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Collection and Morphological Variability in Ecotypes of Indian Pennywort (*Centella asiatica* L.) of Hill Zone of Karnataka, India

C. S. Ravi[1*], K. Umesha[2], K. HimaBindu[3], G. Raviraja Shetty[4] and G. S. Anil Kumar[5]

[1]PSMAC, ZAHRS, Mudigere-577132, India
[2]ICAR-Emeritus Professor, College of Horticulture, GKVK Post, Bengaluru-65, India
[3]Division of Floriculture and Medicinal crops, ICAR-IIHR, Bengaluru, India
[4](PSMAC), College of Horticulture,Mudigere-577132, India
[7]College of Horticulture, GKVK Post, Bengaluru-65, India

**Corresponding author*

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A B S T R A C T

An investigation was carried out on exploration and collection of native germplasm of Indian pennywort for assessment and utilization of existing variability present in the population. Totally thirty nine diverse accessions were collected from 22 talukas of eight districts of hill zone of Karnataka at an altitude ranging from 363 m to 1064 m above mean sea level. The collected accessions were morphologically characterized for 14 qualitative traits as per NBPGR descriptors revealed wide variability for all the morphological traits except leaf surface. Predominantly erect plant growth habit was observed as compared to semi erect and prostrate growth habit. Majority of the accessions exhibited good regenerabilty, rosette type of leaf arrangement medium sized, orbicular shaped with dentate margin and dark green coloured leaves. Considerable differences were also noticed for petiole and flower characteristics. Majority of the accessions were of medium sized, thin petioled with pink colour pigmentation at the base. Greenish pink stolon and pink flower colours were dominant with soft textured stolonsas compared to hard textured.

Introduction

Indian Pennywort (*Centella asiatica* L.) is an important tropical medicinal plant belongs to the family Apiaceae with a somatic chromosome number 2n=18. The plant is native to South East Asian countries including India, SriLanka, China and

Malaysia as well as SouthAfrica and has pantropical in distribution.It occurs throughout India in moist places from plains to hill ranges up to 2000m (Mukherjee and Constance, 1993).The whole herb is economically important and its biological effects have been attributed to the existence of major triterpene derivatives including madecassoside, asiaticoside, madecassic andasiatic acid(Schaneberg *et al.,* 2003). *Centella asiatica* L. is a potential upcoming pharmaceutical, neutraceutical and cosmoceutical herb, gaining importance in the international trade of medicinal plants. In India, it is primarily known as "Brain food" and is often considered as "panacea" for several ailments such as antidiabetic, antimicrobial and antiproliferative properties due to the presence of triterpenoids. Indianpennywort is abundantly distributed throughout the hill zone of Karnataka and is a threatened species, as the genetic resources are markedly depleted because of it's unrestricted over exploitation from the natural habitat.

Variability assessment through morphological markers is considered to be an important step in description and characterization of germplasm. These easily observable morphological traits are useful tools for preliminary evaluation, as they offer a useful approach for assessing the extent of diversity. Hence, the present investigation was carried out to assess the magnitude of genetic diversity present in the native germplasm.

Materials and Methods

An exploration work was carried out during *Kharif* (July-September, 2017) in hill zone districts of Karnataka (Fig.1). The hill zone of Karnataka is situated in Western Ghats extending from Virajpete in the South (Kodagu district) to Khanapur in the North (Belgaum district). The details of accessions collected is furnished in Table 1. The stolons of each germplasm accessions (Designated as Acc.1 to Acc. 39) were collected from every taluk as one unit of exploration site from two different habitats. The collected germplasm accessions from different ecological regions were brought to ZAHRS, Mudigere and planted in nursery beds of 2m x 1m size under poly house conditions for establishment and multiplication.

Observations on morphological characters were recorded on five randomly selected rosettes from each accession at full foliage stageby referring to NBPGR plant descriptors of *Centella asiatica* L. with additions as required. The plant regeneration capacity of each accessions was observed after every harvest, ability of accessions for time taken for sprouting and ground coverage were considered as criteria for categorization. The leaf size of all 40 accessions was considered visually and categorized as small, medium and large based on the visual observations. The leaf, flower and stolon colour and also petiole pigmentation at the base were recorded using RHS colour chart. The classification of accessions based on petiole length into small (less than 10 cm), medium (10-15 cm) and long (more than 15 cm). The list of morphological traits observed are given in Table 3.2.

