



Intraspecific Variations in *Curcuma inodora* Blatt. from Melghat Forest Dist. Amravati (MS), India

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ABSTRACT

Genus *Curcuma* L. (Zingiberaceae) comprising of 120 species is distributed throughout South South-East Asia, with a few species extending to China, Australia and South Pacific. *Curcuma inodora* Blatt. known as 'Jangali Halad' is a common herb of Melghat at higher elevations. It is reported from other districts of Maharashtra also. In Melghat area populations of *C. inodora* were found to show many variations. Twelve distinct accessions were collected for present study and for comparison *C. pseudomontana* J. Graham and *C. longa* L. were also studied. The species shows variations in aerial as well as underground parts. For *Curcuma* species position of spike is considered as key character. Other major variations found are length of spike; shape, size and colour of bracts; length of leaf stalk and shape of root tubers. Cluster analysis of 50 morphological characters was done by UPGMA using PAST software placed 12 variants in to two clusters as per Peldjis method A and in three clusters as per the method D. Careful study of these variations will help to understand evolution within the species.

Key words: *Curcuma inodora* Blatt., Melghat Forests, Population variations, Cluster analysis.

INTRODUCTION

Genus *Curcuma* L. (Zingiberaceae) comprising of 120 species is distributed throughout South and South-East Asia, with a few species extending to China, Austrilia and the South Pacific. *C. inodora* is widely distributed throughout Maharashtra and is very common and abundant in Melghat. It is commonly called 'Jangli halad' and used in traditional medicine by locals. Fresh rhizome paste is applied over cut, as strong antiseptic. The smoke of dried rhizome used to hypnotise the person, some use it in Tantrik, Vashikarana and Mayajal Kriyas (Devarkar ,2001). *C. inodora* Blatt. paste of root stock is applied in glandular diseases and piles (Shah

and Gopal 1982; Mudliyar *et al.* 1987 ; Bhogaonkar and Kadam 2005), psychosomatic disorders and constipation (Rommand-Monnier 2009 and Jagtap *et al.* 2009).

Populations of *C. inodoara* observed in Melghat display tremendous variation in morphological characters such as size of leaf , length and position of inflorescence, bract colour and shape, shape and notching of labellum etc. Intraspecific variations are recently attracting attention of taxonomists

MATERIALS AND METHODS

The study was carried out in two steps: a) Rapid Vegetation Survey and b) Study of plasticity of characters.

a) Rapid vegetation survey

In all three species *Curcuma* species are reported from Melghat of these two are wild viz- , *C. inodora* Blatt. and *C. pseudomontana* J.Graham and one is cultivated i.e. *C. longa* L.

Different localities were visited to note population variations shown by *Curcuma* species growing in Melghat Forests. Survey was carried out to observe the species and species populations of *Curcuma*. Species were identified using standard floras (Hooker,1872; Cooke,1967; Patel, 1968; Dhore, 1986; Naik, 1998 ; Bhogaonkar and Devarkar 1999; Yadav and Sardesai,

2002). However only *Curcuma inodora* populations were found to show great variation in morphology.

Survey was carried out during 2009 to 2015 in the months of June to September. During this survey various locations in Melght forests were visited to note down the population distribution and variations in *C. inodora* . The area and GPS locations of the populations were noted. To study the population variations Survey Sheet was prepared which includes both qualitative and quantitative characters. Since the flowers of *C. inodora* are delicate, most of the floral details were studied on the spot itself. For végétative morphology material was brought to laboratory. Routine permission of Biodiversity board and local bodies were availed.

b) Study of Plasticity of Character:

The species populations showing distinct variations were selected for diversity assessment.

For quantitative variation studies 14 morphometric vegetative and floral characters were selected. For biometric analysis in all twelve distinct variants were selected from different populations. For each variants ten plants were studied. Mungikar (1997) was followed to calculate mean, range, standard deviation, coefficient of variation. (Table 1). Population variation was also studied by considering the qualitative characters.

