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RESEARCH ARTICLE

Assessment of Morphological Characterization and Genetic Variability of Mandukaparni (*Centella asiatica* L.) Accessions

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Centella asiatica L., commonly known as Indian pennywort is one of the chief herbs having a lot of medicinal properties and is used extensively both in traditional and modern medicine. However, the unrestricted exploitation coupled with the limited cultivation has threatened their survival and is now included in the list of endangered species. In the present study, fifteen accessions of *Centella asiatica* have been assessed for the morphological characterization and genetic variability to identify superior genotypes. Genetic variability parameters (phenotypic coefficient of variation, genotypic coefficient of variation, heritability, and the genetic advance) have been evaluated using ANOVA for twelve morphological characters such as shoot length, leaf length, leaf width, rosette diameter, petiole length, fresh leaf weight, and dry leaf weight, etc. Genetic divergence using D^2 analysis has been performed by dividing the accessions into five clusters based on the morphological characters. The results showed that the maximum intra-cluster distance has been found in cluster IV (24.45) followed by cluster I (22.13) and cluster III (21.67), whereas the maximum inter-cluster distance has been found between clusters IV and III (40.45) followed by cluster V and IV (36.81). Thus, the germplasm which includes clusters III and IV can be used in crop improvement using breeding programs.

Key Words: *Centella asiatica* L., Genetic variability, GCV, PCV, Yield

Introduction

Centella asiatica L. is a faintly aromatic creeping evergreen in tropical and subtropical countries growing in swampy areas, including parts of India, Pakistan, Sri Lanka, Madagascar, South Africa, South Pacific, and Eastern Europe. It is an important medicinal herb used in both traditional Chinese medicine and Indian Ayurvedic medicine for thousands of years. It is one of the chief herbs for treating skin problems, healing wounds, and revitalizing the nerves and brain cells, hence primarily known as a "brain food" (Singh *et al.,* 2010). It is effective in the treatment of stomach ulcers, digestive disorders, mental fatigue, diarrhea, epilepsy, hepatitis, syphilis, and asthma (Goldstein and Goldstein, 2012), enhancing memory and longevity (Subathra *et al.,* 2005; Singh *et al.,* 2008), blood purifier (Anjana and Jha, 2008). In the light of its versatile medicinal properties, the pharmaceutical industry's demand for *Centella asiatica* has been sharply increasing thus leading to the overexploitation of the plant, which may have led to losses of genetic diversity and elite accessions. Moreover, it has been listed as threatened plant species by the International Union for Conservation of Nature and Natural Resources (IUCN) and also as an endangered species (Sharma and Kumar, 1998).

It is one of the important medicinal plants in the International market of medicinal plant trades. However, large-scale collection from wild habitats increases the threat of genetic erosion and does not result in the quality of the raw material. Therefore, there is a need to evolve high-yielding cultivars for commercial cultivation. Variability present in the natural wild populations of *Centella asiatica* offers ample scope for a breeder to select and identify new superior accessions in terms of more growth, yield, and quality for commercial cultivation. Selection based on only phenotypic differences will often mislead the breeder as it is influenced by the environment on the expression of traits. Hence, the knowledge of genetic parameters such as genotypic coefficient of variation, heritability, genetic advance as percent of the mean (GAM) is vital to judging the best genotype. The best genotype should

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**Author for Correspondence: Email: luwangjameshort@gmail.com* possess a high heritability of desired characters coupled with a high genetic advance. For the improvement of any trait, the information on its association with other traits is very crucial because selection for a particular trait invariably affects its associated traits. Morphological characterization is considered to be an important first step in the description and classification of germplasm since the breeding program in any crop is mainly based on the magnitude of genetic variability (Smith and Smith, 1989). Genetic variability for agronomic traits as well as quality tests is important in all crop improvement programmes since this component is transmitted to the next generation (Singh *et al.,* 1996; Bhandari *et al.,* 2017). In the present study, an attempt has been made to identify the better performance genotypes among the given accessions in terms of morphological growth, yield, and vegetative traits of *Centella asiatica* crop.

Materials and Methods

The present study was conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru-89 during *Kharif* 2015. The size of the plot was 6m × 1.2 m and the germplasms were planted in a randomized block design and replicated three times at a spacing of 30 cm × 10 cm. The material comprised 15 accessions (Table 1) along with one check variety Vallabh Medha, procured from different regions of the state, India, and maintained the germplasm at ICAR-IIHR. Observations were recorded from five randomly selected plants of each germplasm and in each replication for twelve traits: shoot length (cm), number of primary branches per plant, number of leaves per plant, leaf length (cm), leaf width (cm), number of nodes per plant, rosette diameter (cm), petiole length (cm), specific leaf weight (g/cm^2), internodal length (cm), fresh leaf yield per hectare and dry leaf yield per hectare.