Results and Discussion

Collection of accessions

A total of 39 accessions were collected from various ecological regions of Kodagu, Hassan, Chikkamagalur, Shivamogga, Uttara Kannada, Haveri, Belgaum and Dharwad districts of hill zone of Karnataka (Table 2). Maximum number of accessions were collected from five talukas of Chikkamagaluru district (11) followed by six talukas of Uttara Kannada (09), four talukas of Shivamogga (08) and three talukas of Kodagu (06). While, two

accessions were collected from Sakaleshpura taluk of Hassan district and one accession each from Haveri, Belgaum and Dharwad district from one taluk each. Totally 22 talukas from eight districts of hill zone of Karnataka were explored at an altitude ranging from 363 m (Acc. 29) to 1064 m (Acc.11) above mean sea level for collection of the *Centella asiatica* L. accessions for assessment of morphological variability exists for further exploitation in crop improvement programmes.Similar exploration and collection work has been carried out by Prasad *et al.,* (2014), Kundu *et al.,* (2015), Singh *et al.,* (2015), Thapa *et al.,* (2016) and Lal *et al.,* (2017) collected diverse genetic stocks from different locations of India in *Centella asiatica*.

Morphological characterization of accessions

In the present study, considerable variations were observed in plant growth habit, among 40 accessions studied, 31 accessions (Acc. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 24, 25, 26, 27, 28, 29, 30, 34, 35, 37, 38 and Vallabh Medha) exhibited erect growth habit and six accessions exhibited (Acc. 21, 22, 23, 31, 32 and 33) semi erect growth habit. The rest of accessions (Acc. 16, 39 and 36) recorded prostrate growth habit. Since, herbage is the main economic part which is largely comprised of leaves, the accessions with erect growth are highly preferred.

Erect growing accessions are ideal than semi erect and prostate types as it allows maximum and uniform exposure to sunlight and would result in an increased dry matter production and subsequently the yield. The variations noticed in plant growth habit among the accessions are due to differences in their genetic makeup. Similar variation in different genotypes was observed by Tripathi *et al.,* (2012) in brahmi. Among 40 accessions studied for plant regeneration capacity 16 accessions have recorded good regenerability (Acc. 1, 3, 4, 6, 7, 8, 12, 14, 18, 19, 22, 24, 28, 30, 38 and Vallabh Medha) and accessions such as Acc. 2, 5, 10, 11, 13, 15, 20, 25, 27, 29, 31, 33, 34, 35 and 37 recorded medium regenerability. On the other hand, rest of the accessions (Acc. 9, 17, 26, 16, 36, 21, 23, 32 and 39) showed poor regeneration capacity. *Centella asiatica* L. is a herbaceous perennial, multiple harvests with higher biomass are possible if the accessions possess good regenerability and also due to early ground cover after every ratooning and weeds could be managed by smothering effect of the crop. The variations in ratoonability among the accessions are could be due to differences in their genetic makeup.

With regard to leaf arrangement, all the accessions exhibited rosette type except Acc. 07, 08 and Vallabh Medha which were of spreading type. The variation in leaf arrangement could be attributed purely to the influence of genetic constitution of the accessions. Kaur and Saggoo (2010) observed similar variations in leaf arrangement among *Aloe vera* accessions of North India.

Considerable phenotypic variations were registered for leaf size *viz.*, small, medium and large. The leaf size determines the photosynthetic efficiency of the accessions, on which growth, yield and synthesis of secondary metabolites depends greatly.

The accessions Acc.07, 11, 12, 16, 26 and Vallabh Medha had larger leaves. While, rest of the accessions had medium sized leaves, except Acc.03 and 39 which recorded small leaves.

The variations in leaf size is expected among the accessions as the attribute is genetic in a given set of environmental conditions, as the accessions interact with the environmental conditions, specially soil moisture supply and light.

Table.1 Details of exploration and collection districts and talukas of *Centella asiatica* L. germplasm accessions under hill zone of Karnataka

