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Studies on morphological and genetic diversity of Jasmine ecotypes of coastal Karnataka

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ABSTRACT

In the present investigation, morphological differences among the three jasmine ecotypes *viz.,* Bhatkal mallige, Udupi mallige, and Mangalur mallige cultivated commercially in coastal districts of Karnataka *viz.,* Uttara Kannada, Udupi and Dakshina Kannada respectively. The qualitative character of the three jasmine ecotypes was recorded as per the DUS character of jasmine given by PPV and FRA, whereas quantitative traits of the plant vegetative characters and floral parts were recorded and statistically analysed. The dendrogram constructed on the quantitative characters represented two clusters. The cluster analysis clearly suggested that Bhatkal mallige and Udupi mallige are morphologically similar and are distinctively different from Mangalur mallige. The genetic diversity was evaluated using RAPD markers for which 10 random primers were used. The clustering of the three jasmine ecotypes based on RAPD analysis through the dendrogram revealed two major clusters where Bhatkal mallige and Udupi mallige formed together one cluster showing more similarity and these two ecotypes were found to have maximum genetic diversity with Mangalur mallige. The present study showcased the morphological and genetic characters of the three commercially important jasmine ecotypes of coastal Karnataka. The phenotypic studies and RAPD analysis of the three ecotypes clearly show that there are less morphological and genetic variations among Bhatkal mallige and these ecotypes show maximum diversity from Mangalur mallige.

Key words: *Dendrogram, DUS characters, Ecotypes, Mallige, Primers, RAPD analysis*

Introduction

*Jasminum samba*c L. (Aiton) member of the family Oleaceae is a small shrub grows to a height of 3m and is being cultivated commercially for its flowers. The genus *Jasminum* is known to have 40 species cultivated in India, among them 20 species are cultivated in south India (Bhatacharjee, 1980). The coastal Karnataka which is covered throughout its length by the rich heritage of Western Ghats is known for its wide range of biodiversity and it is considered as the eighth hottest biodiversity hot spot of the world by UNESCO. The three districts of coastal Karnataka comprising of Uttar Kannada, Udupi and Dakshina Kannada are known for the cultivation of jasmine especially by the female members of the family and its one of major earnings for the family. The jasmine flower is commercially grown for its fresh flowers for adoring hair and for decoration during religious functions. Among the different jasmine ecotypes cultivated in coastal Karnataka *viz.,* Bhatkal mallige cultivated in Uttar Kannada district, Udupi mallige in Udupi district and Mangalur mallige in Dakshina Kannada dis-

trict, a distinctive morphological diversity is observed. The study of this diversity with respect to morphological traits will be the base for conducting a breeding program. Suitable knowledge of genetic diversity and the adaptability of the plants to various agro-climatic situations is a pre-requisite for the conservation of plant genotype and for future use (Ramanathan and Hodgkin, 2002). As there is a need to evaluate morphological characters among the different jasmine ecotypes of coastal Karnataka for future breeding work and conservation, a study was initiated to know the morphological characters of important jasmine ecotypes of *Jasminum* sp. of coastal Karnataka.

Materials and Methods

A study on morphological traits in different ecotypes of *Jasminum sambac* was carried out at Zonal Agricultural and Horticultural Research Station, Brahmavar, Udupi Dist. Karnataka State, from 2015 to 2018. In the present study, an extensive survey was conducted in coastal districts of Karnataka from Uttar Kannada, Udupi and Dakshina Kannada Dist. The ecotypes collected from their natural habitat were propagated through cuttings and were planted in the gene bank maintained at Zonal Agricultural and Horticultural Research Station. Three ecotypes of *Jasminum sambac viz.*, Bhatkal mallige, Udupi mallige and Mangalur mallige collected from Uttar Kannada, Udupi and Dakshina Kannada respectively. The morphological traits with respect to plant growth characters both qualitative and quantitative were evaluated from the above ecotypes. The observations for morphological characters were recorded as per the DUS character of Jasmine given by the PPV&FRA of Jasmine (Anon. 2015). Morphological characterization of jasmine ecotypes was recorded as per the distinctive character which is differentiated into qualitative and quantitative character. The current season shoots were selected to record the morphological characters with respect to primary and lateral shoots. The qualitative morphological characters *viz.*, plant growth type, plant growth habit, plant height at flowering, young shoot anthocyanin colouration), young shoot intensity of anthocyanin colouration, ridges on the stem, leaf arrangement/ phyllotaxy, leaf size, intensity of green colour, leaf anthocyanin colouration, leaf glossiness on upper surface, shape of the leaf blade, leaf tip and the base of leaf blade, flower-bearing habit and position, calyx length, size of calyx lobes, boldness of flower bud, flower bud shape, flower bud colour, tinge of flower bud, flower colour on opening, flower petal tip, reflexing of flower, seed setting, season of flowering and flower type were recorded. The quantitative phenotypic characters with respect to leaves included petiole length, length and breadth of the leaf. The quantitative traits recorded in the flowers of the three jasmine ecotypes included length and breadth of the bud, flower diameter, flower stalk length, calyx length, number of calyx teeth, corolla tube length, number of petals, length and breadth of the petal, number of stamens, length of the stamen and the pistil.