Table 1: Morphometric character in *Curcuma inodora* Blatt. (12 Selected variants)

SN	Character	Mean	Range	SD	CV
1	Plant height	63.14	46-105	17.78	0.281
2	Leaf stalk length	25.78	15-40	7.10	0.285
3	Leaf Lamina Length	28.71	20-42	6.07	0.219
4	Leaf Lamina Breadth	13.42	8-30	5.12	0.395
5	Length of Spike	25.78	18-35	4.07	0.163
6	Length Spike Stalk	15.57	11-25	3.77	0.251
7	Calyx length	1.07	1-1.3	0.11	0.112
8	Flower length	4.721	4-5	0.42	0.0936
9	Corolla length	3.92	2-5	1.43	0.379
10	Corolla lobe length	1.82	1.5-2.4	0.327	0.186
11	Comma bract length	3.64	1.5-5	0.92	0.259
12	Flowering bract length	3.35	2.5-4.2	0.498	0.153
13	Lip Length	1.99	1.5-2.5	0.2644	0.137
14	Tuber Stalk length	7.85	4-10	1.84	0.243

SD- Standard deviation, CV – Coefficient of variation.

Twelve variants of *C. inodora* and one population of *C. pseudomontana* and *C. longa* each were selected for characterization. In total 50 morphological characters (36 qualitative and 14 quantitative) were selected. (Table- 2)

In phenetic analysis the means of quantitative characters was used, but for qualitative characters, binary and multistate codes were applied. Character coding was done as per Chatfield and Collins. (1995) and Sheidai *et al.* (2000),

This study deals with the numerical taxonomy of 14 populations based on 50 qualitative and quantitative characters of 130 descriptions coded in to positive (1) and negative (0) state in a two state coding system in N x T table, where N stands for number of characters and T stands for OTUS (Operational Taxonomic Units). Two methods of coding proposed by Peldji cited in Forey and Kitching (2000) were selected. As per this coding system there are two methods – 1. method A: multistate qualitative characters is coded as multistate (also called conventional coding) in this case, each characteristic state was given a unique number i.e.0,1,2 etc and quantitative data are entered as continuous (Table no.- 2).

2. Method D: each character state of multistate character was considered as a variable and coded as 0 or 1. Binary characteristics and quantitative characters were coded as in method A.

Cluster Analysis:

Cluster analysis was done using UPGMA (Unweighted Paired Group Mean Average) method. Dendrogram was constructed based on UPGMA-Jaccard similarity indices method by using PAST software (Rohlf, 2000).

RESULTS AND DISCUSSION

Rapid vegetation survey

Rapid vegetation survey was conducted every year from 2009- 2015 during June to September. Maximum extent of Melghat was explored except reserved core areas.

C. inodora was found to be growing at several locations. GPS locations of 55 populations observed were noted. Of

these 12 populations were selected for collection of different variants to be studied.

Taxonomy :

***Curcuma inodora* Blatt.** in Journ and Proceed. Asiat. Soc. Bengal 26: 357. 1931; Santapau in JNBH 51: 135. 1952; Lakshminarsimhan in Sharma *et al.*, Fl. Maharashtra (Monc.) 75, 1996; Yadav and Sardesai, Fl. Kolhapur Dist. 2002.

