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Germplasm collection and characterization of Mandukaparni (*Centella asiatica* L.) accessions of coastal zone of Karnataka

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Abstract

An investigation was carried out on exploration and collection of local germplasm of *Centella asiatica* to ascertain the variability existed in coastal Karnataka. Twenty accessions were collected from different geographical regions of coastal Karnataka representing nineteen talukas of Dakshina Kannada, Udupi and Uttara Kannada districts at an altitude ranging from 06 m to 129 m above mean sea level were morphological characterized for 13 qualitative traits using NBPGR descriptors. The results of the study revealed that all morphological traits showed high variability except leaf surface. The majority of the accessions (14 accessions) were observed to have semi-erect growth habit with only eight accessions being erect and none a prostrate growth habit. Twenty accessions exhibited a predominantly rosette type of leaf arrangement. There were significant differences in leaf morphology among the accessions studied, with the majority of the leaves being medium in size (18 accessions), orbicular shape (18 accessions) with crenate margin (14 accessions) with predominant strongly yellowish green coloured leaves (11 accessions). Petiole and flower characters showed significant variations as well. Most of the accessions had medium length (16 accessions) with thin petioles (12 accessions) and light pink coloration at the base (09 accessions). Light purplish pink colour stolon (07 accessions) with the hard texture of stolon (13 accessions) were dominant. The majority of the accessions exhibited light pink colour flower (08 accessions).

Keywords: *Centella asiatica*, triterpenoids, exploration, characterization and variability

Introduction

Mandukaparni (*Centella asiatica* L.) is an important tropical and sub-tropical medicinal plant, belongs to the family Apiaceae having somatic chromosome number $2n=18$, a widely available Indian herb has been used for centuries in Indian systems of medicine. It has been used for the purposes like boosting memory hence referred as “Brain food”, one of the important rejuvenating herbs for nerves and brain cells and believed to be capable of increasing intelligence, longevity of memory, increasing concentration and alertness (Lal *et al.*, 2017) [5]. The whole herb is economically important and used as medicinal herb as well as leafy vegetable consumed in South-East Asian countries including India, Malaysia, China and Sri Lanka. The herb contains triterpenoids such as madecassoside, asiaticoside, madecassic acid and Asiatic acid (Schaneberg *et al.*, 2003) [13]. *Centella* can be a potential herbal plant in many health care, functional properties like anti-fungal activity, anti-oxidant activity, cardiovascular, anti-ulcer activity, anti-diabetic activity, anti-depressant, anti-cancer, gastric ulcer and dermatologic activity (Kant *et al.*, 2019) [3].

It grows profusely and abundantly distributed in marshy areas throughout coastal zone of Karnataka. The evaluation of variation using morphological markers is seen to be a crucial step in describing and characterising germplasm. These readily visible morphological characteristics serve as effective tools for first assessment for determining the level of diversity. This study was aimed at collection and systematic characterization of this species of native germplasm, which helps in knowing the diversity present.

Materials and methods

The exploration work was carried out during *Rabi* (October, 2021) in coastal zone of Karnataka which consists of 19 talukas belonging to 3 districts. The coastline of Karnataka called as Karavali which is situated along the eastern shore of Arabian sea, stretches to 320 km between Mangalore in Dakshina Kannada district in South and Karwar in Uttar Kannada district in North.

The stolons of each germplasm accessions from different ecological regions of coastal zone of Karnataka were brought to ZAHRS, Mudigere and planted in nursery beds of 2 m x 1 m size under poly house conditions for well establishment and multiplication, designated as Acc.40 to Acc.59. The details of accessions collected is furnished in Table 1 and 2.

Observations were recorded on five randomly selected rosettes from each accession at full foliage stage by referring to NBPGR plant descriptors of *Centella asiatica* L. The leaf size of all 20 accessions was considered visually and categorized as small, medium and large size. The leaf arrangement, shape, size, margin and surface were recorded. The leaf, flower and stolon colour and also petiole length, thickness and its pigmentation at the base were recorded using RHS colour chart. The list of morphological traits observed are given in Table 3.