Accessions	Collection site			Habitat	Geo reference		
	Village	Mandal/Taluk/ Tehsil	District		Latitude (N)	Longitude (E)	Altitude (m)
Acc. 1	Halekote	Mudigere	Chikkamagaluru	Paddy field	13^0.07'	75^0.37'	916
Acc. 2	Kademadkal	Mudigere	Chikkamagaluru	Paddy field	13^0.09'	75^0.40'	873
Acc. 3	Heggadde	Sringeri	Chikkamagaluru	Up land arecanut	13^0.27'	75^0.15'	558
Acc. 4	Nagalapura	Koppa	Chikkamagaluru	Paddy field	13^0.30'	75^0.18'	531
Acc. 5	Kuppali	Thirthahalli	Shivamogga	Paddy field	13^0.35'	75^0.18'	569
Acc. 6	IthigeSeegodu	N.R. Pura	Chikkamagaluru	Paddy field	13^0.20'	75^0.27'	639
Acc. 7	Kymanahalli (Rakshidi Estate)	Sakaleshpura	Hassan	Coffee + Pepper plantation	13^0.01'	75^0.4'	859
Acc. 8	Kallarahalli	Sakaleshpura	Hassan	Low land coffee + arecanut plantation	12^0.56'	75^0.42'	820
Acc. 9	Madaravalli	Somwarapet	Kodagu	Paddy field	12^0.44'	75^0.53'	830
Acc. 10	Balagunda	Somwarapet	Kodagu	Coffee + Pepper	12^0.34'	75^0.50'	1040
Acc. 11	Makandur	Madikeri	Kodagu	Coffee + Pepper	12^0.27'	75^0.46'	1062
Acc. 12	Madikeri	Madikeri	Kodagu	Paddy field	12^0.25'	75^0.43'	1024
Acc. 13	Ammathi	Virajpet	Kodagu	Coffee plantation	12^0.15'	75^0.52'	831
Acc. 14	Puliyeri	Virajpet	Kodagu	Low land Coffee + Erythrina	12^0.14'	75^0.51'	854
Acc. 15	Doona	Hosanagara	Shivamogga	Paddy field	14^0.00'	75^0.17'	573
Acc. 16	Menase	Hosanagara	Shivamogga	Forest land	14^0.01'	75^0.10'	543
Acc. 17	Henigere	Sagara	Shivamogga	Low land arecanut plantation	14^0.71'	75^0.04'	527
Acc. 18	BhramanaManchale	Sagara	Shivamogga	Forest	14^0.10'	75^0.51'	531

Table 1.Contd….

Accessions	Collection site			Habitat	Geo reference		
	Village	Mandal/Taluk/ Tehsil	District		Latitude (N)	Longitude (E)	Altitude (m)
Acc. 19	Tugur	Sorabha	Shivamogga	Low land arecanut	14^0.15'	75^0.06'	519
Acc. 20	Hosabale	Sorabha	Shivamogga	Forest	14^0.19'	75^0.02'	529
Acc. 21	Siralagi	Siddapura	Uttara Kannada	Upland arecanut	14^0.20'	75^0.57'	486
Acc. 22	Siddapura	Siddapura	Uttara Kannada	Paddy field	14^0.11'	74^0.53'	512
Acc. 23	Terakanahalli	Sirsi	Uttara Kannada	Low land arecanut	14^0.36'	74^0.50'	619
Acc. 24	Isaluru	Sirsi	Uttara Kannada	Low land banana field	14^0.40'	74^0.53'	559
Acc. 25	Balehalli	Hanagal	Haveri	Up land arecanut	14^0.42'	75^0.41'	508
Acc. 26	Hudelakoppa	Mundagod	Uttara Kannada	Rainfed paddy field	14^0.48'	75^0.02'	492
Acc. 27	Khalaghatagi	Khalaghatagi	Dharwad	Arecanut plantation	15^0.10'	74^0.58'	470
Acc. 28	Aralikoppa	Yellapura	Uttara Kannada	Forest	14^0.59'	74^0.43'	487
Acc. 29	Kogilaban, Dandeli	Haliyal	Uttara Kannada	Paddy field	15^0.14'	74^0.37'	363
Acc. 30	Usoda	Joida	Uttara Kannada	Low land arecanut	15^0.18'	74^0.34'	434
Acc. 31	Jagalpete	Joida	Uttara Kannada	Forest	15^0.19'	74^0.30'	615
Acc. 32	Londa	Khanapura	Belgaum	Paddy field	15^0.29'	74^0.28'	602
Acc. 33	Badagabailu	N.R. Pura	Chikkamagaluru	Rubber	13^0.37'	75^0.29'	708
Acc. 34	Mavinahalli	Chikkamagaluru	Chikkamagaluru	Acasia (Forest)	13^0.14'	75^0.41'	1002
Acc. 35	Arenoor	Chikkamagaluru	Chikkamagaluru	Paddy field	13^0.17'	75^0.36'	679
Acc. 36	Mundagod	Sringeri	Chikkamagaluru	Forest	13^0.23'	75^0.18'	687
Acc. 37	Agumbe	Thirthahalli	Shivamogga	Forest	13^0.30'	75^0.06'	583
Acc. 38	Ammadi	Koppa	Chikkamagaluru	Coffee plantation	13^0.33'	75^0.20'	660
Acc. 39	Gubgal	Mudigere	Chikkamagaluru	Coffee plantation	13^0.15'	75^0.29'	742