Statistical analysis of parameters

The collected data has been subjected to analysis of variance (ANOVA) by making use of means of replication, as suggested by Goulden (1959) and the test of significance was worked out by referring to the standard "F" table suggested by Snedecor (1967). The GCV and PCV were calculated as per the method suggested by Burton and Devane in 1953 and were classified as suggested by Sivasubramaniam and Madhava 1973. Heritability (h^2) in a broad sense was calculated as per the formula suggested by Allard 1960 and expressed in percent as Low (<30%), moderate (30-60%), and high (>60%). The genetic advance as percent mean was categorized into low (<10), moderate (10-20), and high (>20) as per the formula suggested by Johnson *et al*. (1955). The genetic diversity was assessed by using Mahalanobis D^2 statistics 1936 and the grouping of accessions into clusters using Tocher's method, as suggested by Rao 1952.

Table 1. List of germplasm used in the experimental study

S.No.	Germplasm	Place of collection	State
1.	Vallabh Medha (Check)	DMAPR, Anand	Gujarat
2.	IIHR CA-1	Jalgaon	Maharashtra
3.	IIHR CA-2	Bengaluru	Karnataka
4.	IIHR CA-4	Jalgaon	Maharashtra
5.	IIHR CA-5	Mangalore	Karnataka
6.	IIHR CA-6	Jabalpur	Madhya Pradesh
7.	IIHR CA-7	Jabalpur	Madhya Pradesh
8.	IIHR CA-8	Khasi hills	Meghalaya
9.	IIHR CA-9	Khanapur	Karnataka
10.	IIHR CA-10	Khanapur	Karnataka
11.	IIHR CA-11	Honnavar	Karnataka
12.	IIHR CA-12	Gonikoppal	Karnataka
13.	IIHR CA-13	Shivamogga	Karnataka
14.	IIHR CA-14	Polyploid-1 (IIHR)	Karnataka
15.	IIHR CA-15	Polyploid-2 (IIHR)	Karnataka

Results and Discussion

The knowledge of genetic variation is important for selection in crop improvement programmes and the success of any crop improvement programme is dependent not only on the amount of genetic variability present in the population but also on the extent to which it is heritable, which sets the limit of progress that can be achieved through selection. From the analysis of variance, it was observed that the genotypes varied significantly for all the twelve characters studied which are presented in Table 2. The mean squares due to genotypes were highly significant for all the traits indicating thereby the presence of genetic variability in the experimental material. The analysis of variance revealed the existence of significant differences among the germplasm studied for most of the traits indicating the presence of a considerable amount of variability. Hence there is a scope for the selection of potential genotypes for the breeding program**.**

Table 2. Analysis of variance for the 12 characters of *Centella asiatica* L.

Source of variation (df)	Mean sum of squares											
	SL (cm)	NPB (no.)	NN (no.)	NL (no.)	LL (cm)	LW (cm)	IL (cm)	RD (cm)	PL (cm)	SLW (m^2. kg^{-1})	FLW (q .h^{-1})	DLW (q .h^{-1})
Replication (2)	1.177	23.402	17.276	469.242	0.043	0.004	0.116	0.863	0.625	0.001	130.609	3.825
Genotype (14)**	25.708	12.568	17.390	216.679	1.280	4.896	4.738	31.712	19.172	0.060	67.219	2.626
Error (28)	0.722	1.612	1.836	21.629	0.047	0.042	0.092	0.446	0.629	0.000	5.847	0.167
CV (%)	9.914	8.213	9.085	6.917	8.029	4.690	4.313	5.658	9.800	7.390	17.115	15.429
GCV (%)	33.66	12.36	15.26	11.99	23.63	29.15	17.71	27.36	30.73	36.22	33.48	35.03
PCV (%)	35.09	14.84	17.76	13.85	24.96	29.52	18.23	27.95	32.25	73.15	33.66	35.78
(h^2)	92.02	69.37	73.84	75.04	89.65	97.47	94.4	95.9	90.79	24.52	98.88	95.84
GA	66.52	21.21	27.02	21.4	46.1	59.28	35.45	55.21	60.31	36.94	68.57	70.64

** significant at $P<0.01$ SL= Shoot length; NPB= No. of primary branches/plant; NN= No. of nodes/plant; NL= No. of leaves/plant; LL= Leaf length; LW= Leaf weight; IL= Internodal length; RD= Rosette diameter; PL= Petiole length; SLW= Specific leaf weight; FLW= Fresh leaf weight (q/ha); DLW= Dry leaf weight (q/ha).