Results and Discussion

Collection of accessions: Twenty germplasm accessions of *C. asiatica* were collected through exploration in 19 talukas across the three districts of coastal zone of Karnataka (Figure 1). All the taluk as in entire coastal zone was explored viz., Dakshina Kannada (7 talukas), Udupi (7 talukas) and Uttara Kannada (5 talukas) (Table 2). The highest number of germplasms were collected from the Dakshina Kannada district (eight accessions) followed by seven from Udupi and five from Uttara Kannada district from different habitats like open field, Arecanut plantation, coconut plantation, paddy field, rubber plantation, cashew and mango orchard. Exploration and collection of germplasm was carried out an altitude range from 06 to 129 m above mean sea level with the range 12° 69' to 14° 78' North latitude and 74° 11' to 75° 39' East longitude. Similar work has been carried out in mandukaparni to assess variability for future use in crop development programmes by Ravi *et al.* (2019) ^[10], Chandrasekara *et al.* (2020) ^[2], Chachai *et al.* (2021) ^[1], Mathavaraj *et al.* (2021) ^[6] and Rohini and Smitha (2021) ^[11].

Morphological characterization of accessions: In the current study, significant differences in plant growth habit among the accessions was noticed. Out of the 22 accessions, eight (Acc. 42, 44, 46, 47, 48, 49, 52 and 58) exhibited erect growth habit and fourteen (Acc. 40, 41, 43, 45, 50, 51, 53, 54, 55, 56, 57, 59, Arka Divya and Arka Prabhavi) exhibited semi-erect growth. The none of the accession exhibited prostrate growth habit. For the maximal and uniform exposure to sunlight, erect growing accessions are preferable to semi-erect because they would boost the yield and dry matter production. The variations in plant growth habit among the accessions are caused by their underlying genetic structure. Comparable variation in different genotypes were also noticed by Ravi *et al.* (2019) ^[10] and Chachai *et al.* (2021) ^[1] in *Centella asiatica*.

Two types of leaf arrangement on the stolon were identified among the accessions studied *i.e.*, rosette and spreading. All

the accessions exhibited rosette type except Arka Divya and Arka Prabhavi which were of spreading type. The diversity in leaf arrangement could only be explained by the genetic makeup of the accessions. Similar variations in leaf arrangement were noticed by Ravi *et al.* (2019) ^[10] and Chachai *et al.* (2021) ^[1] in mandukaparni.

A variation in leaf size consisting of small, medium and large was observed among 22 accessions. Arka Divya had larger leaves than the all-other accessions. The accession Acc. 57, 58 and 59 which had small leaves and the rest of the accessions produced medium-sized leaves. The photosynthetic efficiency of accessions is determined by the size of their leaves, which has a significant impact on growth, yield and the production of triterpenoids content. The leaf variability is genetically determined under a specific set of environmental conditions, especially soil moisture, nutrient supply and light that interacts with the accessions. Similarly, variations in leaf size were also observed by Ravi *et al.* (2019) ^[10] and Rohini and Smitha (2021) ^[11] in mandukaparni.

Leaf shape varied significantly among *Centella* accessions which varied from orbicular, reniform and orbicular reniform (Plate 1). The majority of the accessions (18) recorded orbicular shape. While, accessions Acc. 41, 50 and 57 had reniform shapes and Arka Divya had orbicular reniform shape. The leaf shape is a morphological feature for identifying and classifying a specific accession. Distinct variability in leaf shapes was observed by Ravi *et al.* (2019) ^[10] and Chandrasekara *et al.* (2020) ^[2] in *Centella asiatica*.

The phenotypic variation in leaf margin of *Centella* accessions was either crenate or dentate (Plate 2). Among the 22 accessions studied, eight had dentate leaf margins (Acc. 40, 43, 44, 45, 48, 49, 54 and 55), while rest (14) of the accessions had crenate leaf margin (Acc. 41, 42, 46, 47, 50, 51, 52, 53, 56, 57, 58, 59, Arka Divya and Arka Prabhavi). The existence of distinctive leaf margins is a morphological indicator for identifying and characterizing a specific accession. The genetic make-up is responsible for the variation in leaf margin among accessions. Similar differences in leaf margin among the accessions were also noted by Prasad *et al.* (2014) ^[9] and Ravi *et al.* (2019) ^[10] in *Centella asiatica*.