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

Sl. No.	District	Taluk	Number of accessions collected	Total
1	Kodagu	Virajpet	02	06
		Madikeri	02	
		Somwarapet	02	
2	Hassan	Sakaleshpura	02	02
3	Chikkamagaluru	Mudigere	03	11
		Sringeri	02	
		Koppa	02	
		N.R. Pura	02	
		Chikmagalur	02	
4	Shivamogga	Thirthahalli	02	08
		Hosanagara	02	
		Sagara	02	
		Sorabha	02	
5	Uttara Kannada	Siddapura	02	09
		Sirsi	02	
		Mundagod	01	
		Yellapura	01	
		Haliyal	01	
		Joida	02	
6	Haveri	Hanagal	01	01
7	Belgaum	Khanapura	01	01
8	Dharwad	Khalaghatagi	01	01
Total	**08**	**22**	**39**	**39**

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

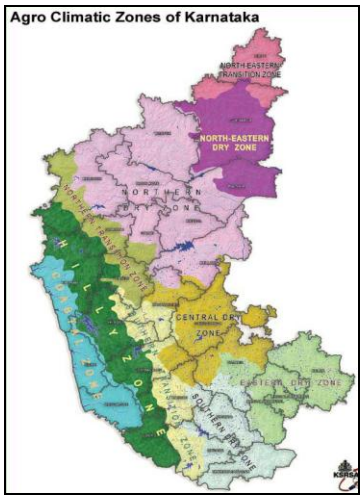


Table.3 Variability in plant growth habit, regenerability and leaf arrangement in *Centella asiatica* L. accessions.

Accessions	Plant growth habit	Regeneration capacity	Leaf arrangement
Acc. 1	Erect	Good	Rosette
Acc. 2	Erect	Medium	Rosette
Acc. 3	Erect	Good	Rosette
Acc. 4	Erect	Good	Rosette
Acc. 5	Erect	Medium	Rosette
Acc. 6	Erect	Good	Rosette
Acc. 7	Erect	Good	Spreading
Acc. 8	Erect	Good	Spreading
Acc. 9	Erect	Poor	Rosette
Acc. 10	Erect	Medium	Rosette
Acc. 11	Erect	Medium	Rosette
Acc. 12	Erect	Good	Rosette
Acc. 13	Erect	Medium	Rosette
Acc. 14	Erect	Good	Rosette
Acc. 15	Erect	Medium	Rosette
Acc. 16	Prostate	Poor	Rosette
Acc. 17	Erect	Poor	Rosette
Acc. 18	Erect	Good	Rosette
Acc. 19	Erect	Good	Rosette
Acc. 20	Erect	Medium	Rosette
Acc. 21	Semi erect	Poor	Rosette
Acc. 22	Semi erect	Good	Rosette
Acc. 23	Semi erect	Poor	Rosette
Acc. 24	Erect	Good	Rosette
Acc. 25	Erect	Medium	Rosette
Acc. 26	Erect	Poor	Rosette
Acc. 27	Erect	Medium	Rosette
Acc. 28	Erect	Good	Rosette
Acc. 29	Erect	Medium	Rosette
Acc. 30	Erect	Good	Rosette
Acc. 31	Semi erect	Medium	Rosette
Acc. 32	Semi erect	Poor	Rosette
Acc. 33	Semi erect	Medium	Rosette
Acc. 34	Erect	Medium	Rosette
Acc. 35	Erect	Medium	Rosette
Acc. 36	Prostrate	Poor	Rosette
Acc. 37	Erect	Medium	Rosette
Acc. 38	Erect	Good	Rosette
Acc. 39	Prostate	Poor	Rosette
Vallabh Medha*	Erect	Good	Spreading