Rhizome conical, pale yellow in the centre, whitish towards the periphery; root tubers many, at the tips of root fibres, ovoid, white inside. Leafy shoot 20–50 cm tall. Leaves distichous, 3–6, petiolate; petiole 20–25 cm long; lamina 15–30 × 7–12 cm, elliptic, base oblique, tip acuminate, minutely hairy along the prominent side veins on the upper side, lower side glabrous; ligule 2 mm long, hyaline. Inflorescence appearing before shoot is lateral, later one central; peduncle 5–10 cm long; spike 10–16 × 4–5 cm with a prominent, violet coma. Coma bracts large, 4–5.5 × 1.5 cm, ovate or lanceolate tip obtuse, Fertile bracts 3.5–4 × 1.5 cm, pale green with a purple patch at the tip, tip slightly acute or round, not recurved. Bracteoles small, outer 1.5 × 0.6 cm, inner 4 × 2 mm, linear, purple, minutely pubescent. Flowers 4.5–5.5 cm long, equal to the bracts. Calyx tubular, 1–1.2 cm long, shortly and irregularly tri-lobed at apex, white, tube minutely pubescent. Corolla tube 2.5 cm long, deep purple, pubescent; lobes unequal, dorsal lobe c.1.6 × 1 cm, apiculate, lateral lobes 1–1.3 × 0.5 cm, ovate-lanceolate, very thin, deep purple, glabrous. Labellum obovate, obscurely trilobed, tip emarginate, purple with a median bright yellow band. Lateral staminodes oblong, 1.5–1.6 × 0.5–0.7 cm, deep purple. Stamen white with pink base; anther 4 mm long, spurs bent inwards. Epigynous glands two, 5 mm long, linear. Ovary 2.8–3.2 × 2–2.2 mm diameter, trilobular, with many ovules on axile placenta, densely pubescent; style long, filiform, pink; stigma fringed, bilobed. Fruit globose to ovoid, 1–1.2 × 0.9 cm, brown, hairy. Seeds many, 3 × 2 mm, brown, aril white.

Flowering: July to September

Note: Underground part of the plant is described as rhizome or rootstock or as tuber by different authors. Root fibres are also tuberous at tip. Hence, it is confusing to describe the underground part as tuber. In the present study it is mentioned as rhizome and swollen root tips as

tuber; tuber at the tip of root fibre is described as stalked tuber.

***Curcuma pseudomontana* J.Graham** Cat . Bombay Pl.210, 1839; Dalz and Gibs., Bombay Fl.275 1861; Lisboa in Journ. Bombay Nat. Soc.2: Soc.2: 144, 1887; Cook, Fl.Pres. Bombay 2: 730,1907; Bole and Almeida, JBNH 83:585, 1986; Mangaly and Sabu in J.Econ. Tax.Bot.10;160, ff,A-G. 1987; Lakshminarsimhan in Sharma *et al.*, Fl. Maharashtra (Monoc.) 76, 1996; Yadav and Sardesai, Fl.Kolhapur Dist 478,2002 .

Rhizome conical, pale yellow in the centre, whitish towards the periphery; root tubers many, ovoid, white inside. Leafy shoot 53 cm tall. Leaves distichous, 3–6, petiolate; petiole 22 cm long; lamina 26 X13 cm, elliptic, base oblique, tip acuminate, minutely hairy along the prominent side veins on the upper side, lower side glabrous; ligule 2 mm long, hyaline. Inflorescence both lateral and central; peduncle 12 cm long; spike 18 cm with a distinct, pink coma. Coma bracts large, linear, obtuse. Fertile bracts, light green, ovate, obtuse. Flowers 5 cm long, equal to the bracts. Calyx tubular, 1–1.2 cm long, shortly and irregularly tri-lobed at apex, white, tube minutely pubescent. Corolla tube .2.5 cm long, deep violet, tip obtuse. Labellum becoming bilobed because of deep notch, dark yellow; staminode yellow, lip longer than other staminode. Anther 4 mm long, thecae parallel, white with a pink base, spurs bent inwards. Ovary hairy, trilobular, stigma fringed.

Flowering: July to September

***Curcuma longa* L.**, Sp.Pl. 2: 1753; Graham, Cat. Bombay Pl.209, 1839; Lindl., Bot Reg.t.886, 1825; Voight, Hort. Suburb. Cal.565, 1847; Baker in Hook, f.,Fl. Brit. Ind. 6:214, 1892; Dalz and Gibs., Bombay 2 : 732, 1907; Burt in Notes Roy.Bot. Garden. Edinberg 35: 212, 1976; Burt in Notes Roy.Bot.Gard. Edinb. 35: 212, 1977; Lakshminarsimhan in Sharma *et al.*, Fl. Maharashtra (Monoc.) 77,1996; Yadav and Sardesai, Fl. Kolhapur Dist. 476, 2002.