The evaluation of genetic variation parameters, viz., PCV, GCV, heritability, and genetic advance as percent of the mean for different characters. In this study, the germplasm revealed a significant amount of variability for all the twelve traits studied. Statistically, the GCV for all characters studied was lesser than PCV suggesting that the variability is due to an interaction with the environment up to some extent (Pushpa *et al.,* 2013). The difference between GCV and PCV was observed to be highest in specific leaf weight as it is strongly influenced by the environment. Low GCV and PCV were found for most of the characters indicating lesser environmental influence in the expression of a particular character (Verma *et al.,* 2014). Specific leaf weight, leaf dry weight, shoot length, petiole length, leaf width, rosette diameter, and leaf length were recorded with high GCV and PCV. It was indicated that these traits were governed by additive gene effects with low environmental effects. A similar result was obtained for shoot length in *Andrographis paniculata* (Misra *et al.,* 2001). Moderate GCV and PCV were recorded for the number of primary branches, number of nodes, number of leaves, and internodes length. A similar result was found for primary branches in sweet basil (*Ocimum basilicum*) (Ibrahim *et al.,* 2013).

The coefficient of variation indicates only the extent of variability present for different characters and does not consider the heritable portion. The heritability estimates devoid of environmental influence from the total variability indicate the accuracy with which superior segregants in a population can be selected by their phenotypic performance, thus making the selection more effective. However, heritability estimates itself is not an indication of the amount of genotypic progress that would result from selecting the superior segregants (Johnson *et al.,* 1955). Genetic advance is an important selection parameter that helps plant breeders in the selection of elite germplasm from a genetically diverse population. Estimates of heritability with genetic advances are more reliable and meaningful than individual consideration of the parameters (Nwangburuka and Denton, 2012). Therefore, heritability estimates along with high genetic advances are more useful criteria in predicting the resultant effects for selecting the best individual. This is since a character may have very high heritability but very less phenotypic variation gives rise to the very low genetic gain. Most of all the characters recorded high heritability along with genetic advance as percent of mean except specific leaf weight with low heritability (24.52%). Higher heritability indicated that these characters were less influenced by the environment and direct selection for these traits would be effective for further improvement (Abou El-Nasr *et al.,* 2013). The traits such as shoot length, leaf length, leaf width, rosette diameter, petiole length, fresh leaf weight, and dry leaf weight recorded high GCV, PCV, heritability, and genetic advance as percent of the mean. A similar report was found in stevia for leaf yield per plant (Gaurav *et al.,* 2008). Low heritability was observed for specific leaf weight which may be due to the character being highly influenced by environmental effects and genetic improvement through selection will be difficult due to masking effects of the environment on the genotypic effects. It can be concluded that traits such as shoot length, leaf length, leaf width, rosette diameter, petiole length, fresh leaf weight, and dry leaf weight had higher GCV, PCV, heritability, and genetic advance as percent of the mean are agreeable for the selection and can be effectively used for genetic improvement of breeding programs.

In the present study, D^2 analysis was carried out on fifteen germplasm using twelve characters showed significant variability for all characters evaluated and were further confirmed through the pattern of distribution of fifteen *Centella asiatica* germplasm into five clusters. Among five clusters, cluster I had six germplasm and formed the largest cluster followed by clusters III and IV had three germplasm in each cluster, cluster II with two germplasm and cluster V was unique, as it had only one germplasm which indicates a wider divergence among the germplasm. In Mahalanobis D^2 analysis, among six different clusters, the maximum intracluster distance was shown by cluster IV (24.45), followed by cluster I (22.13) and cluster III (21.67), whereas the inter-cluster distance was found between cluster IV and III (40.45) followed by cluster V and IV (36.81). It showed that germplasm which includes clusters III (IIHR CA-7, IIHR CA-14, and IIHR CA-15) and IV (IIHR CA-8, IIHR CA-9, and IIHR CA-11) can be used in hybridization programmes to generate a wide range of transgressive segregants in population for developing new varieties.

Conclusion

The present investigation provides that *Centella asiatica* germplasm such as IIHR CA-7, IIHR CA-14, and IIHR CA-15 and IIHR CA-8, IIHR CA-9, and IIHR CA-11, are genetically diverse by cluster distance and it can be concluded that there is a wider variability among these promising germplasms of *Centella asiatica* in terms of morphological characters which can be used in future crop improvement work.

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