The quantitative characters were analysed statistically and the clustering of three ecotypes was done using NTSYS-PC, Version 2.11w and Past 3.x software package (Hammer *et al.*, 2001). The cluster analysis was performed to know the similarity between the three ecotypes of jasmine. The genetic diversity among the three ecotypes was evaluated by using RAPD markers. DNA was extracted and the protocol was standardized using Cetyltrimethyl Ammonium Bromide (CTAB) method (Porebski *et al*., 1997). The ecotypes which are genetically similar are estimated using a similar matching coefficient.

Results and Discussion

The morphological variations with respect to plant growth characters and floral characters were observed among the three ecotypes of jasmine to a greater extent. The three cultivars of jasmine have been classified under shrubs having an intermediate plant growth habit and the plants bear flowers at a medium plant height of 45-100 cm. The anthocyanin coloration on the young shoot was absent in the case of Bhatkal mallige and Mangalur mallige, whereas in the case of Udupi mallige anthocyanin coloration with a low intensity was recorded. The three ecotypes of jasmine recorded opposite leaf arrangement and the intensity of the leaf colour on the upper surface of the leaf was medium green in the case of Bhatkal mallige, light green in the case of Udupi mallige and dark green in case of Mangalur mallige. The anthocyanin coloration on the leaf was absent in all the ecotypes, whereas the leaf glossiness on the upper surface of matured leaf was present only in Bhatkalmallige.

Three ecotypes exhibited the lanceolate shape of the leaf blade, having sharp leaf tip and acute shape

of the leaf blade base. Cluster bearing of flowers was observed in Bhatkal mallige whereas Udupi mallige and Mangalur mallige bear flowers that are both solitary and cluster inhabit. The three ecotypes bear flowers at the terminal and axillary position with a well-developed calyx and medium sized calyx lobes. The genetic diversity with respect to morphological characters among *Jasminum sambac* was studied by Mukundan *et al.,* (2008) and he had reported morphological differences in floral and leaf characters among different genotypes of *Jasminum sambac.*

The boldness of flower buds in all the three ecotypes was thin, with pointed and long in shape in Bhatkal mallige and Mangalur mallige whereas Udupi mallige flower buds were pointed and short in shape. The floral buds having pointed and conical shape has a high market preference and is used for making garlands as it looks more attractive and appealing (Champa, 2012). The character with respect to flower bud colour is observed to be white in case of ecotypes Bhatkal mallige and Udupi mallige whereas cream colour in case of Mangalur mallige and it remains cream colour even when the flowers are open. Bhatkal mallige and Udupi mallige do express blunt tip of flower petal whereas the sharp tip of flower petal is observed in Mangalur mallige. The reflexing of the flower is absent in all the three cultivars which bear flowers of single whorl and do produce seeds. Seasonal flowering was observed in Mangalur mallige, whereas flowering throughout the season is observed in Bhatkal mallige and Udupi mallige. Some ecotypes belonging to *Jasminum sambac* which flower throughout the year was ear-

Table 1. Morphological characters recorded in three cultivars of *Jasminum sambac*