Rootstock large ovoid, with sessile, cylindric tubers; tuber orange inside. Leafy shoot 105 cm tall. Leaves distichous, 3–8, petiolate; petiole 37 cm long; lamina 42 X 15 cm, elliptic, base oblique, tip acuminate, minutely hairy along the prominent side veins on the upper side, lower side

glabrous. Inflorescence central; peduncle 25 cm long; spike 35 cm with a distinct, white coma. Coma bracts large, ovate, obtuse. Fertile bracts broadly ovate, pale green, obtuse. Flowers 4 cm long, equal to the bracts. Calyx tubular, 1 cm long, shortly and irregularly tri-lobed at apex, white. Corolla tube 1.5 cm long, white, tip acute. Lip becoming bilobed because deep notch, white with dark yellow blotch staminode, relative length of lip and other staminode more. Stamen; white with light pink base, spur converging. Ovary hairy, trilobular; stigma fringed.

Flowering: August to November

Plasticity of characters:

Character variations in 55 populations were observed in field. However, these are continuous variations.

Populations showing distinct variations were selected for diversity assessment. 12 accessions amongst the observed populations were selected for further study.

Here they are mentioned as variants and not as accessions.

The variants selected for further study are coded as CI-1, CI-2, CI-3, CI-4, CI-5, CI-6, CI-7, CI-8, CI-9, CI-10, CI-11 and CI-12. Morphological character variations in 12 selected accessions are as per the characters mentioned in survey sheet.

Along with the 12 variants other two species of *Curcuma* reported during rapid vegetation survey were also selected for further study and are coded as CP-13 (*Curcuma pseudomontana*) and CL-14 (*Curcuma longa*) Photoplate -1.

Cluster Analysis:

50 qualitative and quantitative characters were coded. Two methods of coding proposed by Peldji (Forey and Kitching , 2000) were selected.

All variants were analysed using PAST software (version 1.0.0.0) cluster programme that performed UPGMA (Hammer *et al.* 2001). The similarity matrix upon which clusters are based computed using Jaccard indices.

The similarity and distance indices produced by method A are presented in Table no. 3.

Dendrogram formed by UPGMA method (Method -A) indicates that the 14 accessions studied (12 variants of one species and 2 distinct species) clearly get grouped in to two clusters (Fig. 1).

Table 2: Qualitative and Quantitative morphological characters and their coding key

SN	Description of Characters	Coding key for the characters
1	Tuber at the end of root	Absent (0) Present (1)
2	Tuber Stalk Length	Stalk short (0) Stalk long (1)
3	Tuber at the end of root	Tuber sub apical (0) Tuber apical (1)
4	Shape of tuber	Rounded (0) Oval (1)
5	Colour of tuber from inside	Yellow (0) White (1)
6	Leaf apex	Obtuse (0) Acute (1) Mucronate (2) Acuminate (03)
7	Leaf surface Upper	Rough (0) Smooth (1)
8	Leaf surface Lower	Rough (0) Smooth (1)
9	Leaf surface colour Upper	Light green (0) Dark green (1)
10	Leaf surface colour Lower	Dark green (0) Light green (1)
11	Spike position	Lateral (0) Central (1)
12	Sterile bract spread pattern	Horizontal (0) Obliquely (1)
13	Colour sterile of bracts	White (0) White with purple tip (1) White base with pink line (2) Whitish with purple tinge dark purple towards apex (3) Purple tip darker (4) Light purple with dark tip (5) Faint purple with dark purple tip (6) Purplish white with dark middle strip (7) Light pinkish purple with dark pink apex (8) Purple pink (9) Violet (10) Magenta (11)
14	Sterile of bracts tip-	Acute (0) Obtuse (1) Mucronate (2)
15	Sterile bract margin-	Straight (0) Undulate (1)
16	Sterile bract shape	Ovate (0) Obovate (1) Linear (2) Linear Lanceolate (3)
17	Fertile bract Colour	White green with purple tip (0) White with green tip (1) Green (2) Light Green (3) Light green with violet tinge (4) Light green with violet tip (5) Green with violet tip (6) Pale green (7) Light violet (8) Pale yellowish green with purple tinge (9)
18	Fertile bract Shape-	Ovate (0) Broadly ovate (1) Obovate (2) Linear (3) Lanceolate (4)
19	Fertile bract Tip-	Acute (0) Obtuse (1) Mucronate (2)
20	Fertile bract length in relation to flower	Shorter (0) Longer (1) Equal (2)
21	Fertile bract Spread of bract	Horizontal (0) Oblique (1)
22	Flower	Inserted (0) Exerted from bract (1) Equal (2)
23	Calyx Colour	White (0)Light pink (1) Yellow (2)
24	Calyx Shape	Oval (0) Tubular (1)
25	Corolla Colour	Violet (0) Yellow (1) White (2) Light purple (3) Pale violet red (4)

