J.calophyllum; 4-J.flexile; 5-J.grandiflorum; 6-J.pubescens

mic profiles revealed the diversity existing at biochemical level between the six selected Jasmine species. According Hamrick and Godt[1], isozymes are practical, useful genetic and biochemical markers as well as good estimators of genetic variability in plant populations. In the present study we used the isoperoxidase as a tool for inter specific variation studies. Here, the presence and absence of bands has been used to categorize the six species belongs to one genus by the biochemical compositions. In zone / region one *J. officinale*, *J. calophyllum*, *J. flexile* and *J. pubescens*, In zone / region two *J. grandiflorum* and Zone / region four *J. sambac* showed more than one band in this enzyme system. Each zone / region is occupied a particular isozyme in the form of band and is representative of the expression of a particular gene locus coding for that isozyme. In certain species, in a particular zone / region more than one distinct band is resolved. These bands represent allelic isozymes, coded by different alleles of the same gene at locus and thus occupy that particular zone / region in the enzyme system. The present profile system confirmed the multiple allele presence in the selected six species.

Pairing affinity or similarity index analysis revealed the similarity between the six selected Jasmine species. Highest percentage of similarity was observed in *J. flexile* and *J. sambac*. *J. calophyllum* and *J. flexile* showed 28.6% of similarity, *J. calophyllum* and *J. pubescens* also showed 28.6% of similarity, *J. calophyllum* and *J. grandiflorum* showed the second highest similarity 50%. *J. calophyllum* and *J. sambac* showed 25% of similarity. *J. flexile* and *J. pubescens* also showed 25% of similarity. Lowest percentage similarity and highest percentage of variation was observed in *J. flexile* and *J. grandiflorum*. The cladogram of Jasmine revealed the genetical similarity and variation between the selected six species. The cladogram shown that two clusters, of which cluster 2 includes only one species viz., *Jasminum officinale* showed 100% of divergence with other species. Cluster 1 (C_1) showed two nodal (N) branches (C_1N^1 and C_2N^2). Nodal 1 showed two branches (B), $C_1N^1B_1$ was *J. sambac* and $C_1N^1B_2$ was *J. grandiflorum* (Figure 2). Nodal 2 (C_1N^2) showed 3 branches viz., $C_1N^2B_1$, $C_1N^2B_2$and $C_1N^2B_3$. $C_1N^2B_1$ was demonstrated by *J. calophyllum*, $C_1N^2B_2$ was restricted to *J. flexile* and $C_1N^2B_3$ was unique to *J. pubescens*. The enzymatic variation between the six species shows that this gene pool is a good resource for breeding studies and programme. This profile system can use for identifying the correct species for future breeding and other studies. They can also be used toward a better understanding of phylogentic relationships of different species. The electrophoretic separation constructed a pave for the biochemical and molecular studies on the six selected species in near future. However it is necessary to use molecular marker to generate transgenic hybrids.

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