Sl. No.	Plant character	Bhatkal mallige	Udupi mallige	Mangaluru mallige
1	Plant growth type	Shrub	Shrub	Shrub
2.	Plant growth habit	Intermediate	Intermediate	Intermediate
3.	Plant height at flowering	Medium	Medium	Medium
4.	Young shoot anthocyanin colouration	Absent	Present	Absent
5.	Young shoot intensity of anthocyanin colouration	Weak	Weak	Weak
6.	Ridges on the stem	Absent	Absent	Absent
7.	Leaf arrangement/ Phyllotaxy	Opposite	Opposite	Opposite
8.	Leaf size	Medium	Medium	Medium
9.	Intensity of green colour (Upper surface of mature leaf)	Medium green	Light	Dark green
10.	Leaf anthocyanin colouration	Absent	Absent	Absent
11.	Leaf glossiness on upper surface (mature leaf)	Present	Absent	Absent
12.	Shape of leaf blade	Lanceolate	Lanceolate	Lanceolate
13.	Leaf tip	Sharp	Sharp	Sharp
14.	Shape of base of leaf blade	Acute	Acute	Acute
15.	Flower bearing habit	Cluster	Solitary & Cluster	Solitary and Cluster
16.	Flower bearing position	Terminal & Axillary	Terminal & Axillary	Terminal & Axillary
17.	Calyx	Well developed	Well developed	Well developed
18.	Size of calyx lobes	Medium	Medium	Medium
19.	Boldness of flower bud	Thin	Thin	Thin
20.	Flower bud shape	Pointed and Long	Pointed and Short	Pointed and Long
21.	Flower bud colour	White	White	Cream
22.	Tinge of flower bud	Absent	Absent	Absent
23.	Flower colour on opening	White	White	Cream
24.	Flower petal tip	Blunt	Blunt	Sharp
25.	Reflexing of flower	Absent	Absent	Absent
26.	Seed setting	Present	Present	Present
27.	Season of Flowering	Throughout the season	Throughout the season	Seasonal
28.	Flower type	Single	Single	Single

lier reported by Bhatnagar (1956) and Raman *et al.*, (1955).

Any flower crop to be cultivated commercially depends on the quantitative character of the flower in terms of bearing, number of petals, length of the flower bud, number of whorls, corolla tube length, flower diameter and the size of the petals.

The length and breadth of the flower bud was recorded highest in Bhatkal mallige (1.425 cm and 0.431 cm) and was significantly different from the other two ecotypes Udupi mallige (1.365 cm and 0.428 cm) and Mangalur mallige (1.398 cm and 0.377 cm), whereas non-significant difference was recorded with respect to flower diameter. The ecotype Udupi mallige recorded maximum stalk length and calyx length (1.202 cm and 1.124 cm respectively) which was significantly superior to the stalk length and calyx length of Bhatkal mallige (1.148 cm and 1.047 cm respectively) and Mangalur mallige (0.974 cm and 0.988 cm respectively). The parameter with respect to the maximum number of calyx teeth was recorded in Mangalur mallige (5.262) which was found to be significantly higher when compared with Udupi mallige (4.484) and Bhatkal mallige (4.202).

The length of the corolla tube was recorded maximum in the case of Bhatkal mallige (0.851 cm) and was statistically significant from Udupi mallige

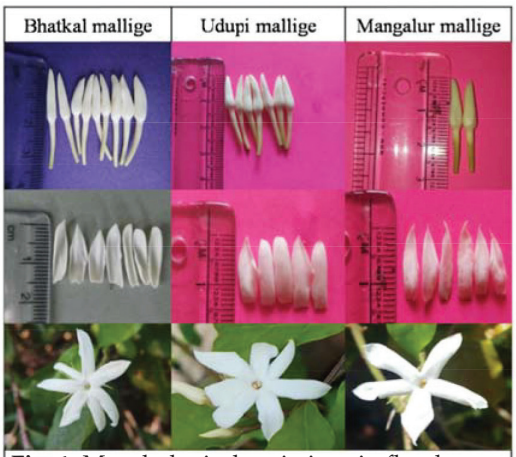


Fig. 1. Morphological variations in floral parts

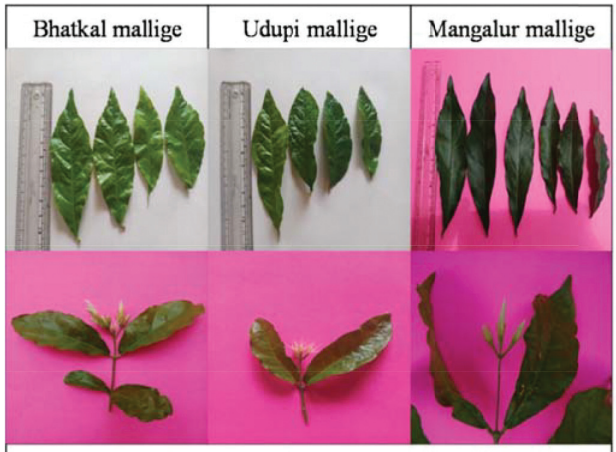


Fig. 2. Morphological variations in leaves

Table 2. Quantitative traits recorded in leaves and buds of three ecotypes *Jasminum sambac*

