CHARACTERIZATION AND EVALUATION OF MANDUKAPARNI (*Centellaasiatica*L.) GERMPLASM)

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2014- 2016

DEDICATED

TO MY

BELOVED PARENTS

UNIVERSITY OF HORTICULTURAL SCIENCES, BAGALKOT
DEPARTMENT OF PLANTATION, SPICES,
MEDICINAL AND AROMATIC CROPS
COLLEGE OF HORTICULTURE, BENGALURU-65

CERTIFICATE

This is to certify that the thesis entitled **"Characterization and evaluation of Mandukaparni (*Centellaasiatica* L.) germplasm"**submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (HORTICULTURE) in PLANTATION, SPICES, MEDICINAL & AROMATIC CROPS** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried out by **Mr. LUWANGSHANGBAM JAMES SINGH, ID No. UHS14PGM526,** during the period of his study in this University under my guidance and supervision, and this thesis has not previously formed the basis of the award of any other degree, diploma, associateship, fellowship or any other similar titles.

Bengaluru
May, 2017

(**T. VASANTHA KUMAR**)
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Bengaluru
May, 2017 ***(L. JAMES SINGH)***

Characterization and Evaluation of Mandukaparni (*Centellaasiatica* L.) germplasm

Abstract

Mandukaparni (*Centellaasiatica*(L.) Urban) is a perennial, prostrate, stoloniferous herb grown commonly in damp areas in different tropical countries and is used both in traditional and modern medicine. The main active principles are Asiaticoside, Madecassoside, Asiatic acid and Madecassic acid. The study was undertaken to assess the genetic diversity by morphological, biochemical and molecular methods within the 15 germplasm accessions maintained at ICAR-Indian Institute of Horticultural Research, Bengaluru. Morphological characterization of the accessions showed differences among the qualitative traits in terms of leaf shape, leaf margin, leaf petiole colour and flower colour. For quantitative characters, higher estimates of phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance was observed for shoot length, leaf length, leaf width, rosette diameter, petiole length, fresh leaf weight and dry leaf weight. The genetic divergence studies using Mahalanobis D^2 analysis divided the accessions into five clusters based on morphological characters. Biochemical analysis for terpenoids showed wide variation among the accessions with the highest asiaticoside content in IIHR CA-13 followed by IIHR CA-14. The madecassoside content was found highest in IIHR CA-14 followed by IIHR CA-7, IIHR CA-1 and IIHR CA-13. The molecular characterization was done using 20 SSR markers out of which 18 primers showed 100 per cent polymorphism. PIC values of primers ranged from 0.20 to 0.34 with a mean of 0.26. Thus, this study showed that there is a wide variation among the genotypes in terms of morphological, biochemical and molecular characters and offers scope for future crop improvement programmes.

L. JAMES SINGH (Signature of student)

T.VASANTHA KUMAR (Signature of Major Advisor)

ಮಂಡುಕಪರ್ಣಿ (*ಸೆಂಟೆಲ್ಲಾಏಷಿಯಾಟಿಕ್‌ಎಲ್.*) ಯ ಪ್ರಮೆಶನುಮತಿಗಳ ಗುಣಲಕ್ಷಣ ಮತ್ತು ಮೌಲ್ಯಮಾಪನ

ಸಾರಾಂಶ

ಮಂಡುಕಪರ್ಣಿಒಂದು ಬಹುವಾರ್ಷಿಕ, ಸುರಕ್ಷಿತ, ಸ್ಟೋಲೊನಿಫೆರಸ್ ಮೂಲಿಕೆಯಾಗಿದ್ದುಉಪವಲಯದ ರಾಷ್ಟ್ರಗಳಲ್ಲಿತೇವ ಪ್ರದೇಶಗಳಲ್ಲಿ ಸಾಮಾನ್ಯವಾಗಿಇರುತ್ತವೆ. ಇದನ್ನು ಸಾಂಪ್ರದಾಯಿಕ ಮತ್ತುಆಧುನಿಕಔಷಧಿ ಪದ್ದತಿಗಳಲ್ಲಿ ಬಳಸಲಾಗುತ್ತದೆ. ಇದರಲ್ಲಿರುವ ಮುಖ್ಯಾಂಶಗಳಿದರೆ ಏಷಿಯಟೀಕೊಸೈಡ್, ಮೇಡಿಕಾಸೋಸೈಡ್, ಏಷಿಯಟಿಕ್ ಮತ್ತು ಮೇಡಿಕಾಸಿಕ್ ಆಮ್ಲಗಳು. ಭಾರತೀಯತೋಟಗಾರಿಕ ಸಂಶೋಧನಾ ಸಂಸ್ಥೆ, ಬೆಂಗಳೂರಿನಲ್ಲಿ 15 ಜರ್ಮಪ್ಲಾಂಸ್ಮಗಳ ಆಕೃತಿವಿಜ್ಞಾನ, ಜೀವರಾಸಾಯನಿಕ ಮತ್ತುಅಶ್ವತ ವಿಧಾನಗಳಿಂದ ಅನುವಂಶಿಕ ವ್ಯವಿಧ್ಯತೆಯನ್ನು ನಿರ್ಣಯಿಸಲು ಈ ಅಧ್ಯಯನವನ್ನು ಕೈಗೊಳ್ಳಲಾಯಿತು. ಪ್ರವೇಶಾನುಮತಿಗಳ ಆಕೃತಿವಿಜ್ಞಾನದಗುಣಲಕ್ಷಣವು ಎಲೆ ಆಕಾರ, ಎಲೆಯ ಅಂಚು, ಎಲೆಯತೊಟ್ಟು, ಬಣ್ಣ ಮತ್ತು ಹೂವಿನ ಬಣ್ಣದ ಪರಿಭಾಷೆಯಲ್ಲಿಗುಣಾತ್ಮಕ ಲಕ್ಷಣಗಳ ನಡುವೆ ಬಿನ್ನತೆಗಳನ್ನು ತೋರಿಸಿದೆ. ಅಂದರೆ ಬದಲಾವಣೆಯ ಫೀನೋಟ್ಯಪಿಕ್‌ಗುಣಾಂಕದ ಹೆಚ್ಚಿನ ಅಂದಾಜುಗಳು, ಬದಲಾವಣೆಯಜೀನೊಟ್ಯಪಿಕ್‌ಗುಣಾಂಕದ, ಆನುವಂಶಿಕತೆ ಮತ್ತು ಅನುವಂಶಿಕ ಮುಂಗಡವನ್ನುಉದ್ದಕಾಂಡ, ಉದ್ದ ಎಲೆ, ಅಗಲ ಎಲೆ, ರೋಸೆಟ್ ವ್ಯಾಸ, ತೊಟ್ಟಿನಉದ್ದ, ಹಸಿ ಎಲೆ ತೂಕ ಮತ್ತು ಒಣ ಎಲೆಯತೂಕವನ್ನುಇದರಲ್ಲಿ ಗಮನಿಸಲಾಗಿದೆ. ಈ ಒಂದುಜೈವಿಕ ವಿಜ್ಞಾನವನ್ನು ಉಪಯೋಗಿಸಿಕೊಂಡು ಮಹಾನೊಬಲಿಸ್ D^2ಅಂಶ ವಿಶ್ಲೇಸಿಸಲಾಗಿದೆ. ಟರ್ಪೆನೊಯೊಡ್ಗಳ ವಿಶ್ಲೇಷಣೆಅತ್ಯುನ್ನತ ವಿಷಯಧಾರಿತ ವಿಷಯದೊಂದಿಗೆ ಪ್ರದೇಶಗಳ ನಡುವೆ ವ್ಯಾಪಕ ವ್ಯತ್ಯಾಸವನ್ನು ತೋರಿಸಿದೆ. ಅವುಗಳಂದರೆ **IIHR CA-13,IIHR CA-14, IIHR CA-7, IIHR CA-1, IIHR CA-13** ಮತ್ತು **IIHR CA-14** ದಲ್ಲಿ ಮ್ಯಾಡೆಕ್ಯಾಸೊಸೈಡ್ ಅಂಶವು ಅತಿ ಹೆಚ್ಚುಕಂಡು ಬಂದಿದೆ. ಅಣ್ವೆಕ ಪಾತ್ರವನ್ನು ಪರಿಶೀಲಿಸಲು **20 SSR** ಮಾರ್ಕರ್ಸ್ ಬಳಸಲಾಯಿತು. ಅದರಲ್ಲಿ **18** ಪ್ರೈಮರ್‌ಗಳು, **100** ರಷ್ಟು ಪಾಲಿಮೂರ್ಫಿಜಮನ್ನುತೋರಿಸಲಾಯಿತು. ಪ್ರೈಮರ್ಸ ಪಿ.ಐ.ಸಿ ಮೌಲ್ಯಗಳು **0.20** ರಿಂದ **0.34** ರವರೆಗೆ ಸರಾಸರಿ **0.26** ರಷ್ಟಿತ್ತು. ಅದ್ದರಿಂದ ಈ ಅಧ್ಯಯನವುರೂಪ ವಿಜ್ಞಾನ, ಜೀವರಾಸಾಯನಿಕ ಮತ್ತುಅಣ್ವೆಕ ಪಾತ್ರಗಳ ವಿಷಯದಲ್ಲಿ ಜೀನೋಟ್ಯಪ್ಗಳಲ್ಲಿ ವ್ಯಾಪಕ ವ್ಯತಾಸವಿದೆ ಮತ್ತು ಭವಿಷ್ಯದ ಬೆಳೆಗಳ ಸುಧಾರಣೆ ಕಾರ್ಯಕ್ರಮಗಳಿಗೆ ಉಪಯೋಗಿಸಬಹುದುಎಂದು ತೋರಿಸಿದೆ.