Table 2: Continued...

SN	Description of Characters	Coding key for the characters
26	Corolla Shape of lobes	Tubular (0) Oval (1)
27	Corolla Apex of lobes	Obtuse (0) Acute (1) Mucronate (2)
28	Petaloid staminodes lip	Lip not lobed, with distinct notch. (0)
		Lip becoming bilobed because of deep notch. (1)
		Lip distinctly 3 lobed; central lobe larger, protruding. (2)
		Lip obscurely or not lobed, without notch.(3)
		Lip-trilobed central lobe protruding, obovate, shallowly notched. (4)
		Lip indistinctly three lobed central lobe distinctly notched. (5)
		Lip obscurely or not lobed some what notched. (6)
		Lip almost oval completely splitting from center up to base the 2 units produced diverge. (7)
29	Staminode colour	Reddish purple (0) Almost entire lip yellow (1) Purple with yellow blotch (2) Brownish purple with yellow blotch (3) Upper half almost yellow (4) Yellow (5) White with yellow blotch (6)
30	Yellow blotch on lip	Absent (0) Present (1)
31	Yellow blotch on lip spread	Half band (0) Complete band (1)
32	Relative length of lip and other staminode	Less (0) More (1) Equal (2)
33	Stamen	White base (0) White pink light base (1)
34	Stamen Spur	Diverging (0) Converging (1)
35	Stigma	Glabrous (0) Fringed (1)
36	Ovary	Glabrous (0) Hairy (1)
	Quantitative characters in cm	
37	Tuber Stalk length	Cm
38	Plant height	Cm
39	Leaf stalk length	Cm
40	Leaf Lamina Length	Cm
41	Leaf Lamina Breadth	Cm
42	Length of Spike	Cm
43	Length Spike Stalk	Cm
44	Calyx length	Cm
45	Flower length	Cm
46	Corolla length	Cm
47	Corolla lobe length	Cm
48	Comma bract length	Cm
49	Flowering bract length	Cm
50	Lip Length	Cm



Photoplate 1: *Curcuma* accessions

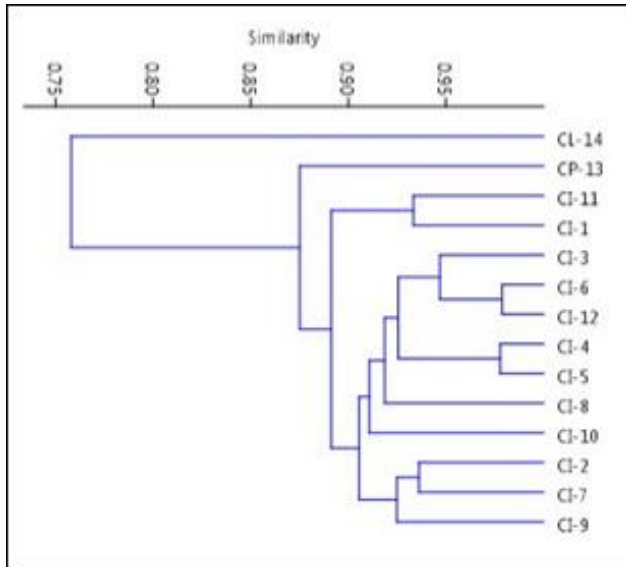


Fig: 1- Dendrogram (Method A)

