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Genetic variability and associations in the accessions of Manduk parni {*Centella asiatica* (L)}

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ABSTRACT

The present study was conducted to estimate the nature and amount of genetic variation in genotypes in order to understand genetic, phenotypic and environmental associations and character contribution. Genetic variability, co-heritability and path coefficients were studied in the six economic traits of eight genotypes of mandukparni (*Centella asiatica* (L). Genotypic and phenotypic coefficient of variations was largest for dry herb yield and asiaticoside content followed by madecassoside content, madecassic acid and fresh herb yield. Path coefficient under study revealed that the highest direct contribution to dry herb yield was made by fresh herb yield (0.687) and madecassic acid (0.545) followed by madecassoside content (0.155) in per cent. All traits expressed high heritability ($\hat{h}^2_{BS}$) 88.89–99.40% except madecassoside content low heritability (39.92%). Genetic advance was high for the three traits namely, fresh, dry weight and asiaticoside content. Fresh and dry herb yield was highly significant and positively correlated with each other at both genetic and phenotypic levels. Amongs the accessions CIM Medha followed by RK-2 and CA-7 and CA-4 was found suitable for cultivation on the commercial scale.

1. Introduction

Centella asiatica (L) (Mandukparni) is a small herbaceous creeping plant of the family 'Apiaceae' and native of India/Sri Lanka. Mandukparni a widely available Indian herb has been used for centuries in both traditional Chinese and Indian systems of medicine. *Centella asiatica* also has a historical reputation for boosting mental activity and for helping a variety of systemic illnesses, such as high blood pressure, rheumatism, fever, and nervous disorders. In traditional African system of medicine it has been used for the treatment of leprosy, bronchitis, asthma, syphilis and wound healing (Husain et al., 1988; Wang et al., 1997). In India for the last 3000 years of Ayurvedic medicine, it has been used for the purposes like boosting memory, wound healing, a mild diuretic, increasing concentration, alertness, as well as anti-anxiety and anti-stress. The isolated steroids from the plant have been used to treat leprosy and re-vitalize the brain and nervous system. To make its cultivation cost-effective in India especially, North Indian Plains requires genetic study amongst available genetic variability in genotypes. As varietal development is depends upon existing and/or induced genetic variability. The present study was conducted to estimate the nature and amount of genetic variation for different traits of a number of genotypes/accessions in order to understand genetic, phenotypic and environmental associations and contribution of various yield components with centelloside for crop improvement.

Keeping in mind the importance of *Centella*, there are needs to study genetic variability, associations genetic and path analysis and develop a strain with better plant type and quality. Therefore, the planned breeding and selection process was undertaken at CSIR-CIMAP, Lucknow to identification of such strains.

2. Materials and methods

Eight diverse genetic stocks of Mandukparni {*Centella asiatica* (L)} were evaluated in field trials for fresh and dry herb yield and centelloside (Table 1). The accessions were grown in a randomized block design with five replications, with plot size = 100 cm^2 at the research farm of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India in the two consecutive years 2006–2007 and 2007–2008. The experimental site at the institute research farm was located at 26.5° N latitude and 80.50° E longitude, and 120 m above mean sea level. The climate is semiarid to subtropical in nature. Each row to row and plant to plant distance in plot was 15 × 15 cm. The plants received normal cultural operations, irrigation, and fertilizer applications (60 kg N, 30 kg P_2O_5, and 30 kg K_2O per hectare). Observations were recorded on six eco-

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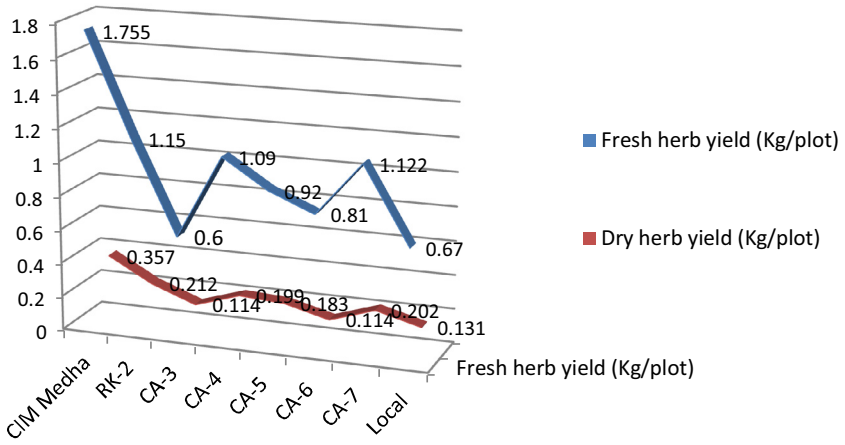

Fig 1. Centelosides in the accessions and new cultivar CIM Medha.