ವಿದ್ಯಾರ್ಥಿಯ ಸಹಿ
(ಎಲ್. ಜೇಮ್ಸ್ ಸಿಂಗ್)

ಮುಖ್ಯ ಸಲಹೆಗಾರರ ಸಹಿ
(ಟಿ. ವಸಂತ್‌ಕುಮಾರ್)

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ABBREVATIONS USED IN THE TEXT

%	Per cent
° C	Degree Celsius
viz	Namely
et al	And others
cm	Centimeter
g	Gram
ha	Hactare
Sl. No.	Serial number
ANOVA	Analysis of Variance
C.D	Critical difference
S.Em.	Standard error of the mean
C.V	Co-efficient of variation
GA	Genetic advance
GAM	Genetic advance as percentage mean
GCV	Genotypic coefficient of variation
PCV	Phenotypic coefficient of variation
h^2	Heritability
w/w	Weight by weight
qt	Quintal
Kg	Kilograms
HPLC	High performance liquid chromatography
ng	Neno-gram
mg	Milligram
µg	Microgram

µl	Micro liter
ppm	Parts Per million
O.D	Optical density
DNA	Deoxyribonucleic acid
SSR	Simple sequence repeat
EDTA	Ethylenediaminetetraacetic acid
pH	Potential of hydrogen
CTAB	Cetyl Trimethyl Ammonium Bromide
NaCl	Sodium chloride
PVP	Polyvinylpyrrolidone
dNTP	Deoxynucleotide Triphosphate
$MgCl_2$	Magnesium chloride
PCR	Polymerase chain reaction
UV	Ultraviolet
UPGMA	Unweighted pair group method with arithmetic average
SAHN	Sequential agglomerative hierarchial and nested
PIC	Polymorphic information content
Ho	Observed heterozygosity

I. INTRODUCTION

Centella asiatica (Linn.), is commonly known as Indian Pennywort or Mandukaparni or Gotukola or Pegaga belongs to the family Apiaceae and subfamily Mackinlaya (Liu *et al*., 2003). It is native to Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar *etc*. It is distributed throughout tropical and sub-tropical regions of India upto an altitude of 600m and also at high altitudes of 1550m in Sikkim and 1200m in Mount Abu (Rajasthan). It is abundantly found during rainy season and also found in most of tropical and subtropical countries growing in swampy areas, including parts of India, Pakistan, Sri Lanka, Madagascar, South Africa, South pacific and Eastern Europe.

Centella asiatica L. is a prostrate faintly aromatic, stoloniferous, perennial, herbaceous creeper flourishes abundantly in moist areas in shady, marshy, damp and wet places such as paddy fields, river banks forming a dense green carpet (Anon., 1992). It is clonally propagated by producing stolons that are characterized by striated glabrous stem, long internodes and rooting at the nodes, on which are borne orbicular or reniform-cordate leaves and sessile flowers in simple umbels (Zheng and Qin, 2007), each umbel consisting of 3-4 white to purple or pink flowers and fruits are oblong, globular in shape and strongly thickened pericarp. Seeds have pendulous embryo which are laterally compressed, the form and shape of the *Centella asiatica* plant can differ depending on environmental conditions (Adamson, 1950).

It is used both in traditional and modern medicine and used in the ancient traditional Chinese Shennong Herbal about 2000 years ago and in Indian Ayurvedic medicine about 3,000 years ago as a Medhya Rasayana (Psychotropic drugs). It is one of the chief herbs for treating skin problems, to heal wounds, for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" in India and effective in treatment of stomach ulcers, digestive disorders, mental fatigue, diarrhoea, epilepsy, hepatitis, syphilis and asthma (Goldstein and Goldstein, 2012), enhancing memory and longevity (Subathra *et al*., 2005 and Singh *et al.*, 2008), blood purifier (Anjana and Jha, 2008), antidiabetic (Chauhan *et al.,*2010), gastrointestinal disease, gastric ulcer, and eczema (Brinkhaus *et al*., 2000), antidepressant (Chen *et al.*, 2003), antibacterial, antifungal (Ullah *et al*., 2009), antipsoriatic (Sampson *et al*.,2001), anti-cancer agent (Babu *et al*., 1995), anti-inflammatory (Somchit *et al*., 2004; George and Joseph, 2009), cardio protective (Gnanapragasam *et al*., 2004 and Raghavendra *et al*.,2009), antidiabetic (Venu Gopal Rao and Mastan, 2007), immunostimulant (Wang *et al*., 2003), antioxidant (Hamid *et al*., 2002; Bajpai *et al*., 2005), venous deficiency treatments (Pointel *et al*., 1987; Cesarone *et al*., 2001). It is also commonly used as a vegetable and in drinks as in tea or juice (James and Dubery, 2009).

There are seven main groups of chemical compounds that present in *Centella asiatica.* They are Amino acids *viz*., aspartic acid, glycine, glutamic acid, α-allanine and phenylalanine, (Malhotra *et al*., 1961), carbohydrates, phenols, terpenoids, volatile oils fatty oils and vitamins and minerals. Among these triterpenoids and saponins groups are primary constituents of *Centella asiatica* such as triterpenes, asiaticoside, centelloside, madecassoside, brahmoside, brahminoside (saponinglycosides), asiatic acid, centellic acid, centoic acid, madecassic acid, terminolic acid and betulic acid (Barnes *et al*., 2007 and Jamil *et al*., 2007).

According to the reports of Export and Import Bank of India, *Centella asiatica* is one of the important medicinal plants in the International market of medicinal Plant trade. However, the wild stock of this plant species has been markedly depleted, because of its large scale and unrestricted exploitation coupled with limited cultivation and insufficient attempts for its replacement made. Moreover, now it has been listed as threatened plant species by the International Union for Conservation of Nature and Natural Resources (IUCN) (Pandey *et al.,*1993) and also as an endangered species (Singh 1989; Sharma and Kumar, 1998).

Though *Centella asiatica* L. is widely distributed, only limited attempts have been made in exploiting the diversity in conservation and crop improvement. Sporadic efforts have been made in collecting region specific germplasm for assessing diversity. However, extensive collection and systematic evaluation to identify superior accessions for yield and quality and their characterization needs immediate attention.

Characterization of plants with the use of morphological and molecular markers is an ideal approach for the conservation of plant genetic resources and genetic improvement (Rout and Mohapatra, 2008). Both molecular and morphological markers are valuable for the identification of distinct populations or germplasm for conservation, optimum sites for germplasm collection, and ongoing changes in the pattern

of diversity over time, the study of diversity of pre-breeding and breeding programme for the protection of the breeder's intellectual property rights (Franco *et al*. 2001; Newbury and Ford-Lloyd, 1997). The morphological variability alone may not be efficient in assessing the diversity. So, the use of molecular markers for genetic diversity assessment and genotypic characterization are of prime importance.

Molecular markers are widely used in various applications for improvement of the crop. The various applications include diversity analysis, mapping, gene tagging, QTL analysis, paternity analysis, finger printing and germplasm characterization of plant, using markers namely RFLP, RAPD, AFLP, ISSR and SSR. Among these, SSR markers have particularly important because of their abundant distribution, highly polymorphic, reliable, and co-dominance in nature. They are useful for both genetic diversity analysis and genetic mapping. SSR markers are attractive for DNA finger printing studies, as they are co-dominant (Beckmann and Soller, 1990; Brown *et al*., 1996; Senior *et al.,* 1998).

In the past studies, SSR marker from *Centella asiatica* were developed and characterized to promote genetic and molecular studies (Rakotondralambo *et al.,* 2012). Analysis of phenotypic performance of *Centella asiatica* germplasm in combination with molecular analysis provides useful information on the actual genetic diversity and could be useful to increase the efficiency of Plant breeding programmes.

In the light of the above, the present study "Characterization and evaluation of *Centella asiatica* L. germplasm" was aimed with following objectives.

1. To assess the morphological, yield and quality variation in *Centella asiatica* L. germplasm.

2. To evaluate the biochemical variability in *Centella asiatica* L. germplasm.

3. To estimate the molecular diversity of *Centella asiatica* L. germplasm employing SSR marker.

II. REVIEW OF LITERATURE

Centella asiatica L. is one of the important medicinal plants in the International market of medicinal plant trade. However, the wild stock of this plant species has been markedly depleted, due to large scale and unrestricted exploitation coupled with limited cultivation and insufficient attempts for its replacement, nowadays 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 (Singh, 1989: Sharma and Kumar, 1998). Therefore, it is important to undertake extensive collection, systematic evaluation and characterization of this species for conservation and crop improvement programmes. Identification of superior germplasm for yield and quality benefit the drug industry in terms of more yield with higher biochemical content. In this regard, the present research has been taken up to carry out the morphological and molecular characterizaton of *Centella asiatica* germplasm along with its yield and quality analysis for active ingredient content.

The available brief review of the work done in *Centella asiatica* in relation to the objectives of the research programme is presented here.

2.1 Morphological characterization of *Centella asiatica* L.

Variation in different growth traits of *Centella asiatica* was investigated using vegetative clone of genome from one population in Kirtipur, Kathmandu, Nepal. Plants were raised in each of six soil compositional type and assessed for vegetative traits like number of leaves, petiole length, specific leaf area, number of primary branches and plant biomass. Most of the observed growth traits demonstrated significant variation in response to soil type. The *Centella asiatica* plant was found to maximize growth and yield in sandy loam rather than clayey soil (Anjana and Jha, 2009).