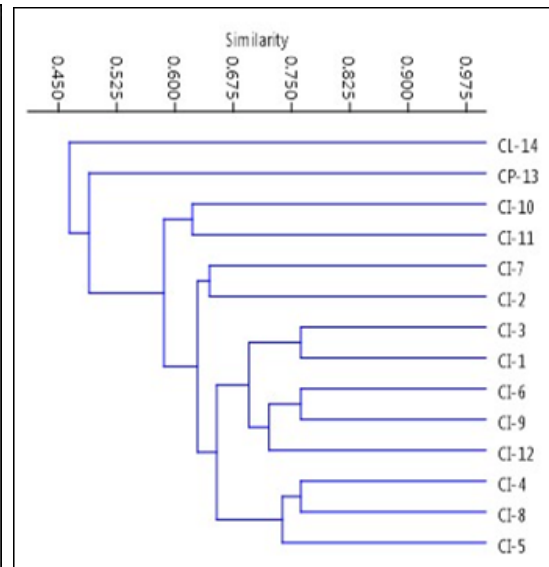


Fig: 2 Dendrogram (Method D)

Cluster 1- : CL-14
 Cluster 2 : This is further divided in to subclusters.
 Sub cluster 2-a - CP-13
 Sub cluster 2-b - CI-1 and CI-11
 Sub cluster 2-c – CI-3 , CI-4, CI-5, CI-6, CI-8 CI-10 and CI-12
 Sub cluster 2-d – CI-7 CI-2 and CI-9

Cluster -1- CL-14 it seems to be an outlier. Even to human eye *C. longa* is very different from the rest of the accessions studied.

Cluster -2 – It includes CP-13 and CI-1 to CI-12. In this group also CP-13 is an outlier which is a distinct species – *C. pseudomontana* closely related to *C. inodora*. From similarity indices (Table no -3) it is clear that the variants of *C. inodora* show more than 85 % similarity. The similarity and distance indices produced by method D are presented in Table no. 4

Dendrogram formed by UPGMA method (Method –D) indicates that the 14 accessions studied (12 variants of one species and 2 distinct species) clearly get grouped in to three clusters (Fig. 2).

Cluster 1- : CL-14
 Cluster 2 : CP-13
 Cluster 3 : Further divided in to three subclusters.
 Sub cluster 3-a - CI-10 and CI-11
 Sub cluster 3-b – CI-7 and CI-2
 Sub cluster 3-c –CI-1, CI-3 , CI-4, CI-5, CI-6, CI-8 , CI-9 and CI-12

Cluster -1- CL-14 it seems to be an outlier. Even to human eye *C. longa* is very different from the rest of the accessions studied.

Cluster -2 – It includes CP-13 in this group also CP-13 is an outlier which is a distinct species – *C. pseudomontana*. Cluster -3: It includes CI-1 to 12 which are variants of *C. inodora*

From similarity indices (Table no -4) it is clear that the variants of *C. inodora* show similarity range from 51 % to 76 %. This means that even the variants studied here are quite distinct from each other.

During exploration of *Curcuma inodora* populations it was found that all the variations are continuous variations; no discontinuous variations were observed. However, for the study distinct stages of variations were considered as distinct variants.