The leaf colour of *Centella* accessions revealed a significant variation among the accessions, which were grouped into deep yellowish green, moderate yellowish green and strong yellowish green. In six accessions, the leaf coloration was deep yellowish green (Acc. 42, 46, 49, 57, Arka Divya and Arka Prabhavi). The accessions Acc. 44, 52, 53, 54 and 59 produced moderate yellowish green leaves. The rest of the accessions exhibited strong yellowish green colour. The genetic make-up of the accessions as well as the environmental interaction may be responsible for the variations in leaf colour. Variability in the leaf colour among the accessions was recorded in *Centella asiatica* by Mathur *et al.* (2003) ^[7] and Ravi *et al.* (2019) ^[10] in *Centella asiatica*.

Table 1: Details of exploration of *Centella asiatica* L. accessions in coastal zone of Karnataka

Accessions	Collection site			Habitat	Geo reference		
	Village	Mandal/Taluk/Tehsil	District		Latitude (N)	Longitude (E)	Altitude (m)
Acc. 40	Vitla	Bantwal	Dakshina Kannada	Open field	12 ^o .76'	75 ^o .11'	99
Acc. 41	Puddhu	Bantwal	Dakshina Kannada	Areca nut plantation	12 ^o .84'	74 ^o .96'	26
Acc. 42	Kannuru	Mangalore	Dakshina Kannada	Coconut plantation	12 ^o .86'	74 ^o .89'	16
Acc. 43	Munduru	Puttur	Dakshina Kannada	Areca nut plantation	12 ^o .75'	75 ^o .25'	98
Acc. 44	Kudmar	Kadaba	Dakshina Kannada	Areca nut plantation	12 ^o .73'	75 ^o .32'	97
Acc. 45	Murulya	Sulya	Dakshina Kannada	Up land areca nut	12 ^o .69'	75 ^o .39'	96
Acc. 46	Naikuli	Belthangadi	Dakshina Kannada	Areca nut plantation	13 ^o .00'	75 ^o .23'	104
Acc. 47	Bannadka	Moodbidri	Dakshina Kannada	Open field	13 ^o .09'	75 ^o .00'	129
Acc. 48	Neere	Karkala	Udupi	Paddy field	13 ^o .30'	74 ^o .90'	50
Acc. 49	Puttige	Udupi	Udupi	Coconut garden	13 ^o .36'	74 ^o .87'	18
Acc. 50	Shivapura	Hebri	Udupi	Areca nut plantation	13 ^o .42'	74 ^o .96'	40
Acc. 51	ZAHRS	Brahmavara	Udupi	Cashew orchard	13 ^o .52'	74 ^o .75'	37
Acc. 52	Koravadi	Kundpura	Udupi	Coconut garden	13 ^o .56'	74 ^o .69'	17
Acc. 53	Shirva	Kapu	Udupi	Coconut garden	13 ^o .22'	74 ^o .73'	18
Acc. 54	Jadkal	Byandoor	Udupi	Rubber plantation	13 ^o .82'	74 ^o .80'	43
Acc. 55	Sharada hole	Bhatkal	Uttara Kannada	Coconut and areca nut plantation	14 ^o .03'	74 ^o .51'	11
Acc. 56	Konabagilu	Honnavaara	Uttara Kannada	Areca nut plantation	14 ^o .21'	74 ^o .48'	52
Acc. 57	Kadekodi	Kumta	Uttara Kannada	Coconut garden	14 ^o .38'	74 ^o .40'	06
Acc. 58	Ankola	Ankola	Uttara Kannada	Coconut garden	14 ^o .66'	74 ^o .31'	20
Acc. 59	Binaga	Karwar	Uttara Kannada	Mango orchard	14 ^o .78'	74 ^o .11'	08

Table 2: Details of exploration and collection sites of *Centella asiatica* L. accessions in coastal zone of Karnataka

Sl. No.	District	Taluk	Number of accessions collected	Total
1	Dakshina Kannada	Bantwal	02	08
		Mangalore	01	
		Puttur	01	
		Kadaba	01	
		Sulya	01	
		Belthangadi	01	
		Moodbidri	01	
2	Udupi	Karkala	01	07
		Udupi	01	
		Hebri	01	
		Brahmavara	01	
		Kundpura	01	
		Kapu	01	
		Byandoor	01	
3	Uttara Kannada	Bhatkal	01	05
		Honnavaara	01	
		Kumta	01	
		Ankola	01	
		Karwar	01	
Total	03	19	20	20