The growth patterns and yield of *Centella asiatica* plant under different levels of shading 0 % (full sunlight), 30 %, 50 % and 70 % of solar radiation interception were investigated in Kirtipur, Kathmandu, Nepal. The plantlets were grown in earthen pots containing soil, sand and vermicompost (1:2:1) and subjected to different levels of shading and of solar radiation and full sunlight as control. The results suggested that plants subjected to 30 % shading showed higher plant biomass. However, the plantlets root system showed higher dry biomass under full sunlight (Anjana and Jha, 2010).

Variation in different growth traits of *Centella asiatica* was investigated using vegetative clones of one population in Kirtipur, Kathmandu, Nepal. Plantlets were grown in earthen pots containing soil, sand and vermicompost and treated with different levels of water stress (30 %, 70 %, 100 % and 125 % of pot capacity by mass). Vegetative traits viz., number of leaves, petiole length, specific leaf area, number of primary branches, and plant biomass were examined. The study revealed that plants irrigated with 100 % pot water capacity showed highest growth and plant biomass production (Anjana and Jha, 2011).

Srithongkul *et al*. (2011) studied the effects of different light intensities on leaf area, petiole length and accumulation of asiaticoside, madecassoside, asiatic acid and madecassic acid in three germplasm of *Centella asiatica,* collected from three locations in Thailand i.e, Nakon SiThammarat, Rayong and Ubon Ratchathani. The Nakhon Si Thammarat germplasm had the largest leaf area followed by the Ubon Ratchathani and Rayong germplasm respectively, while the longest petioles were found in Rayong.

Effect of integrated manuring on growth of *Centella asiatica* was investigated using vegetative clones from Kirtipur, Kathmandu. Plantlets were grown in earthen pots containing soil, with integrated manuring [Urea (%): FYM (%),75:25; 50:50; 25:75], individual manuring (100 % Urea, 100 % FYM) and control conditions (no manure) and examined for vegetative traits like number of leaves per ramet, petiole length, specific leaf area, number of primary branches, number of flowers per ramet and plant biomass. The number of leaves per ramet, leaf area and number of flowers per ramet were observed significantly higher in integrated manuring than other treatments (Anjana and Jha, 2013).

2.1.1 Morphological characterization of other medicinal plants

Arrigoni-Blank *et al*. (2005) studied accessions of six *Hyptis pectinata* for their morphological parameters such as plant height, canopy diameter, leaf length (L) and width (W), L/W relation and dry weight of leaves and stem. The results showed that the accession SAM006 had highest leaf dry matter yield when the three harvests were combined. It was concluded that the accessions SAM004, SAM005 and SAM006 were promising germplasm for plant breeding programs to develop a high yielding cultivar.

Sharma *et al*. (2009) studied fifteen accessions of Kalmegh from different locations of Chhattisgarh and adjoining states for their morphological parameters. The plant height ranged from 21.44 (AP-5) to 58.67 cm (KI-2) with a mean height of 39.89 cm, number of branches ranged from 18.44 (AP-22) to 33.44 (AP-6) with a mean value of 22.72, number of leaves ranged from 83.11 (AP-5) to 149.78 (AP-6) with an average of 111.68, leaf width ranged from 1.07 (AP-6) to 2.96 cm (KI-2) with a mean value of 2.18, leaf length ranged from 5.88 (AP-9) to 7.28 cm (AP-5) with a mean of 6.54, dry weight of plant ranged from 30 (AP-5) to 50g (KI-2) with a mean value 40.53. The quantitative data indicated that germplasm KI-2 gave the best performance. Hence they revealed that morphological characters showed usefulness of selecting the germplasm for commercial cultivation.

Gupta *et al*. (2009) evaluated fifty germplasm accessions of *Tagetes erecta* (African marigold) for genetic variation in xanthophylls and other morphological traits. Large variation was noticed for morphological traits viz., plant height (ranging from 38 to 123 cm). Primary (2 to 25) and secondary (24 to 82) branches.

Singh *et al*. (2010) collected eight genotypes of *Aloe vera* from different parts of Himalayan hills and evaluated morphological, biochemical and genetic variations. Evaluation showed variation in plant height from 46.2 to 62.6 cm, leaf length from 41.25 cm to 60.50 cm and leaf fresh weight from 134.2 to 305 g.

Panday and Mandal (2010) studied accessions of kalmegh (*Andrographis paniculata*) from five locations of Madhya Pradesh and Chhattisgarh for their morphological variability. The maximum plant height reported was 60.20 cm, leaf length 6.22 cm and width of 1.98 cm from Amarkantak source. Maximum height was 52.30 cm, collar diameter 4.96 mm, number of branches 16.80, leaf length 5.84 cm, width 1.83 cm from Dhamtari source.

A study was conducted to evaluate stevia clones and characterize genetic divergence where the Plant height, production of fresh and dry matter, number of branches per plant, concentration of total edulcorant and relation between rebaudioside A/stevioside were evaluated. ST75, ST86, ST141 and ST145 clones were presented high mean genetic divergence in relation to the whole genotypic pool studied and most promising for rebaudioside A/stevioside relation (79 %), total edulcorant concentration (12 %) and dry matter production. It showed that plant height had significantly positive correlation with rebaudioside A relation and the number of branches per plant exhibited significant positive correlation with fresh matter and dry matter yields (Anami *et al*., 2010).

Six *Gymnema sylvestre* (Gudmar) germplasm collected from various regions of Madhya Pradesh were evaluated for their morphological traits such as habit, leaf length, leaf width, and leaf shape. Average leaf length ranged from 3.58 (Panna) to 4.92 cm (Chitrakoot) and the average leaf width varied from 1.67 (Panna) to 2.68 cm (Amarkantak). The leaf base shape was mostly subcordate, but truncate, rounded, and obtuse. The leaf tip shape was either acute or acuminate. However, a few accessions showed cuspidate shape. They revealed that there is wide variation in the morphological characters of *Gymnema sylvestre* germplasm collected from various locations, which can be exploited for further crop improvement programmes (Pandey and Yadav, 2010).

Abhila and Jessykutty (2011) evaluated morphological variability of thirty accessions of *Aloe barbadensis* collected from different parts of Kerala and Tamil Nadu and recorded morphological variation. The height of accessions varied from 32.70 to 69.74 cm. Wide variation of plant spread was observed for the accessions, which varied from 0.318 to 1.101 m^2. Length of leaf ranged from 27.14 to 50.6 cm while breadth of leaf ranged from 4.54 to 7.14 cm.

Barfa *et al.* (2012) studied morphological and yield attributing parameters in sixteen Isabgol germplasm under Madhya Pradesh state of Jabalpur district. The study revealed that germplasm accession Gujarat-1 was best performing in terms of mean plant height (38.77cm) specific leaf area (67.74 cm^2/mg), specific leaf weight (0.55 mg/cm^2), crop growth rate (13.65 g/m^2/day), ear number (52.95/plant), ear length (5.36 cm) and number of seeds per ear (102.81). The germplasm Gujarat-1 and RI-157 are recommended for commercial cultivation under ecological conditions of Jabalpur.

Fifty genotypes of *Aloe vera* were collected from Iran and evaluated their morphological characters where the number of plant leaves, mature leaf length, mature leaf width, mature leaf weight etc. It showed that Plant height and leaf width varied from 55.0 to 69.7 cm and from 10.8 to 24.0 cm, respectively, plant height was positively correlated with the width of plant and with the number of leaves ($r = 0.452$ and $r = 0.409$, respectively. Based on Cluster analysis the fifty genotypes arranged into three main Groups.

Genotypes in Group A had the botanical potential, which could be considered for future breeding programmes (Nejatzadeh-Barandozi *et al*., 2012).

An investigation was conducted to assess the genetic parameters in respect of yield and yield determining characters of nine germplasm lines of Coriander (*Coriandrum sativum*). The quantitative traits like plant height, number of secondary branches per plant, number of primary branches per plant, number of umbel per plant, number of umbellets per umbel and number of seeds per umbel exhibited wide range of variability, maximum genotypic and phenotypic coefficient of variability, broad sense heritability and genetic gain as per cent of mean. It was found that the genotypes 9106, 2007, 2108 and 2015 were promising (Singh and Singh, 2013).

Chhaya *et al*. (2013) had sixteen accessions of basil and maintained at day and night temperatures of 28-33°C and 20-25°C for analysis of quantitative and qualitative characters. The results showed that location had significant effect on all the six characters and the interaction between genotype and environment was also pronounced for all the characters.

Tripathi *et al.* (2013) collected eighteen *Coleus forskohlii* genotypes from different places of central India and evaluated morphological characters. The plant height of accessions varied from 43.2 to 61.0 cm, root length varied from 6.1 to 9.7 cm. The fresh root weight ranged from 102.1 to 149.6 g and the dry root weight ranged from 14.3 to 29.8 g.

Tomar *et al*., 2014, attempted the morphological characterization in twenty-five genotypes of coriander and found largest variation with respect to days of 50% flowering (27 to 33 days), plant height (75 to 80 cm), number of basal leaves (5 to 9) and longest basal leaf (5 to 7).

Forty-nine accessions of cultivated Holy basil (*Ocimum tenuiflorum*) representing four phytogeographical regions of India were characterized for quantitative traits where, the number of leaves ranged from 71 in IC 583279 to 18 in IC 583306 and IC 583312. Leaf size ranged from 3.36 cm (medium) in KCB-25 to 8.84 cm (large) in PM/12/6. Number of primary branches ranged from 14 in IC583281 to 7 in IC583300, IC583304 and KCB-25. Petiole length varied from 1.16 to 3.36 sscm. The result showed that high degree of variation among the accessions indicating rich diversity representing within the populations from different phytogeographical regions and relatedness among the morphotypes (Pavan *et al*., 2015).