* Check variety

Table.4 Variability in leaf morphology of *Centella asiatica* L. accessions

Accessions	Leaf				
	Size	**Shape**	**Margin**	**Colour**	**Surface**
Acc. 1	Medium	Orbicular	Crenate	Green	Glabraous
Acc. 2	Medium	Reniform	Dentate	Green	Glabraous
Acc. 3	Small	Orbicular	Crenate	Dark green	Glabraous
Acc. 4	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 5	Medium	Orbicular	Dentate	Green	Glabraous
Acc. 6	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 7	Large	Orbicular	Dentate	Light green	Glabraous
Acc. 8	Medium	Reniform	Dentate	Light green	Glabraous
Acc. 9	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 10	Medium	Reniform	Dentate	Dark green	Glabraous
Acc. 11	Large	Reniform	Dentate	Dark green	Glabraous
Acc. 12	Large	Reniform	Dentate	Dark green	Glabraous
Acc. 13	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 14	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 15	Medium	Orbicular	Crenate	Light green	Glabraous
Acc. 16	Large	Orbicular	Dentate	Dark green	Glabraous
Acc. 17	Medium	Orbicular	Dentate	Green	Glabraous
Acc. 18	Medium	Reniform	Dentate	Light green	Glabraous
Acc. 19	Medium	Orbicular	Dentate	Light green	Glabraous
Acc. 20	Medium	Orbicular	Dentate	Light green	Glabraous
Acc. 21	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 22	Medium	Reniform	Dentate	Dark green	Glabraous
Acc. 23	Medium	Reniform	Dentate	Green	Glabraous
Acc. 24	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 25	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 26	Large	Orbicular	Dentate	Light green	Glabraous
Acc. 27	Medium	Reniform	Dentate	Light green	Glabraous
Acc. 28	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 29	Medium	Orbicular	Dentate	Light green	Glabraous
Acc. 30	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 31	Medium	Orbicular	Dentate	Dark green	Glabraous
Acc. 32	Medium	Reniform	Dentate	Light green	Glabraous
Acc. 33	Medium	Orbicular	Crenate	Light green	Glabraous
Acc. 34	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 35	Medium	Reniform	Crenate	Dark green	Glabraous
Acc. 36	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 37	Medium	Orbicular	Crenate	Dark green	Glabraous
Acc. 38	Medium	Orbicular	Dentate	Light green	Glabraous
Acc. 39	Small	Reniform	Dentate	Light green	Glabraous
Vallabh Medha*	Large	Orbicular reniform	Dentate	Greenish yellow	Glabraous

***Check variety

Table.5 Variability in petiole and stolon characteristics of *Centella asiatica* L.accessions