Table 3: Similarity Indices (Multistate character)

	CI-1	CI-2	CI-3	CI-4	CI-5	CI-6	CI-7	CI-8	CI-9	CI-10	CI-11	CI-12	CP-13	CL-14
CI-1	1													
CI-2	0.8936	1												
CI-3	0.9348	0.9149	1											
CI-4	0.8936	0.9149	0.9149	1										
CI-5	0.8723	0.8936	0.8936	0.9778	1									
CI-6	0.9362	0.9167	0.9574	0.9574	0.9362	1								
CI-7	0.875	0.9362	0.8958	0.9362	0.9149	0.9375	1							
CI-8	0.8723	0.8542	0.8936	0.9348	0.913	0.9362	0.875	1						
CI-9	0.8723	0.9348	0.8936	0.9348	0.913	0.9362	0.9149	0.913	1					
CI-10	0.8723	0.8542	0.8936	0.8936	0.913	0.9362	0.875	0.913	0.913	1				
CI-11	0.9333	0.8723	0.913	0.8723	0.8511	0.9149	0.8542	0.8913	0.8913	0.8913	1			
CI-12	0.9149	0.8958	0.9362	0.9362	0.9149	0.9787	0.9167	0.9149	0.9149	0.9149	0.935	1		
CP-13	0.8723	0.8936	0.8936	0.8936	0.8723	0.8958	0.875	0.8723	0.8723	0.8333	0.851	0.875	1	
CL-14	0.7292	0.7143	0.75	0.7872	0.766	0.7917	0.7708	0.766	0.7292	0.7292	0.745	0.809	0.766	1

Table 4: Similarity indices (Binary characters)

0	CI-1	CI-2	CI-3	CI-4	CI-5	CI-6	CI-7	CI-8	CI-9	CI-10	CI-11	CI-12	CP13	CL-14
CI-1	1													
CI-2	0.6444	1												
CI-3	0.7619	0.6444	1											
CI-4	0.6818	0.6444	0.6087	1										
CI-5	0.6222	0.6222	0.6977	0.7381	1									
CI-6	0.6818	0.6087	0.7209	0.6818	0.7381	1								
CI-7	0.6818	0.6444	0.6087	0.7209	0.587	0.6818	1							
CI-8	0.6087	0.5745	0.5745	0.7619	0.7381	0.6444	0.609	1						
CI-9	0.6818	0.6087	0.7209	0.6444	0.7381	0.7619	0.644	0.7209	1					
CI-10	0.5417	0.5417	0.5745	0.5745	0.6977	0.6444	0.51	0.6087	0.6818	1				
CI-11	0.6222	0.5532	0.587	0.4898	0.5319	0.5532	0.553	0.5208	0.6222	0.6222	1			
CI-12	0.6444	0.5745	0.7209	0.6087	0.6591	0.7209	0.609	0.5745	0.7209	0.6818	0.6222	1		
CP-13	0.5652	0.5	0.5	0.5	0.5106	0.4694	0.44	0.5	0.4694	0.4694	0.4792	0.469	1	
CL-14	0.4118	0.44	0.4118	0.5	0.5106	0.4694	0.44	0.4694	0.44	0.4694	0.449	0.532	0.489	1

CONCLUSION:

In phenetic analysis both coefficient of similarity and methods of coding of multistate characters affect the clustering pattern. According to Gonclaves *et al.* (2008) cophenetic correlation score measures the degree fit and also have been used as suitability of index to select dendrogram that represent the classification be used.

Dendrogram based on UPGMA- Jaccard similarity indices as per the method- A produced two distinct clusters . *C. longa* stands distinct while *C. pseudomontana* clusters

with all the accessions of *C. inodora*. Dendrogram prepared as per method D produced three clusters keeping all the three species separate, but still showing very close relation between *C. pseudomontana* and *C. inodora*.

Some of the variants are very distinct; so much so that if suddenly one comes across only single variation he may collect the specimen as distinct species. Out of three species studied *C. longa* stands distinct from other two species *C. inodora* and *C. pseudomontana* have shown the clubbing on the basis of characters studied here.

Limitations of the conventional taxonomic tools for resolving the taxonomic confusion prevailing within the species suggests the need of molecular marker studies in conjunction with morphotaxonomic study. Careful study of these variations will help to understand evolution within the species.

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Conflict of interest:

The Authors declare no conflict of interest.

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