Table 1
Mean performance and origin/places of collections of eight accessions of *Centella aisatica* maintained at CSIR-CIMAP, Lucknow.

S. No.	Entries	Places of collection	Fresh herb yield (Kg/plot)	Dry herb yield (Kg/plot)	Asiaticoside mg/g dry weight	Madecassoside mg/g dry weight	Madecassic mg/g dry weight	Asiatic acid mg/g dry weight
1.	CIM Medha	Released variety by CSIR CIMAP on dated: 26-9-2015	1.755	0.357	52.620	15.340	4.540	3.000
2.	RK-2	Gauhati (Assam)	1.150	0.212	21.660	11.176	0.740	1.640
3.	CA-3	Mohan Lal Ganj (U.P.)	0.600	0.114	38.320	4.120	0.752	0.762
4.	CA-4	Shilang (Meghalay)	1.090	0.199	12.630	1.000	0.490	0.390
5.	CA-5	Barabanki (U.P.)	0.920	0.183	11.550	1.002	0.228	0.270
6.	CA-6	Khozekhode (Kerala)	0.810	0.114	10.420	1.384	0.190	2.252
7.	CA-7	Dehradoon (Uttranchal)	1.122	0.202	11.840	1.520	0.180	1.380
8.	Local	Asthi, Lucknow (U.P.)	0.670	0.131	9.680	0.444	0.208	1.650
		Range	0.600–1.755	0.131–0.337	9.680–52.620	0.444–15.340	0.180–4.540	0.270–3.000
		CD $_{5\%}$	0.160	0.031	3.201	0.565	0.197	1.282
		CD $_{1\%}$	0.215	0.042	4.310	0.761	0.265	1.727

nomic traits, i.e., two quantitative fresh herb yield kg/plot (weight of fresh areal parts (stem + leaves) and dry herb yield (shad dried fresh herb kg/plot) and four qualitative components or in total centellosides (asiaticoside, madecassoside, madecassic acid and asiatic acid mg/g dry weight/in percent).

2.1. Extraction and quantification of centellosides (asiaticoside, madecassoside, madecassic acid and asiatic acid in percent

For centellosides extraction 100 mg of lyophilized powdered shoot tissue was extracted thrice with 5 ml of methanol: water (80:20) for 24 h each. The pooled methanol extract was concentrated in Rotavapor to 5.0 ml and defatted twice with 20 ml *n*-hexane for 4 h. The *n*-hexane layer was discarded and the defatted methanol extract was collected, dried under vacuum and dissolved in 1.0 ml HPLC grade methanol for further analysis. The HPLC quantification of centellosides was carried out using the procedure described earlier (Verma et al., 1999). A gradient liquid chromatographic system (model LC-10A series, Shimadzu, Japan) fitted with two LC-10AD pumps controlled by CMB-10A interface module equipped with multi-dimesional UV–vis detector (SPD-10A), 7725i manual injector (Rheodyne), 20 ll sample loop and a CLCODS (M) column (250 9 4.6 mm id; Shimadzu) was used. The elution was performed using the solvent system composed of 1% trifluoroacetic acid (TFA): methanol (30:70 v/v) at a flow rate of 1 ml min-1 (column temperature of 26 °C). Detection was done at 220 nm wavelength. Reference compound of centellosides (asiaticoside, madecassoside, madecassic acid and asiatic acid) was purchased from Fluka Analytical, France.

2.2. Statistical analysis

Statistical analyses was done using the Statistical Software 0.3 version, available in the Division of Genetics and Plant Breeding of the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India, that is based on the standard methods in (Panse and Sukhatme, 1967) and (Singh and Chaudhary, 1979). The pooled mean values of two years for the all nine characters were subjected to correlation and path coefficient analyses (Dewey and Lu, 1959; Lal et al., 2001; Lal, 2014).

3. Results and discussion

Variation among the pooled mean of accessions – eight diverse collections for the nine economic traits was highly significant ($p < 0.01$) for the six traits examined (Table 2). The meticulous study of analysis of variance, means and critical difference (C.D.) revealed highly significant differences among the genotypes for all six char-