2.2 Yield and quality traits of *Centella asiatica* L.

Mathur *et al*, (2000) collected sixteen accessions of *Centella asiatica* from different parts of India and were screened for their herb and asiaticoside yields at different levels of shading under sub-tropical field conditions of Indo-Gangetic plains at Lucknow during winter season. The herb and asiaticoside yields of accessions ranged from 470 to 2730 $kgha^{-1}$ and 1.0 to 9.8 $kgha^{-1}$ respectively. Considering all the accessions together, 50 % shading of plants resulted in higher yields of herbage and asiaticoside. The accession CaShT was identified as very high herbage and asiaticoside yielding under 50 % shade. The other accessions CaBp and CaCl gave high herb and asiaticoside yields under full light.

In a study at Zandu Foundation for Health Care, Gujarat, five genotypes of *C. asiatica* (named ZFB1, ZFB2, ZFB3, ZFB4 and ZFB5) collected from natural populations were evaluated for yield and tri-terpenoid content. A superior line (ZFB 4) was selected and designated as cv. Zandu Brahmi. This line was assessed under artificial shade net condition, with 100:50:0 kg/ha N:P:K and it showed improved dry yield of 2.16 mt/ha/year from 3 coppicing with 1.351 % total Triterpenoids content in about 90 days (Krishnamurthy *et al*., 2006).

A comparative quantitative analysis of the active triterpenoids in *Centella asiatica* samples collected in different locations in Madagascar was carried out to evaluate the natural variability in triterpenoid content. The Asiaticoside content ranged from 2.67 to 6.42 %, and madecassoside from 2.38 to 5.89 % on dry weight basis. The highest total triterpenoid content (12.69 %) was observed in CA-1 sample and the lowest content (5.83%) was found in CA-4 sample (Randriamampionona *et al*., 2007).

Sixty *Centella asiatica* accessions collected from various locations in south India and the Andaman Islands were estimated by HPTLC densitometry. The highest detected madecassoside and asiaticoside contents were 5.67 ± 0.08 % (dry wt. of whole plant) and1.70 ± 0.02 %, respectively. The variations in madecassoside and asiaticoside content between them are due to variations in their genetic make-up,

expression levels and other biosynthetic steps. Therefore they revealed that genetic traits are critical factors in deciding the biosynthesis of potential terpenoids in *Centella asiatica*. (Thomas *et al.,* 2010).

Srithongkul *et al.* (2011) studied the effects of different light intensities on leaf area, petiole length and accumulation of asiaticoside, madecassoside, asiatic acid and madecassic acid in three accessions of *Centella asiatica*, Nakon Si Thammarat, Rayong and UbonRatchathani from three locations of Thailand. UbonRatchathani had the highest fresh weight per unit area and highest asiaticoside and madecassoside contents. madecassic acid was highest in Nakhon Si Thammarat followed by Ubon Ratchathani and Rayong. Asiatic acid content decreased in Nakhon Si Thammarat under full sunlight while in UbonRatchathani, under both 50 % and 80 % shading. Madecassic acid contents increased in Rayong under 50 % shading.

High Performance Liquid Chromatography (HPLC) analysis of the bioactive centellosides in the crude triterpenoids extract of the hydroponically grown plants harvested leaves showed the presence of 11 mg, 1.7 mg, 36.6 mg and 6.3 mg of madecassoside, asiaticoside, madecassic acid and asiatic acid per gram on dry weight basis respectively (Prasad *et al*., 2012).

Rahajanirina *et al*. (2012) identified two foliar morphotypes in *Centella asiatica*. Morphotype A with small reniform leaves (leaf area 4.5 cm^2) found in the east of Madagascar, and morphotype B with large round leaves (up to 7.5 cm^2) found in the west, with sympatric zones in the central part of the island. Morphotype A produced higher biomass, and was twice as rich in asiaticosides as morphotype B. Significant variations in biomass yield and asiaticoside content were observed depending on the date of collection higher during the rainy season (December to April) and lower during the dry season (June to August). Inter-annual variations were also observed.

Effect of integrated manuring on growth of *Centella asiatica* was investigated using vegetative clones from Kirtipur, Kathmandu. Plantlets were grown in earthen pots containing soil, with integrated manuring [Urea (%): FYM (%), 75:25; 50:50; 25:75], individual manuring (100 % Urea, 100 % FYM) and control conditions (no manure). Biomass production in integrated manuring (50 % Urea and 50 % FYM) was seven times higher than in control and five times higher than in complete organic manuring (100 % FYM) and 1.5 times higher than in inorganic manuring (100 % Urea) (Anjana and Jha, 2013).

Prasad *et al.* (2014) studied *Centella asiatica* germplasm for biomass and centellosides productivity under uniform agro-climatic conditions of the Indo-Gangetic plains at Lucknow. The highest biomass accumulation 411.9 g FW/m^2 area was recorded in accession A from north India, followed by 284.0 g, 135.7 g and 29.2 g FW/m^2 in accessions M, B and E from southern, eastern and north-eastern regions, respectively. Accession M possessed the highest asiaticoside content of 52.1 mg/g DW. The madecassoside level in leaves of accessions B and M was 28.9 and 25.7 mg/g DW. The madecassic and asiatic acid content in leaf tissue of all four accessions remained low.

Gupta *et al.* (2014) evaluated two morphologically distinct accessions of *Centella asiatica* through its morphology, quantitative microscopy and physico-chemical tests. The metabolites included madecassoside, asiaticoside and its sapogenin asiatic acid analyzed and quantified by HPTLC. The Concentration of asiatic acid, asiaticoside and madecassoside found in SL accession were 0.04 %, 0.34 % and 0.38 %, respectively, while in LL it was 0.05 %, 0.31 % and 0.31 %, respectively.

Srivastava *et al.* (2014) collected distinct accessions of *Centella asiatica* from Nilgiri range of India and investigated chemotypic variations for madecassoside, asiaticoside, and its sapogenin, asiatic acid by high-performance layer chromatography (HPTLC). The study revealed highest content of asiaticoside, madecassoside and asiatic acid in accessions CA-45, CA-51 and CA-47, respectively.

Alqahtani *et al.* (2015) collected Australian *Centella asiatica* from a designated area in different months and studied the impact of harvesting time on the contents of major triterpenoid (asiaticoside, madecassoside, asiatic acid and madecassic acid), phenolic acids, Flavonoid compounds and Chlorogenic acid. The total content of the four triterpenes reached its highest levels in January (83.15 ± 0.16 mg/g) and February (78.41 ± 0.16 mg/g) and their lowest values in June (35.65 ± 0.20 mg/g) and October (35.50 ± 0.55 mg/g). Similarly, the contents of chlorogenic acid and kaempferol were highest in December, January and the lowest in June.

Rahajanirina *et al.* (2016) evaluated the effects of time and frequency of collection on biomass yield and content of active ingredients in *Centella asiatica*. Six collection frequencies were considered (monthly,

bi monthly, quarterly, four-monthly, semi-annual and annual). The yield of leaf biomass acquired maximum during the rainy season (November to April) and the minimum during the driest season (June/August) and highest total triterpenoids contents were obtained during the months of November and April, and the lowest between May and October.

2.2.1 Yield and quality traits of other medicinal plants

Bhan *et al*. (2005) screened ten kalmegh accessions for yield and its components besides andrographolides. The results revealed that last week of October is ideal for obtaining maximum dry herbage yield (931.3 kg/ha) and total andrographolide yield (61.83 kg/ha) in subtropical region of Jammu. The dry herbage yield per plant varied from 32.50 to 49.50 g. Accession Acc.1 and Acc.9 have been identified as the best sources for obtaining higher drug yield.

Hegde *et al.* (2005) estimated tuber and forskolin content in thirteen IIHR accessions of *Coleus forskohlii* by using HPLC and found that forskolin content to vary from 0.025 % (IIHR-1) to 0.798 % (IIHR-12). Maximum number of tubers per plant (12.0), highest fresh (870 g) and dry mass of tubers (88.75 g) per plant were observed in IIHR-59. Forskolin yield per plant was highest in IIHR-7 (85.00 mg). Based on tuber yield and forskolin content, they concluded that the accession IIHR-80 with medium tuber yield and higher forskolin content (0.715 %) can be promoted for commercial cultivation as the crop is propagated through vegetative means.

Gupta *et al*. (2005) estimated anticancerous drugs vincristine, vinblastine, and their precursors catharanthine and vindoline in ten accessions of *Catharanthus roseus* using RF-HPLC method and found that highest vincristine, vinblastine, catharanthine and vindoline content was recorded in accessions 98 (0.0012 %), 243 (0.0048 %), 112 (0.0363 %), 76 (0.1346 %) and lowest in accession number 14 (0.0001 %), 49 and 151 (0.0001 %), 49, 151 ,176 and 183 (0.0002 %), 183 (0.0142 %), respectively.

HPLC procedure was used to screen the presence of HCA (Hydroxy Citric Acid) in extracts collected from roselle accessions and their mutant lines such as Acc.3, Acc.12 HSO3100-29-2-1-17-15-1 (red calyx), HSO3100-29-7-1-6-3-1 (white calyx), HS1250-18-18-1-1-1-1 (red calyx) and HS1250-1-18-1-1-1-1 (white calyx) while *Garcinia atroviridis* and *Garcinia cambogia* which were known to contain HCA were used as control. Result revealed that roselle accession HS1250-1-18-1-1-1-1 (white calyx) showed highest (14.6 %) HCA content and HS1250-18-18-1-1-1-1 (red calyx) showed less (10.1 %) HCA content (Mohamad *et al*., 2006).