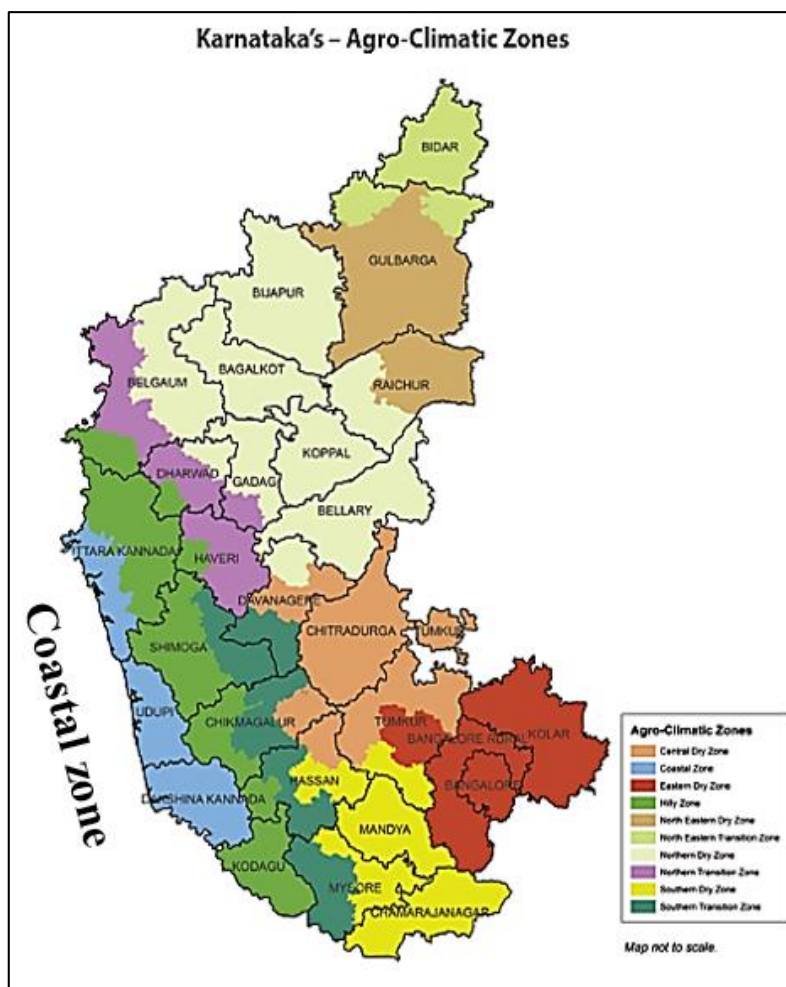


Fig 1: Map showing details of *Centella asiatica* L. accessions collection from coastal zone of Karnataka

Table 3: Variability in plant growth habit, leaf arrangement and morphology in *Centella asiatica* L. accessions of coastal zone of Karnataka

Accessions	Leaf morphology						
	Plant growth habit	Leaf arrangement	Size	Shape	Margin	Colour	Surface
Acc. 40	Semi erect	Rosette	Medium	Orbicular	Dentate	Strong yellowish green	Glabrous
Acc. 41	Semi erect	Rosette	Medium	Reniform	Crenate	Strong yellowish green	Glabrous
Acc. 42	Erect	Rosette	Medium	Orbicular	Crenate	Deep yellowish green	Glabrous
Acc. 43	Semi erect	Rosette	Medium	Orbicular	Dentate	Strong yellowish green	Glabrous
Acc. 44	Erect	Rosette	Medium	Orbicular	Dentate	Moderate yellowish green	Glabrous
Acc. 45	Semi erect	Rosette	Medium	Orbicular	Dentate	Strong yellowish green	Glabrous
Acc. 46	Erect	Rosette	Medium	Orbicular	Crenate	Deep yellowish green	Glabrous
Acc. 47	Erect	Rosette	Medium	Orbicular	Crenate	Strong yellowish green	Glabrous
Acc. 48	Erect	Rosette	Medium	Orbicular	Dentate	Strong yellowish green	Glabrous
Acc. 49	Erect	Rosette	Medium	Orbicular	Dentate	Deep yellowish green	Glabrous
Acc. 50	Semi erect	Rosette	Medium	Reniform	Crenate	Strong yellowish green	Glabrous
Acc. 51	Semi erect	Rosette	Medium	Orbicular	Crenate	Strong yellowish green	Glabrous
Acc. 52	Erect	Rosette	Medium	Orbicular	Crenate	Moderate yellowish green	Glabrous
Acc. 53	Semi erect	Rosette	Medium	Orbicular	Crenate	Moderate yellowish green	Glabrous
Acc. 54	Semi erect	Rosette	Medium	Orbicular	Dentate	Moderate yellowish green	Glabrous
Acc. 55	Semi erect	Rosette	Medium	Orbicular	Dentate	Strong yellowish green	Glabrous
Acc. 56	Semi erect	Rosette	Medium	Orbicular	Crenate	Strong yellowish green	Glabrous
Acc. 57	Semi erect	Rosette	Small	Reniform	Crenate	Deep yellowish green	Glabrous
Acc. 58	Erect	Rosette	Small	Orbicular	Crenate	Strong yellowish green	Glabrous
Acc. 59	Semi erect	Rosette	Small	Orbicular	Crenate	Moderate yellowish green	Glabrous
Arka Divya*	Semi erect	Spreading	Large	Orbicular Reniform	Crenate	Deep yellowish green	Glabrous
Arka Prabhavi*	Semi erect	Spreading	Medium	Orbicular	Crenate	Deep yellowish green	Glabrous