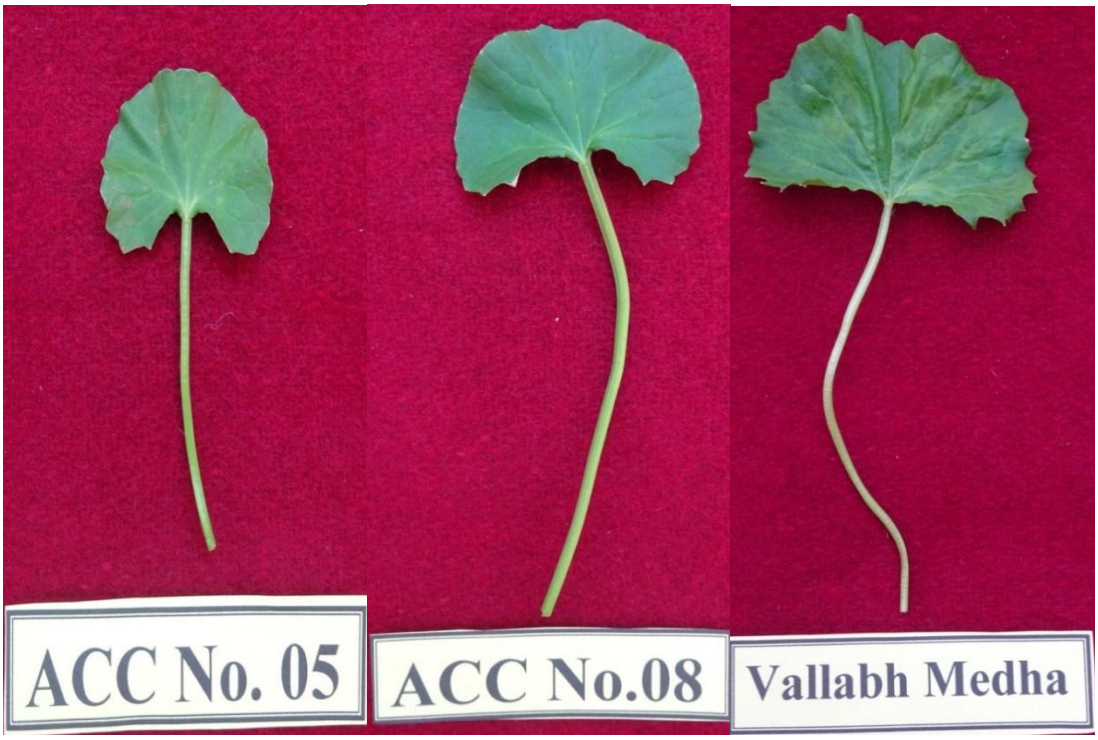
Accessions	Petiole length	Petiole thickness	Petiole pigmentation	Stolon colour	Flower colour	Stolon texture
Acc. 1	Medium	Thin	Light pink	Greenish pink	Pink	Soft
Acc. 2	Medium	Thick	Pink	Greenish pink	Pink	Soft
Acc. 3	Long	Thick	Pink	Greenish pink	Pink	Hard
Acc. 4	Medium	Thin	Light pink	Greenish pink	Pink	Soft
Acc. 5	Medium	Thin	Pink	Greenish pink	Pink	Soft
Acc. 6	Medium	Thin	Pink	Light pink	Pink	Soft
Acc. 7	Long	Thick	Pink	Light pink	Light pink	Hard
Acc. 8	Medium	Thin	Pink	Pink	pink	Hard
Acc. 9	Medium	Thin	Light pink	Greenish pink	Light pink	Soft
Acc. 10	Medium	Thick	Pink	Pink	Pink	Hard
Acc. 11	Medium	Thick	Light pink	Greenish pink	Pink	Hard
Acc. 12	Long	Thin	Pink	Pink	Pink	Hard
Acc. 13	Medium	Thin	Pink	Greenish pink	Light pink	Soft
Acc. 14	Long	Thin	Pink	Greenish pink	Dark pink	Soft
Acc. 15	Medium	Thin	Pink	Greenish pink	Pink	Soft
Acc. 16	Medium	Thin	Pink	Greenish pink	Pink	Soft
Acc. 17	Medium	Thin	Light pink	Light pink	Greenish pink	Soft
Acc. 18	Long	Thin	Light Pink	Light pink	Pink	Soft
Acc. 19	Medium	Thin	Dark pink	Dark pink	Light pink	Soft
Acc. 20	Medium	Thin	Light pink	Greenish pink	Light pink	Soft
Acc. 21	Long	Thin	Light pink	Light pink	Pink	Soft
Acc. 22	Medium	Thick	Pink	Dark pink	Pink	Soft
Acc. 23	Long	Thin	Light pink	Dark pink	Pink	Soft
Acc. 24	Medium	Thick	Light pink	Greenish pink	Pink	Hard
Acc. 25	Medium	Thick	Light pink	Greenish pink	Pink	Hard
Acc. 26	Medium	Thin	Light pink	Pink	Pink	Soft
Acc. 27	Medium	Thin	Greenish pink	Greenish pink	Greenish pink	Soft
Acc. 28	Medium	Thin	Pink	Pink	Pink	Soft
Acc. 29	Medium	Thick	Pink	Pink	Pink	Hard
Acc. 30	Medium	Thick	Pink	Light pink	Pink	Hard
Acc. 31	Long	Thick	Pink	Dark pink	Pink	Hard
Acc. 32	Medium	Thick	Pink	Greenish pink	Pink	Hard
Acc. 33	Medium	Thin	Light pink	Greenish pink	Pink	Soft
Acc. 34	Medium	Thin	Light pink	Dark pink	Pink	Soft
Acc. 35	Medium	Thick	Light pink	Pink	Pink	Hard
Acc. 36	Small	Thick	Light pink	Light pink	Pink	Soft
Acc. 37	Medium	Thin	Greenish pink	Greenish pink	Pink	Soft
Acc. 38	Medium	Thin	Greenish pink	Greenish pink	Greenish pink	Soft
Acc. 39	Small	Thin	Pink	Greenish pink	Pink	Soft
Vallabh Medha*	Long	Thin	Dark pink	Light pink	Pink	Soft

*Check variety

Table.6 Summary statistics of morphological characterization of *Centella asiatica* L. accessions used in the present study