Abhila and Jessykutty. (2011) evaluated morphological variability of thirty accessions of aloe (*Aloe barbadensis*) collected from different parts of Kerala and Tamil Nadu and recorded morphological variation. The average fresh leaf weight ranged from 61.00 to 192.70 g while, not limited variation was found in leaf shape and leaf colour among the accessions.

Fifty genotypes of *Aloe vera* were collected from Iran and evaluated for phytomedical constituents by HPLC and showed that the aloenin content of genotype 39, from Hormozgan was twice as high as in all other genotypes. Based on Cluster analysis arranged the fifty genotypes into three main Groups. Genotypes in Group A had the highest aloenin contents and botanical potential, which could be considered for future breeding programmes. (Nejatzadeh-barandozi *et al.,* 2012).

Archana *et al*. (2013) reported variability among thirty accessions of Kalmegh (*Andrographis paniculata*) for andrographolide content and showed that diverse accessions of kalmegh exist in three Indian states of Orissa, Tamil Nadu and Kerala. Promising accessions having high andrographolide content were reported to be IC520361 (2.99 %), IC520395 (2.86 %), IC399125 (2.81 %), IC369404 (2.77 %) and IC520394 (2.61 %).

Tomar *et al*. (2014) studied the morphological characterization in twenty-five genotypes of coriander. Largest variation was exhibited by the character harvest index (36-75 %), seed yield per plant (7-18 g), umbellets per plant (215-268) and seeds per umbel (20-29).

Forty-nine accessions of cultivated Holy basil (*Ocimum tenuiflorum*) representing four phytogeographical regions of India were characterized for qualitative traits where, the Leaf weight (of 100 leaves) varied from 13 g in KCB- 25 to 62 g in IC583296. The purple flower colour was observed in PM/12/4, IC583278, IC583317 and IC583322. An accession AK/ HS/OS-02 recorded with pure white corolla. Calyx colour varied from green (IC75730, IC583310, IC583320 and AK/HS/OS-02) to purple green

(IC381185, IC583299), Variation was observed in leaf margin with smooth serrate types to dentate types with hairiness on midrib and stem. Accessions IC583318 and IC583319 with smooth undulated margins hence they revealed that high degree of variation among studied accessions indicating rich diversity within the populations from different phytogeographical regions and relatedness among the morphotypes. (Malav *et al.,* 2015).

2.3 Molecular characterization of *Centella asiatica* L. using DNA markers

Ruan *et al.* (2008) analyzed the DNA molecular characters of *Centella asiatica* by using RAPD. With the genomic DNA as templates extracted from various source of plant samples, optimized RAPD PCR reaction systems had been used. The random primers had been screened to amplify the specific molecular fragments of *Centella asiatica*. The genetic character bands of *Centella asiatica* amplified with the RAPD method show high homogeneity in several samples from different habitats.

The genetic diversity of *Centella asiatica* populations in China was investigated using ISSR markers. Fourteen natural populations comprising 162 individuals were estimated for genetic diversity. On the basis of Nei's Gst value, more genetic differentiation among populations was determined (Gst = 0.6573). In addition, the fourteen populations were clustered into four groups in view of abundant ISSR data, which further defined the genetic relationship among populations. (Zhang *et al*., 2012).

A GA/GT-enriched genomic library was constructed from an accession from Madagascar. Roughly 75 % of the 768 clones of the enriched library contained microsatellites. Eighty sequences containing microsatellites were obtained from 96 positive clones. Specific primers were designed for 20 loci, and 17 of them displayed polymorphism when screened across 17 *Centella asiatica* accessions. These 17 polymorphic microsatellite markers are a useful resource for this plant, applicable for diversity studies, pedigree analyses, and genetic mapping. (Rakotondralambo *et al*., 2012).

Prasad *et al*. (2014) studied *Centella asiatica* accessions A, M, B and E from north, south, eastern and north east India respectively. Amplified fragment length polymorphism (AFLP) analysis with 23 primers yielded 696 fragments, 563 of which were polymorphic. Accession M out-grouped with genetic dissimilarity indices of 83, 85 and 95 % from accessions A, E and B, respectively.

2.3.1 Molecular characterization of other medicinal plants using DNA markers

Darokar *et al*. (2001) analysed a collection of twenty-four *Bacopa monnieri* accessions from different agro-climatic zones of India and introduction from Malaysia for RAPD variation. Among the 40 primers, 29 primers generated one or more polymorphic bands ranging from 2 to maximum of 8. All the accessions were found to be in the range of 0.8 to 1.0 of similarity, which is indicative of a narrow genetic base among the accessions. The observed low level of genetic variation was attributed to interplay of sexual and vegetative modes of reproduction and similarity of local environments in habitats of *Bacopa monnieri*.

Fifty accessions of indigenous Fennel were collected from different parts of Pakistan and evaluated for characters like seed germination percentage, days to initiation of flowering, plant height, stem girth, nodal distance, umbel diameter, days to 50 % maturity, days to harvesting, seed yield per row, weight of 100 seeds, Harvest index (%) and used RAPD markers to ascertain their genetic diversity. Twenty-four out of 30 decamer primers generated 145 clear bands and 70 (48 %) were polymorphic. Sixteen primers gave polymorphism for different characters (Zahid *et al.,* 2009).

Hashemi *et al*. (2010) analysed fifteen populations of Persian Zira *(Bunium persicum)* from Iran, two populations from India, two populations from Afghanistan, and one population from Europe using RAPD marker with respect to genetic diversity, all 26 primers showed polymorphism. A total of 146 reproducible amplified bands were scored and 96 % (141 bands) of polymorphic bands (*PPB*) was found. The genetic similarity ranged from 0.37 to 0.95. The two distinct subgroups were formed at 0.50 similarities. One subgroup consisted of Iranian populations, and the second, non-Iranian populations. At a higher level of similarity (0.70), Iranian group was divided in two clusters and the populations of each province were classified in the same group.

Laribi *et al*. (2011) investigated genetic variability and differentiation among five annual Caraway (*Carum carvi*) populations from Tunisia, Germany and Egypt by using Random Amplified Polymorphic DNA (RAPD) marker with respect to genetic diversity, population structure and gene flow. Fourteen primers

generated a total of 136 discernible and reproducible bands across the analyzed populations, out of which 56 were polymorphic. The UPGMA based dendrogram grouped all the genotypes into 3 main cluster groups. It showed that Tunisian caraway populations diverged significantly from German and Egyptian ones.

Bahmani *et al*. (2012) studied the genetic diversity in twenty-five different ecotypes of fennel by using seven ISSR primers. 52 amplified fragments were generated out of which 49 were polymorphic. The highest similarity coefficient among the ecotypes was between Chahestan and Haji abad whereas the minimum similarity coefficient was observed between Fozveh and Moqan.

Torabi *et al*. (2012) studied the genetic diversity of thirty accessions of the Fennel from Iran by using 20 selective AFLP primers, a total of 1127 observed bands, 250 were polymorphic, the highest genetic similarity was 97 % found between two genotypes from Hungary and also in Iranian genotypes, similarity of 89 % which was found between Karaj and Kashan genotypes. One genotype from Tabriz with a genetic diversity of 60 % has shown the most amount of diversity in comparison with other genotypes.

Tripathi *et al.* (2012) evaluated fifteen Brahmi (*Bacopa monnieri*) accessions using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers. During RAPD analysis, 22 primers generated 197 fragments, of which 187 were polymorphic with an average of 8.95 bands per primer. The amplified products varied in size from 2,200 to 250 bp. Twenty-five selected ISSR primers produced 284 bands across 15 accessions, of which 270 were polymorphic with an average of 10.80 bands per primer. The PIC value ranges from 0.363 to 0.908 for RAPD primers, while 0.419 to 0.836 in case of ISSR. The size of amplified bands ranged from 2,800 to 240 bp. Similarity index values ranged from 0.16 to 0.95 (RAPD), 0.18 to 0.98 (ISSR) and 0.179 to 0.945 for pooled ISSR and RAPD markers data. It revealed the similar distribution pattern of the polymorphism between RAPD and ISSR markers and the correlation co-efficient (r) was 0.71384.

In a study conducted by Bahraminejad *et al.* (2012) forty-nine Cumin ecotypes, belonging to nine Iranian regional sub-populations were assessed using RAPD markers. Twenty-three RAPD markers were used for diversity assessment, in which 21 showed polymorphism. Molecular variability among and within populations was assessed accordingly. Golestan and Northern-Khorasan populations showed the highest difference while Kerman and Esfahan populations showed the most similarity. Populations of Semnan, Yazd and Golestan showed different reaction rather than the other populations. It is suggested that Kerman, Esfahan and Southern-Khorasan may have the same ancestors.

Pareek *et al.* (2012) studied the genetic variability in different genotypes of three medicinal plants by using RAPD. Highest polymorphism was observed in *Verbesina encelioides* (73.66 %), then in *Foeniculum vulgare* (50 %) and lowest in *Coriandrum sativum* (43.2 %). The PIC (Polymorphism Information Content) value of *Foeniculum vulgare* ranges from 0.081 to 0.281, *Coriandrum sativum* varied from 0.05 to 0.22 and *Verbesina encelioides* ranges from 0.18 to 0.416.

Solouki *et al*. (2012) collected *Anethum graveolens* seeds of thirty-seven accessions from different areas of Iran in addition to one European accession were cultured. Cluster analysis framed by morphological traits was performed using Ward's cluster analysis and upon molecular markers the UPGMA and Jaccard's similarity coefficients was carried out in cluster analysis appeared by amplified fragement length polymorphism (AFLP) markers. Twenty-five primers were used and 355 bands were detected with 138 loci (39.8 %) polymorphic. It showed that genetic diversity expressed by morphological traits and molecular markers did not completely correlate with geographical region.