Jasmine cultivars	Petiole length (cm)	Leaf length (cm)	Leaf Breadth (cm)
Bhatkal mallige	2.225	8.427	3.218
Udupi mallige	2.254	9.207	3.148
Mangaluru mallige	2.102	11.901	2.885
CD@5%	0.21	0.039	0.019
CV (%)	0.476	0.318	0.55

Table 3. Quantitative traits recorded in flowers of three ecotypes *Jasminum sambac*

Jasmine cultivars	Bud length (cm)	Bud Breadth (cm)	Flower Diameter (cm)	Flower Stalk length (cm)	Calyx length (cm)	Calyx teeth (No.s)	Corolla tube length (cm)	No. of whorls
Bhatkal mallige	1.425	0.431	3.101	1.148	1.047	4.202	0.851	1
Udupi mallige	1.365	0.428	3.142	1.202	1.124	4.484	0.772	1
Mangaluru mallige	1.398	0.377	3.188	0.974	0.988	5.262	0.665	1
CD@5%	0.013	0.012	NS	0.011	0.016	0.03	0.021	NS
CV (%)	0.828	2.14	6.848	0.863	1.36	0.628	2.817	NA

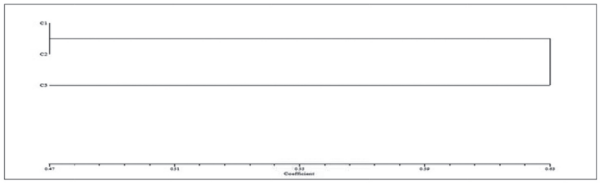
(0.77 cm) and Mangalur mallige (0.65 cm). All three ecotypes of jasmine recorded a single whorl with an almost similar number of petals. Variations with respect to the number of whorls, petal lobes, shape, size and colour in different genotypes of *Jasminum* sp has been reported earlier by Mukundan *et al.*, 2008; Champa, 2012 and Safeena *et al.*, 2017. The observations with respect to petal length were observed maximum in the case of Bhatkal mallige (1.632 cm) followed by Mangalur mallige (1.568 cm), whereas the jasmine ecotype Udupi mallige recorded least petal length (1.301 cm).

The phenotypic variations in floral characters among different genotypes of jasmine under different ago-climatic situations have been reported by Malik Abid Mahamood *et al.,* (2013); Sushant Shekar *et al.,* (2013) and Nirmala *et al.,* (2017).

Morphological diversity

The morphological characters of the three ecotypes of jasmine were statistically analysed and a dendrogram was constructed.

The dendrogram of three ecotypes clustered into two groups which clearly suggested that cluster-1 constituted Bhatkal mallige (C1) and Udupi mallige (C2) which were morphologically similar and were significantly different with respect to quantitative characters from Mangalur mallige (C3).



C1: Bhatkal mallige, C2: Udupi mallige, C3: Mangalur mallige

Fig. 3. Dendrogram of jasmine ecotypes based on morphological data

Analysis of diversity using RAPD markers

Randomly Amplified Polymorphic DNA marker analysis was carried out in 03 jasmine ecotypes *viz.*, Bhatkal mallige, Udupi mallige, Mangalur mallige, and DNA fingerprinting was performed by the Random Amplified Polymorphic DNA (RAPD) using PCR. From the RAPD-PCR, a spectrum of amplified products which represented the characteristics of the selected three ecotypes of jasmine. Five primers, three from OP-B, one from OP-C and another one from OP-F series produced bright and clear bands which ranged from 100bp to 1500bp from the ten decamer primers. Overall 51 amplicons were detected, where 27 amplicons were polymorphic and 24 amplicons were monomorphic. The number of markers or bands scored for each primer varied from 05 to 14. The information on the extent of polymorphism with each primer is given in Table 5. The

Table 4. Quantitative traits recorded in flowers of three cultivars jasmine

Jasmine cultivars	Petal (No.s)	Petal length (cm)	Petal breadth (cm)	Stamens (No.s)	Stamen length (cm)	Pistil length (cm)
Bhatkal mallige	6	1.632	0.575	2	0.7	1.437
Udupi mallige	5	1.301	0.521	2	0.665	1.322
Mangaluru mallige	6	1.568	0.528	2	0.575	1.234
CD@5%	NS	0.026	0.017	NS	0.012	0.015
CV (%)	NA	1.295	2.171	NA	1.61	0.818