Sl. No	Character	Phenotype	Number of accessions	Percentage of distribution
1	Plant growth habit	Erect	31	77.50
		Semi erect	06	15.00
		Prostrate	03	07.50
2	Regenerability	Good	16	40.00
		Medium	15	37.50
		Poor	09	22.50
3	Leaf arrangement	In rosettes	37	92.50
		Spreading	03	07.50
4	Leaf size	Small	02	05.00
		Medium	32	80.00
		Large	06	15.00
5	Leaf shape	Orbicular	27	67.50
		Reniform	12	30.00
		Orbicular-reniform	01	2.50
6	Leaf margin	Crenate	12	30.00
		Dentate	28	70.00
		Wavy	-	0.00
7	Leaf colour	Light green	13	32.50
		Dark green	21	52.50
		Green	05	12.50
		Greenish yellow	01	2.50
8	Leaf surface	Glabrous	40	100.00
		Pubescent	-	00.00
9	Petiole length	Small	02	05.00
		Medium	29	72.50
		Long	09	22.50
10	Petiole thickness	Thick	14	35.00
		Thin	26	65.00
11	Petiole pigmentation at the base	Light pink	16	40.00
		Dark pink	02	05.00
		Greenish pink	03	07.50
		Pink	19	47.50
12	Stolon colour	Light pink	08	20.00
		Dark pink	05	12.50
		Greenish pink	20	50.00
		Pink	07	17.50
13	Flower colour	Light pink	05	12.50
		Dark pink	01	02.50
		Greenish pink	03	07.50
		Pink	31	77.50
14	Texture of stolon	Hard	13	32.50
		Soft	27	67.50

Plate.1 Variation in leaf shapes in ecotypes of *Centella asiatica* L. accessions



Orbicular	Reniform	Orbicular reniform

Plate.2 Variation in leaf margin in ecotypes of *Centella asiatica* L. accessions

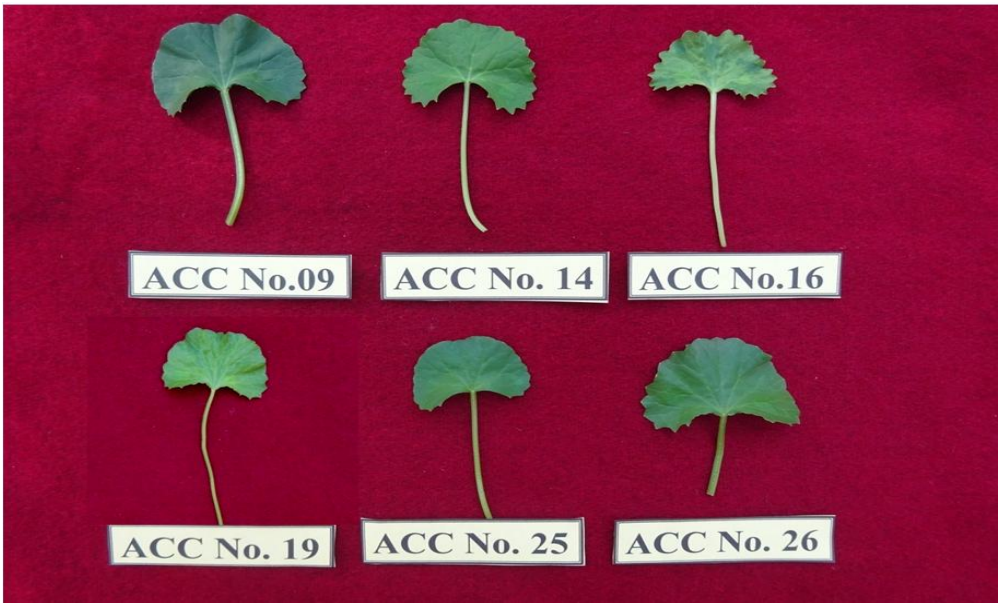


Plate.3 Variation in petiole length in ecotypes of *Centella asiatica* L. accessions

Plate.4 Variation in petiole pigmentation at the base in ecotypes of *Centella asiatica* L. accessions

Similar variations in leaf size were reported by Prasad *et al.,* (2014) in *Centella asiatica* and Tripathi *et al.,* (2012) in brahmi.

Wide phenotypic variations were recorded among the accessions studied for leaf shape (Plate 1) and majority of the accessions (27) had orbicular shape (Acc. 1, 3, 4, 5, 6, 7, 9, 13,

14, 15, 16, 17, 19, 20, 21, 24, 25, 26, 28, 29, 30, 31, 33, 34, 36, 37 and 38). While, the accessions such as Acc. 11, 2, 8, 10, 12, 18, 22, 23, 27, 32, 35 and 39 had reniform shape except Vallabh Medha which had orbicular reniform shape. The presence of characteristic leaf shape is a key morphological marker for identification and characterization of a particular accession. The variations in leaf shape could be ascribed to genetic makeup of the accessions. Variation in leaf shapes were observed by Prasad *et al.*, (2014) in *Centella asiatica* and Roshni *et al.*, (2014) in brahmi.