Randomly Amplified Polymorphic DNA (RAPD) markers were used to access the genetic diversity among five accessions of *Withania somnifera*. Selected 4 RAPD primers generated 156 DNA fragments, 89 of them were found to be polymorphic. The polymorphism generated by the primers were 84.21 % (OPG-09), 40.90 % (OPH-03), 45.23 % (OPC-08) and 62.5 % (OPG-19). UPGMA Dendrogram obtained from cluster matrix revealed two main clusters, wild accessions formed one cluster and the cultivated accessions formed the other. It showed genetic diversity useful in facilitating development of large number of new varieties through hybridization and transferring of useful genes (Towseef *et al.,* 2012).

Simple Sequence Repeat (SSR) markers were used to investigate the genetic variation between forty-nine Cumin ecotypes collected from nine different provinces of Iran. SSR primers Elap1479, Elap040 and Elap1493 showed the highest (89 %), while Elap1340 and Elap 017 (56 %) showed the lowest number of polymorphic bands. The highest and the lowest PIC value were obtained by Elap017, Elap1340 (0.37) and Elap040, Elap149, Elap1479 (0.18), respectively. Based on the clusters analysis the populations

Semnan and Northern-Khorasan showed the highest difference, whereas Kerman and Esfahan exhibited the lowest difference. However, Kerman and Northern- Khorasan showed the closet genetic background (Bahraminejad and Mohammadinejad, 2013).

Tripathi *et al.* (2013) collected eighteen *Coleus forskohlii* genotypes from different places of central India. The genetic variability was investigated using RAPD, ISSR, and AFLP marker systems. RAPD and ISSR showed 61.39 and 68.75 % polymorphism, respectively, while eight AFLP primer combinations produced 70.81 % polymorphism. The UPGMA based dendrogram grouped the genotypes in two different clusters. The results indicated that the RAPD, ISSR, and AFLP approaches, along with morphological trait analysis, seemed to be best-suited for assessing the genetic relationships among distinct *Coleus forskohlii* genotypes with high accuracy.

A study was conducted to evaluate the genetic diversity of *Gymnema sylvestre* collected from twelve different geographical locations of Karnataka state of India using ISSR molecular markers. A total of 80 clear and reproducible bands were amplified with different lengths using 10 selected ISSR primers, of which 68 (85 %) were polymorphic and remaining 12 (15 %) were monomorphic. The high level of genetic variation (85 %) observed among the populations of *Gymnema sylvestre* implied the need to conserve populations within this species (Mouna *et al*., 2014).

Tomar *et al*. (2014) studied twenty-five *Coriandrum sativum* accessions for molecular characterization by using 38 RAPD and 28 ISSR primers, which yielded total 3721 fragments with average of 7.13 number of band. The polymorphism with RAPD primers ranged from 38 % to 100 % with Jaccard's similarity coefficient ranging from 88 % to 56 %. ISSR which yielded 142 total fragments with average of 5.07 number of band. Cluster analysis showed the variability among each genotype.

Madhukar *et al.* (2015) assessed the genetic divergence amongst the accessions of *Adathoda vasica* collected from different sub-climatic zones of India by RAPD (Randomly Amplified Polymorphic DNA) using twenty random decamer primers (OPA 1-OPA 20). Out of the twenty random primers used for studying genetic divergence sixteen primers were found to be polymorphic. Out of 20 primers 3 were found to be 100 % polymorphic generating a total of 313 amplification products with an average of 19.5 products per polymorphic primer and showed that both environmental and genetic factors were effective in observing variations. The degree of genetic variations detected among the accessions of vasica suggested that RAPD approach seemed to be best suited for assessing with high accuracy for genetic relationships among distinct *Adathoda vasica* accessions.

Kumar *et al.* (2015) collected twelve *Aloe vera* accession from twelve states covering all the different agro-climatic zones of India were investigated for its genetic diversity by using SSR marker. A total of 27 SSR primers were screened, out of which 18 primers showed amplification. 15 primers showed polymorphism. The similarity value ranged from 46 % to 100 %. The highest 100 % similarity was noted between Haryana and Uttar Pradesh accessions followed by 93 % similarity between Haryana and Punjab accessions with Rajasthan. Minimum similarity was noted between Gujarat and Kerala accessions. The results suggest that SSR marker analysis can be a useful tool for the assessment of genetic diversity of the medicinal plants.

III. MATERIALS AND METHODS

An experiment was conducted to study on “Characterization and evaluation of Mandukaparni (*Centella asiatica* L.) germplasm” at ICAR-Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bengaluru-89 during 2015-2016. The details of the material used and methodology adopted during the course of investigation are detailed below.

3.1 Geographical location

The field and laboratory experiments were carried out at ICAR - Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru located at an altitude of 930 m above MSL 12º 58’ North latitude and 78º 35’ East longitudes in the Eastern Dry Zone (zone 5) of Karnataka. The meteorological data collected during the crop growth period are presented in Annexure

3.2 Experimental details

An experiment was conducted to study on morphological and molecular characterization of Mandukaparni (*Centella asiatica* L.) germplasm. The details programme of experiment are presented here under.

Number of treatments: 15
Number of replications: 3

Design	: RCBD (Randomized Complete Block Design)
Spacing	: 30 × 10 cm
Plot size	: 6 m × 1.2 m
Location	: ICAR - IIHR, Bangalore, Medicinal block.
Season	: *Kharif* (August to December)
Situation	: Irrigated condition (drip system)
Fertilizers	: 100:50:50 kg N: P: K ha^{-1}

3.3 Analysis of Morphological variability

To analyze the morphological variations among the populations, various quantitative, yield and quality parameters were recorded. The cultural operations were followed as per the package of practices recommended by University of Horticultural Sciences, Bagalkot. The observation data was collected from five randomly chosen plants from each accession at various intervals.

3.4 Cultural practices

3.4.1 Planting material

Centella asiatica L. fifteen germplasm including a released variety Vallabh Medha and two are polyploids were collected by section of Medicinal crops, Indian Institute of Horticultural Research, Bengaluru and were used for present investigation. The germplasm are listed in Table No. 1

3.4.2 Land preparation

The experimental field area was ploughed and brought to fine tilth to make raised beds. Each bed was levelled, cleaned of weeds, stones *etc*. The area was divided into beds of 6×1.2 m size with 15 raised beds in each block and three blocks representing three replications. The distance between each bed was 30 cm.

3.4.3 Application of manures and fertilizers

Well decomposed FYM was applied to individual plots @10-15 t/ha. along with the recommended dose of nutrients @ 100:50:50 Kg urea: DAP (diammonium phosphate): MOP (murate of potash) ha^{-1}. Half dose of nitrogen, full dose of DAP and MOP were applied after land preparation as a basal dose and mixed thoroughly into the soil and remaining half dose of nitrogen was applied as top dressing.

Table 1: List of germplasm used in the experimental study

Sl. 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	—
15.	IIHR CA-15	Polyploid	—

Plate 1: General view of the experimental site

3.4.4 Planting in field

Disease free healthy runners were planted in the beds at 30×10 cm apart under 50 % of shade net.

3.4.5 Irrigation

Drip Irrigations was provided immediately after planting of runners. Initially, the plants are watered daily for better establishment of crop later irrigation was given at 1-2 day's interval according to the soil moisture field condition.

3.4.6 Gap filling and weeding

Gap filling was done to those plants which failed to establish in the field. It was replaced by healthy runner. Gap filling process were continued for period of 1-2 weeks after planting and manual hand weeding was done regularly with one week intervals based on the weed growth.

3.4.7 Plant protection

The crop was not affected by any pest and disease except leaf spot which was observed on grown up plants after the three months of planting and the crop was sprayed three times with fungicide NATIVO®@ 0.5 % at 15 days.

3.4.8 Harvesting

The crop was harvested at 90 days, 120 days and 150 days after transplanting. Mature leaves with petiole were plucked leaving young leaves. The fresh leaf weight was recorded. Leaf was dried under shade for about 10 to 20 days and dry weight was recorded.

3.5 Observations recorded

The plants were randomly selected for recording the observations on various growth parameters. Five plants were selected from each germplasm and in each replication by leaving the border plants. Data were recorded from each germplasm of each replication was taken average and used for statistical analysis. Details of observations recorded are described below.

3.5.1 Shoot length (cm)

Shoot length was measured from ground level upto the highest point of the main shoot at intervals of 30, 60 and 90 days after the planting. The average of shoot length was computed and expressed in centimeter.

3.5.2 Number of primary branches per plant

The number of branches arising from the main stem above the ground level in tagged plants was recorded at 30, 60 and 90 days after planting. The average was computed and expressed in centimeter.

3.5.3 Number of leaves per plant

The total number of leaves in the whole plant was recorded at 30, 60 and 90 days after planting and the mean values were computed.

3.5.4 Leaf length (cm)

The length of leaf from selected plants was recorded at 30, 60 and 90 days after planting. The mean values were computed and expressed in centimeter.

3.5.5 Leaf width (cm)

The width of leaf from selected plants was recorded at 30, 60 and 90 days after planting. The mean values were computed and expressed in centimeter.

3.5.6 Number of nodes per plant

The total number of nodes per plant was recorded at 30, 60 and 90 days after planting. The mean values were computed.

3.5.7 Rosette diameter (cm)

The rosette diameter of plants in each treatment and replication was measured in North-South and East-West directions. The average was computed and expressed in centimeter.

3.5.8 Petiole length (cm)

The petiole length was recorded on fully matured petiole from selected branches.

3.5.9 Specific leaf weight (g/cm^2)

Dry leaf weight per unit leaf area.