*Check varieties

Table 4: Variability in petiole and stolon characteristics and flower colour of *Centella asiatica* L. accessions of coastal zone of Karnataka

Accessions	Petiole characteristics			Stolon characteristics		Flower characteristics
	Petiole length	Petiole thickness	Petiole pigmentation of the base	Stolon colour	Stolon texture	Flower colour
Acc. 40	Medium	Thin	Light pink	Greenish pink	Hard	Moderate Pink
Acc. 41	Medium	Thick	Strong yellowish green	Deep purplish pink	Soft	Deep pink
Acc. 42	Long	Thin	Moderate yellowish green	Light purplish pink	Hard	Greenish pink
Acc. 43	Medium	Thick	Strong yellowish green	Deep purplish pink	Soft	Moderate Pink
Acc. 44	Medium	Thin	Moderate yellowish green	Moderate purplish pink	Hard	Moderate Pink
Acc. 45	Medium	Thin	Strong yellowish green	Greenish pink	Hard	Moderate Pink
Acc. 46	Long	Thin	Light pink	Light purplish pink	Hard	Deep pink
Acc. 47	Medium	Thick	Moderate yellowish green	Greenish pink	Soft	Greenish pink
Acc. 48	Long	Thick	Light pink	Light purplish pink	Soft	Greenish pink
Acc. 49	Medium	Thin	Light pink	Light purplish pink	Hard	Light pink
Acc. 50	Medium	Thick	Light pink	Deep purplish pink	Hard	Deep pink
Acc. 51	Medium	Thin	Light pink	Greenish pink	Hard	Light pink
Acc. 52	Medium	Thick	Moderate yellowish green	Deep purplish pink	Soft	Moderate Pink
Acc. 53	Medium	Thin	Moderate yellowish green	Deep purplish pink	Hard	Greenish pink
Acc. 54	Medium	Thick	Light pink	Light purplish pink	Soft	Light pink
Acc. 55	Medium	Thick	Greenish pink	Light purplish pink	Soft	Moderate Pink
Acc. 56	Medium	Thick	Light pink	Deep purplish pink	Hard	Light pink
Acc. 57	Small	Thin	Deep pink	Greenish pink	Hard	Light pink
Acc. 58	Small	Thin	Strong yellowish green	Moderate purplish pink	Soft	Light pink
Acc. 59	Small	Thin	Deep pink	Moderate purplish pink	Soft	Light pink
Arka Divya*	Medium	Thick	Moderate yellowish green	Moderate purplish pink	Hard	Moderate Pink
Arka Prabhavi*	Medium	Thin	Light pink	Light purplish pink	Hard	Light pink

*Check varieties

Table 5: Summary statistics of morphological characterization of *Centella asiatica* L. accessions of coastal zone of Karnataka