Among the accessions, marked variations in leaf margin (Plate 2) were observed. Most of the accessions (28) had dentate margin (Acc. 2, 5, 6, 7, 8, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 27, 26, 28, 29, 31, 32, 38, 39 and Vallabh Medha) and rest of the accessions (12) exhibited crenate margin(Acc. 1, 3, 4, 9, 15, 25, 30, 33, 34, 36, 37 and 35). The manifestation of characteristic leaf margin is also a prime morphological marker for identification and categorization of a particular accession. The variations in leaf margin among the accessions is due to differences in their genetic makeup. Similarly, the variations in leaf margin among the accessions were reported by Prasad *et al.*, (2014) in *Centella asiatica* andMalav *et al.*, (2015) in holy basil.

The leaf colouration was dark green colour in 21 accessions (Acc. 3, 4, 6, 9, 10, 11, 12, 13, 14, 16, 21, 22, 24, 25, 28, 30, 31, 34, 35, 36 and 37). The accessions such as Acc.1, 2, 5, 17 and 23 produced green leaves while, rest of the accessions possessed light green colour except Vallabh Medha which had greenish yellow leaves. The variations in leaf colour could be attributed to genetic makeup of the accessions as well as the environmental conditions, as these interact and the resultant colour depends on these two. However, under a given set of conditions, any variation could be attributed to the genetic constitution of the plant. Phenotypic variability for the leaf colour among the accessions were reported in C*entella asiatica* (Mathur *et al.*, 2003); brahmi (Tripathi *et al.*, 2012) and *Aloe vera* (Kaur and Saggoo, 2010).

Leaf surface among the accessions did not vary markedly and all the accessions were of glabrous type. The petiole length among the accessions showed remarkable variations. Long, medium and short petioles were recorded in nine, 29 and two accessions, respectively. The accessions Acc.03, 7, 12, 14, 18, 21, 23, 31 and Vallabh Medha were long petioled. Medium to long petioled accessions are desirable as it contributes to higher herbage. The petiole length (Plate 3) is genetically controlled and the variations among the accessions is due to differences in the genetic makeup. Variations in petiole length among the accessions were also noticed by Padmalatha and Prasad (2008) in *Centella asiatica.*

The thick petioled accessions were Acc. 2, 3, 7, 10, 11, 22, 24, 25, 29, 30, 31, 32, 35 and 36. While, rest of the accessions possessed thin petiole. The variations in petiole thickness could be attributed to differences in their genetic makeup.

Wide variations for pigmentation at the base of petiole were noticed (Plate 4), which varied from light pink, pink, dark pink and greenish pink. Among these, pink pigmentation was found to be a dominant trait (15 accessions), followed by light pink (16 accessions), while, greenish pink and dark pink pigmentations were recorded in three (Acc. 27, 37 and 38) and two accessions (Acc. 19 and Vallabh Medha), respectively. Dark pink to pink pigmentation at petiole base is due to differential distribution of anthocyanin pigments and could be attributed to antioxidant properties. The expression of

pigmentation in petiole would aid in characterization of unique accessions, as it serves as colour marker. The variation in pigmentation in petiole base is attributed to differences in the genetic makeup of accessions. Roshni *et al.,* (2014) reported pink coloration in stems of certain accessions of brahmi and Kaur and Saggoo (2010) in spine colour among North Indian *Aloe vera* accessions.

The stolon colour was dark pink (Acc. 19, 22, 23, 31 and 34) and pink in Acc. 8, 10, 12, 26, 28, 29 and 35. While, it was light pink in Acc. 6, 7, 17, 18, 21, 30, 36 and Vallabh Medha. All remaining accessions had greenish pink stolon. The manifestation of stolon colour in accessions would help in identification and characterization of unique accessions as it serves as colour marker and also attributed to antioxidant properties. The differences in the stolon colour of accessions can be attributed to variations in genetic makeup and differential distribution of anthocyanin pigments. Similar variations among the accessions of brahmi for stem colour have been reported (Roshni *et al.,* 2014).

Among 40 accessions studied, Acc.14 alone recorded dark pink flower. The light pink flowers were noticed in five accessions (Acc.7, 9, 13, 19 and 20) and it was greenish pink in Acc. 17, 27 and 38. All remaining accessions had pink coloured flowers. Although difference in flower colour is genetically controlled it does interact with the environmental conditions more particularly light intensity and duration. But the difference in the flower colour in any given set of environmental conditions is always due to differential genetic makeup in various accessions. Roshni *et al.,* (2014) in brahmi reported variation in flower colours in different accessions.

Thirteen accessions (Acc. 3, 7, 8, 10, 11, 12, 24, 25, 29, 30, 31, 32 and 35) had hard texture in the stolon. All other accessions had soft texture. The variation in stolon texture shall be attributed to differences in their genetic makeup.

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