3.5.10 Internodal length (cm)

The length between the two nodes was recorded on fifteen different germplasm

3.5.11 Leaf shape

The leaf shape of the fully matured leaf was recorded by making use of Minimal Descriptors of Medicinal and Aromatics plants by National Bureau of Plant Genetic Resource (NBPGR) and categorized as follows.

1. Reniform
2. Reniform deltoid
3. Orbicular reniform

3.5.12 Leaf colour

Leaf colour at fully matured leaf stage was recorded and visually scored by using RHS (Royal Horticultural Society) colour chart and categorized as follows.

1. Green (143-A)
2. Green (143-B)
3. Green (143-C)

3.5.13 Petiole colour

1. Green (143-A, B, C and 141-C)
2. Yellow green (144-A, B, C)

1.5.14 Flower colour

The flower colour was recorded by making use of Minimal Descriptors of Medicinal and Aromatic plants by NBPGR. When flowers are visible (visual scoring) following RHS colour chart and categorized as follows.

1. Red- purple
2. White

3.5.15 Leaf margin

The leaves were visually observed and recorded as dentate and crenate-dentate among the germplasm.

3.5.16 Fresh leaf yield per hectare

Fresh herb yield per hectare was computed and expressed as quintal per hectare.

3.5.17 Dry leaf yield per hectare

Dry herb yield per hectare was computed and expressed as quintal per hectare.

3.6 Estimation of terpenoids content in fifteen germplasm of *Centella asiatica.*

Ultra High Performance Liquid Chromatographic (UHPLC) analysis was carried out by following the procedure (Agarwal and Murali, 2010) with minor modifications to estimate the total terpenoids *viz*., madecassoside, asiaticoside, medecassic acid and asiatic acid in fifteen germplasm of *Centella asiatica* L.

3.6.1 Procedure:

1. Chromatographic system: Shimadzu, Nexera X_2 Ultra High Performance Liquid Chromatographic system. Detector: SPD-M20A Photo diode array detection combination with Lab solutions software.

Chromatographic conditions

Mobile phase:

1. Dissolved 0.136 g of anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) in 900 ml of HPLC grade water and added 0.5 ml of concentrated Orthophosphoric acid and made the volume to 1000 ml with water, filtered through 0.45 µm membrane and degassed in a sonicator for 3 minutes (solvent A).

2. Acetonitrile (Solvent B).

Details of gradient program followed are given in Table 2.

Flow rate: 1.5 ml/min.

Detection wavelength: 210 nm.

Column: Phenomenex Luna 5µ C_{18} (2) 100Å 250×4.6 mm

Injection volume: 20 µl.

a) Stock solution

Weighed accurately 2.34 mg of Madecassoside, 2.23 mg Asiaticoside, 2.32 mg Medecassic acid and 1.96 mg of Asiatic acid and transferred to a 10 ml volumetric flask and dissolved in 5 ml of methanol by sonicating for 5 min and warming on water bath for

Table 2: Gradient program followed during UHPLC analysis

Time (min.)	Solvent A concentration (%)	Solvent B concentration (%)
00.01	85	15
05.00	75	25
15.00	55	45
20.00	40	60
25.00	40	60
30.00	50	50
35.00	55	45
40.00	85	15
45.00	85	15

10-15 min, cooled and made up the volume to 10 ml with methanol and mixed well. The standard curve obtained is shown in fig. 1.

b) Preparation of different dilutions

From the stock solution, different dilutions like 25%, 50% and 100% were made.

c) Injection of standards

Different concentrations of stock solutions were injected by auto sampler within the UPHLC unit. Performed UPHLC analysis by maintaining above mentioned chromatographic conditions. A sequence of injections were made from lower concentra- tions to higher concentration of standards.

d) Calibration

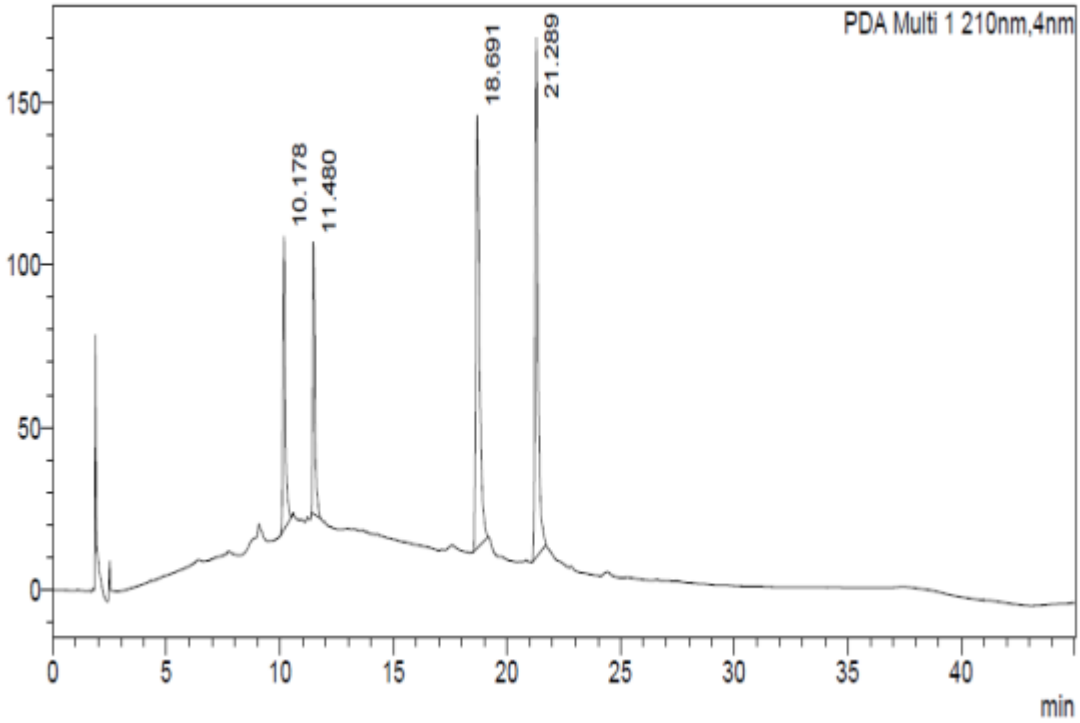
The peaks with retention time of 9.92 min. for Madecassoside, 11.18 min for Asiaticoside, 18.49 min for Medecassic acid and 20.98 min for Asiatic acid were identified as the standard concentration peaks. By the repeated injections of the standards it was possible to get the peak at a particular retention time.

e) Preparation of sample

The shade dried leaf samples were thoroughly ground using Matrix mixer and the powder sieved finely and used for the extraction of total terpenoids *viz.*, Madecassoside, Asiaticoside, Medecassic acid and Asiatic acid.

f) Protocol for sample preparation for terpenoids estimation

Sample of each germplasm in each replication were pooled and used for the estimation of terpenoids. Three grams of powdered leaf sample was taken in a 250 ml beaker, to which 75 ml HPLC grade methanol was added and kept for agitation in water bath at 80°C for half an hour and the supernatant was collected in 100 ml volumetric flask. Again the residue was dissolved in 50 ml HPLC grade methanol and the supernatant was collected and added to the already collected supernatant in 100 ml



PDA Ch1 210nm

Peak	Ret. Time	Name
1	10.178	Madecassoside
2	11.480	Asiaticoside
3	18.691	Madecassic acid
4	21.289	Asiatic acid

Fig.1: HPLC Chromatogram for Standard curve of terpenoids

volumetric flask. Same procedure was repeated until a colourless solution was obtained. In all, the sample was extracted thrice. The extract was cooled and the volume was made upto 100 ml with HPLC grade methanol. The extract was filtered through a Millipore filter (0.2 µm mesh size) and used for UHPLC analysis.

g) Procedure for estimation of terpenoids

The methanol extract of leaf samples was auto sampled in UHPLC column and calculated the mean area and its relative standard deviation (RSD). Injected 20 µl of prepared sample and recorded the chromatogram at 210 nm.

h) Calculations

Calculated the Madecassoside, Asiaticoside, Medecassic acid and Asiatic acid content using the formula.

$$\frac{\text{Area of the sample X Standard weight (mg)X Sample dilution X Purity of standard}}{\text{Area of the standard X Standard dilution X Sample weight (mg)X 100}}\text{X100}$$

After obtaining the individual contents, these contents were added to obtain total terpenoids content.

1.6.2 Total terpenoids yield (kg/ha)

The dry weight of leaf was multiplied by total terpenoids content estimated in leaf samples, to estimate the total terpenoids yield gram per plant and converted to kilo gram per hectare by multiplying with the plant density per hectare.

3.7 Statistical analysis

The observations and data recorded were subjected to statistical scrutiny. The results of the following different statistical parameters were adopted.

3.7.1 Performance

3.7.2 Analysis of Variance (ANOVA)

3.7.3 Coefficient of variability (phenotypic and genotypic).

3.7.4 Heritability (%)

3.7.5 Genetic advance (GA) and Genetic advance as per cent mean (GAM)

3.7.6 Correlation coefficients

3.7.7 Genetic divergence

3.7.1 Performance

The mean performance of the different genotypes for quantitative characters was studied.

i. General mean (GM) = $\frac{\text{Total of all values}}{\text{n}}$

Where (n) = Number of observations

ii. Variance $= \frac{SS - CF}{DF}$

Where, SS = Sum of square of all observations of a variable

CF = Critical Factor = $\frac{(\text{Grand Total})2}{n}$

DF = Degrees of freedom

iii. Standard deviation (SD) = Variance

iv. Standard error (SE) = $\frac{SD}{n}$

v. Coefficient of variation (CV) = $\frac{SD}{Mean} \times 100$

2.7.2 Analysis of Variance (ANOVA)

ANOVA was worked out for all the characters 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).