Sl. No	Character	Phenotype	Number of accessions	Percentage of distribution
1	Plant growth habit	Erect	08	36.36
		Semi erect	14	63.63
		Prostrate	-	0.00
2	Leaf arrangement	Rosette	20	90.90
		Spreading	2	9.09
3	Leaf size	Small	03	13.63
		Medium	18	81.81
		Large	01	4.54
4	Leaf shape	Orbicular	18	81.81
		Reniform	03	13.63
		Orbicular-reniform	1	4.54
5	Leaf margin	Crenate	14	63.63
		Dentate	08	36.36
		Wavy	-	0.00
6	Leaf colour	Deep yellowish green	06	27.27
		Moderate yellowish green	05	22.72
		Strong yellowish green	11	50.00
7	Leaf surface	Glabrous	22	100.00
		Pubescent	-	0.00
8	Petiole length	Small	03	13.63
		Medium	16	72.72
		Long	03	13.63
9	Petiole thickness	Thick	10	45.45
		Thin	12	54.54
10	Petiole pigmentation at the base	Light pink	09	40.90
		Strong yellowish green	04	18.18
		Moderate yellowish green	06	27.27
		Greenish pink	01	4.54
		Deep pink	02	9.09
11	Stolon colour	Greenish pink	05	22.72
		Light purplish pink	07	31.81
		Moderate purplish pink	04	18.18
		Deep purplish pink	06	27.27

12	Texture of stolon	Hard	13	59.09
		Soft	09	40.90
13	Flower colour	Light pink	08	36.36
		Pink	07	31.81
		Greenish pink	04	18.18
		Deep pink	03	13.63



Orbicular Reniform Orbicular reniform

Plate 1: Variations in leaf shape of *Centella asiatica* L. accessions



Plate 2: Variations in leaf margin of *Centella asiatica* L. accessions



Small Medium Long

Plate 3: Variations in petiole length of *Centella asiatica* L. accessions

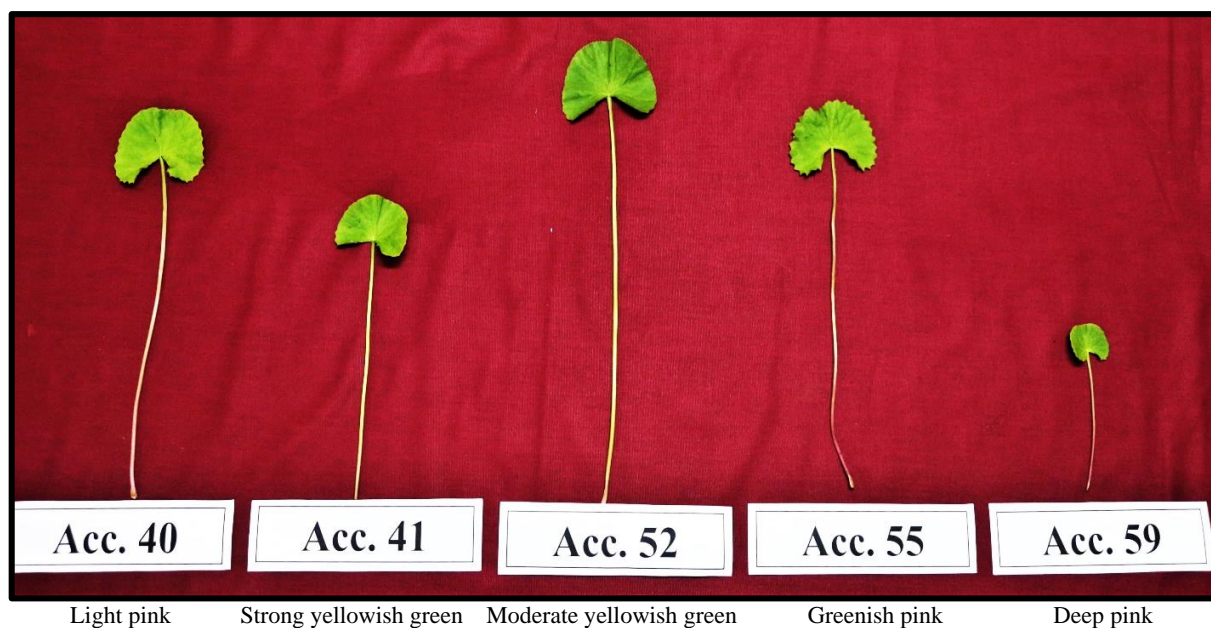


Plate 4: Variations in petiole pigmentation at the base of *Centella asiatica* L. accessions

Two types of leaf surface *viz.*, glabrous and pubescent are generally observed among *Centella asiatica* population. However, in the current study all the accessions were glabrous type and no significant differences in the leaf surface were recorded. Similar findings were reported by Ravi *et al.* (2019)^[10] in *Centella asiatica*.