Table 5. Amplification pattern in jasmine ecotypes with RAPD primers

Sl. No.	Primers	Total no. of amplicons	No. of polymorphic amplicons	No. of monomorphic amplicons	Polymorphism (%)	Size of amplicons (range-bp)
1.	OPB 02	14	10	4	71.42	ˆ1500-100
2.	OPB 05	13	8	5	61.53	ˆ1500-300
3.	OPB 10	9	2	7	22.22	1000-120
4.	OPC 06	5	1	4	20.00	800-100
5.	OPF 01	10	6	4	60.00	>1500-700
	Total	51	27	24	47.03	

primers gave good amplification and differentiated the jasmine ecotypes. The average percentage of polymorphism shown by five primers was 47.03 per cent, whereas it was high (71.42 %) in OPB 02 primer and low (20%) in OPC-06 primer.

RAPD profile analysis

The investigation on diversity analysis among the three jasmine ecotypes revealed higher diversity up to 71.42 per cent and out of the five primers, three primers depicted more than 60 per cent polymorphism indicating moderate variations among the jasmine ecotypes.

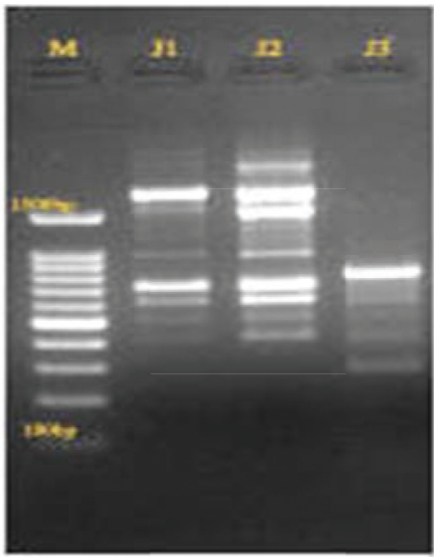


Fig. 4. RAPD-PCR amplified products with primer OPB-05 primer

Genetic diversity and cluster analysis

Dendrogram using Average Linkage (Between Groups)

The clustering of three jasmine ecotypes based on RAPD analysis through dendrogram revealed two major clusters with two jasmine ecotypes grouped under cluster-1 indicating moderate genetic diversity among Bhatkal mallige(J1) and Udupi mallige (J2) compared to high to very high genetic diversity between Mangalur mallige (J3) and Bhatkal mallige (J1). The higher diversity is also noticed between Mangalur mallige (J3) and Udupi mallige (J2). The study reveals that Bhatkal mallige (J1) and Udupi mallige (J2) are closely related whereas Mangalur mallige (J3) is distantly related to Bhatkal mallige (J1) and Udupi mallige (J2). Similar observations with respect to jasmine morphological and genetically studies were recorded by Champa 2012; Malik *et al*., 2013 and Sushanth Shekar *et al*., 2014.

The three ecotypes of viz., jasmine Bhatkal mallige, Udupi mallige and Mangalur mallige in the present study where characterized morphologically based on qualitative and quantitative characters. The genetic variations among the three ecotypes were studied through DNA fingerprinting using RAPD markers. The cluster analysis of the quantitative characters of the three ecotypes of jasmine indicates the morphological characters of Bhatkal mallige and Udupi mallige are very much similar and these two ecotypes of jasmine is a far relative of Mangalur mallige. These results are very much substantiated by the RAPD profile analysis which represents a similar clustering of the three ecotypes.

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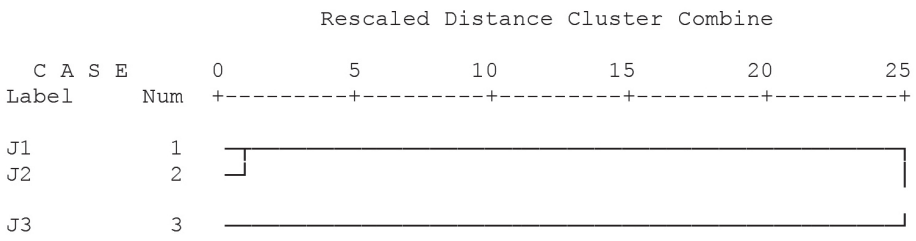

Dendrogram using Average Linkage (Between Groups)

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