Petiole length in *Centella* accessions was classified as small, medium and long (Plate 3). The accession Acc. 42, 46 and 48 were long petioled accessions. Sixteen out of the 22 accessions had medium petiole length, while only three had small petioled leaves (Acc. 57, 58 and 59). Accessions with medium to long petioles are preferred since they increase the quantity of triterpenes and herbage yield. The genetic makeup and their interaction with environment are responsible for the variation in petiole length. Similar variations in petiole size were reported by Padmalatha and Prasad (2008)^[8] and Ravi *et al.* (2019)^[10] in *Centella asiatica*.

Petiole thickness was classified as thick and thin among the *Centella* accessions studied. Acc. 40, 42, 44, 45, 46, 49, 51, 53, 57, 58, 59 and Arka Prabhavi were thin petioled accessions. The rest of the accessions had thick petiole. The genetic makeup account for the variability in petiole thickness, whereas thick petioles imply on herbage yield. A similar study has been reported in *Centella asiatica* by Ravi *et al.* (2019)^[10].

The observations on petiole pigmentation in *Centella* accessions revealed significant phenotypic variability (Plate 4). Acc.40, 46, 48, 49, 50, 51, 54, 56 and Arka Prabhavi accessions had light pink pigmentation at the base of the petiole, whereas Acc.42, 44, 47, 52, 53 and Arka Divya accessions had moderate yellowish green pigmentation. Strong yellowish green petiole pigmentation was also observed in accessions *viz.*, Acc.41, 43, 45 and 58. Deep pink petiole at the base was observed in Acc. 57 and 59. Only Acc. 55 showed greenish pink pigmentation at the base of the petiole. As a colour marker, the expression of pigmentation in the petiole would help to identify particular accessions. The genetic changes across accessions are thought to be the cause of the variation in colour in the petiole base. Wide variability in pink coloration was observed by Ravi *et al.* (2019)^[10] in *Centella asiatica* germplasm; Roshni *et al.* (2014)^[12] reported

pink coloration in stems of brahmi accessions.

A considerable variation in stolon colour were recorded among the accessions. Light purplish pink (Acc.42, 46, 48, 49, 54, 55 and Arka Prabhavi) and greenish pink (Acc.40, 45, 47, 51 and 57) stolon colours were recorded. The accession Acc.44, 58, 59 and Arka Divya exhibited moderate purplish pink stolon colour. Deep purplish pink stolon colour was present in all remaining accessions. Stolon colour is a colour identifier and also reported to have antioxidant properties, hence its manifestation in accessions will aid in identifying and characterizing the accessions. Similar variations in stolon colour were recorded by Ravi *et al.* (2019)^[10] in *Centella asiatica*; Roshni *et al.* (2014)^[12] and Kumar (2017)^[4] reported stem colour in brahmi accessions.

In the stolon texture, nine accessions (Acc.41, 43, 47, 48, 52, 54, 55, 58 and 59) had a soft texture which all other accessions had a hard texture. The genetic variations between them would be cause for the diversity in stolon texture. A similar result has been recorded by Ravi *et al.* (2019)^[10] in *Centella asiatica* L.

Among the 22 accessions examined, the accession Acc.41, 46 and 50 produced deep pink flowers. The flower colour was light pink in eight accessions (Acc.49, 51, 54, 56, 57, 58, 59 and Arka Prabhavi) and greenish pink in the remaining four accession (Acc.42, 47, 48 and 53). All remaining accessions had flowers that were moderate pink in colour. Although flower colours are genetically controlled, they do interact with environmental factors, especially light intensity and duration for its expression. But under any given set of environmental circumstances, the variation in blossom colour is always the result of varied genetic make-up in different accessions. Flower colour variations in *Centella asiatica* was observed by Ravi *et al.* (2019)^[10], Roshni *et al.* (2014)^[12] in brahmi and Tripathi *et al.* (2013)^[14] in coleus.

Conclusion

Collection of 20 accessions of *Centella asiatica* L. from coastal zone of Karnataka, India and morphological characterisation for 13 qualitative variables were recorded significant variation for all traits except leaf surface. Genetic diversity is normally assessed by common morphological

traits. The present investigation was conducted to evaluate the level of genetic diversity found in the local germplasm which can be utilize for further crop improvement programme, conservation needs and also helps in registration of accessions.

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