3.7.3 Coefficient of variability

The Genotypic and Phenotypic coefficients of variation were calculated as per the method suggested by Burton and Devane (1953).

Genotypic coefficient of variation (GCV) = $\frac{\text{Genotypic variance (Vg)}1/2}{\text{General mean of population (X)}} \times 100$

Phenotypic coefficient of variation (PCV) = $\frac{\text{Phenotypic variance (Vp)}\}1/2}{\text{General mean of population (X)}} \times 100$

PCV and GCV were classified as noted below and suggested by Sivasubramaniam and Madhava (1973).

GCV and PCV	Category
Less than 10 %	Low
10-20 %	Moderate
More than 20 %	High

3.7.4 Heritability (h^2)

Heritability in broad sense was calculated as per the formula suggested by Allard (1960) and expressed in per cent.

Heritability (h^2) = $\frac{Vg}{Vp}$ x 100

Where, Vg = Genotypic Variance

Vp = Phenotypic Variance

As suggested by Johnson *et al.* (1955), heritability values are categorized as follows:

Heritability	Category
Less than 30%	Low
30 - 60%	Moderate
More than 60%	High

3.7.5 Genetic advance and genetic advance as per cent mean

Genetic advance was estimated by the following formula as per the method suggested by Johnson *et al*. (1955).

$$\text{Genetic advance (GA)} = \frac{Vg}{Vp\ ½} \times K$$

Where, V_g = Genetic variance

V_p = Phenotypic standard deviation

K = Selection differential, the value of which is 2.06 at 5 % selection index

$$\text{Genetic advance as per cent of mean} = \frac{GA}{\text{General mean}} \times 100$$

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al*, (1955)

Genetic advance	Category
0-10	Low
10-20	Moderate
>20	High

3.7.6 Correlation coefficient

The genotypic and phenotypic correlation coefficient were calculated as per Al- Jibour *et al*. (1958) by using variance and covariance matrix.

a) Genotypic correlation coefficient

$$rg\ XY = \frac{Co\ Vg\ XY}{\sqrt{(VgX + VgY)}}$$

Where, rg XY = Genotypic correlation coefficient between the trait X and Y

CoVg XY = Genotypic covariance between X and Y

Vg X = Genotypic variance of X

Vg Y = Genotypic variance of Y

b) Phenotypic correlation coefficient

$$rp\ XY = \frac{Co\ Vp\ XY}{\sqrt{(VpX + VpY)}}$$

Where, rp XY = Genotypic correlation coefficient between the trait X and Y

Co Vg XY = Genotypic covariance between X and Y

Vp X = Genotypic variance of X

Vp Y = Genotypic variance of Y

3.7.7 Genetic divergence analysis using D^2 analysis.

Genetic divergence is a technique extensively used in plant breeding and genetics for the study of genetic divergence in various plant breeding materials therefore, the genetic diversity among fifteen germplasm of Mandukaparni (*Centella asiatica*) was assessed by using D^2 Mahalanobis statistics (1936). The grouping of accessions was done using Tocher's method, as described by Rao (1952).

a) Test of significance of difference by Wilk's static for aggregate traits.

b) Transformation of correlated variables

In terms of variance and covariance, the D^2 value was obtained by,

$D^2 = W^{ij} (Xi^1 - Xi^2) (Xj^1 - Xj^2)$

W^{ij} is the estimate of variance and covariance matrix.

Transformation was done by pivotal condensation method. Transformation of correlated variables into uncorrelated variables was done by substituting these values of X_1, X_2, X_3 and X_4 in the transformed equation and the corresponding transformed values Y_1, Y_2, Y_3 and Y_4 were obtained (original mean to transformed data).

c) Computation of D^2 values

For each combination of population, the mean of deviation for the characters was computed and the D^2 was calculated as the sum of the squares of these deviations.

i.e., $D^2 = \Sigma (Yi^1 - Yi^2)^2$ Where, i = 1, 2, 3…….p characters.

d) Test of significance of D^2 values

The significance of D^2 values for a pair of population was tested against the table values of X^2 for p degrees of freedom

Where p = Total number of characters

e) Grouping into clusters

The method suggested by Tocher (Rao, 1952) was followed for cluster formation. The germplasm were arranged in the order of their relative distance from each other. The values were arranged in ascending order of magnitude in each column. Two germplasm having smallest distance from each other were considered first to which a genotype having smallest average D^2 value from the first two germplasm was added. If at any stage, the average D^2 of a group appeared to be high from those already included, it was considered that the group does not fit with the former cluster and hence taken outside the first cluster and second cluster was formed. This process was continued and clusters were formed.

(i) Estimation of average of the distances of all possible combinations of germplasm included in a cluster was calculated.

(ii) Estimation of average inter cluster distances

This was calculated by measuring the distance between various combinations of clusters divided by the product of the number of germplasm in the concerned cluster combinations. The cluster were taken one by one and their distance from each other was calculated.

i.e., Average inter cluster distance between cluster i and j = $\frac{\Sigma Di\,2}{ni \times nj}$

n_i = Number of germplasm in cluster i

n_j = Number of germplasm in cluster j

3.8 Assessment of genetic diversity through SSR markers

3.8.1 Plant material collection

Fresh young fully expanded and disease free leaves of different *Centella asiatica* germplasm were collected and were brought to the laboratory in butter paper bags and wiped with distilled water or 76 % of ethanol to remove traces of dirt.

3.8.2 Isolation of genomic DNA

The DNA from fresh young leaves of *Centella asiatica* was isolated using CTAB method described by Doyle and Doyle (1990).

Solutions used in DNA isolation

I M Tris, pH 8	Dissolve 12.14 g Tris HCl in 100 ml distilled water and autoclave
0.5 M EDTA, pH 8	Dissolve 18.612 g EDTA in 100 ml of double distilled water and autoclave. NaOH pellets were added for dissolving EDTA.
10 % CTAB	Dissolve 10 g CTAB (Cetyltrimethyl ammonium bromide or Hexadecyltrimethyl ammonium bromide) in 100 ml of double distilled water
Chloroform : Iso Amyl Alcohol	Mix chloroform and Isoamyle alcohol 24:1 (v/v) ratio
5 M NaCl	Dissolve 29.22 g in 60 ml double distilled water and make up the volume to 100 ml and autoclave
TE Buffer (10:1)	Prepare 10 mMTrisHCl and 1mM EDTA and mix. Adjust the pH to 8
PVP (PolyVinyl Pyrrolidine)	50 mg was used to remove polyphenols
Wash solution	76 % v/v ethanol; chilled

Absolute Alcohol (100 %)	Stored at -20°C
RNase (10 mg/ml)	RNase stock was diluted as per the requirement in Milli-Q water.
3 M Sodium acetate	Dissolve 24.6 g Sodium acetate in 100 ml of double distilled water. Adjust the pH to 8 and autoclave

Extraction buffers used for isolation of DNA

10 % CTAB	3 ml
1M Tris, pH	1 ml
0.5 M EDTA, pH	400 µl
5 M NaCl	2.8 ml
β-Mercaptoethanol	100 µl
Sterile water	2.65 ml
Total volume	10 ml

3.8.3 DNA isolation protocol:

1. 10 ml of extraction buffer (10 % CTAB) was prepared into 50 ml of sterile centrifuge tubes and kept in water bath at 60°C for 10 min.

2. Collected young leaves of 2 g were grounded into a fine powder using liquid nitrogen in a mortar and pestle in which added 50 mg of PVP.

3. Pre warm 10 ml CTAB extraction buffer was poured and 100 µl β-mercapto ethanol was added to it and mixed well then incubated 65ºC in a water bath for one hour with intermittent shaken at every 10 minutes.

4. The tubes were taken out, cooled to room temperature about 10 minutes. Then 10 ml of chloroform: Iso-amylalcohol (24:1) was added and mixed gently. The tubes were centrifuged at 8000 rpm for 15 minutes at 4ºC.

5. The supernatant was transferred to a new tube using pipets, to which 2.5 ml of 5 M Nacl was added followed by 10 ml of chilled isopropanol and mixed gently by vortexing for 5-6 times (Generally the DNA can be seen to precipitate out of solution) and kept in deep freezer -20°C overnight.

6. The tubes were centrifuged at 3000 rpm for 3 minutes followed by 5000 rpm for 5 minutes at 20°C-30°C. Then supernatant was discarded and precipitated DNA appears as pellet

7. To wash the DNA pellet 2.5 ml of cold 70 % ethanol was added and centrifuged at 5000 rpm for 5 minutes. The washing was repeated three times and ethanol was discarded. After washing, the pellet was air dried till the smell of ethanol disappeared

8. After drying, the DNA pellet was dissolved in 250 µl of TE buffer (10:1) and stored at -20ºC.

3.8.4 DNA purification

For obtaining good quality and amplifiable DNA, the crude DNA was purified by the following steps.

Procedure

1. 6 µl of RNase was added to the tubes containing DNA and kept for incubation at 37°C for 1 hour in water bath.

2. Then 7 ml of absolute ice cold ethanol and 0.75 ml of 3 M Sodium acetate (pH 8) were added and mixed well.

3. Then the samples were kept at -20°C for 1 hour.

4. Then centrifuged at 20,000 rpm for 20 minutes, supernatant was discarded.

5. The pellet was washed with 2.5 ml of 70 % ethanol. Then the pellet was air dried till the smell of ethanol disappeared.

6. DNA pellet was dissolved in 250 µl of TE buffer and stored at -20°C.

3.8.5 DNA Quantification

1. DNA concentration in the sample was estimated by recording absorbance at 260 nm using UV/VIS spectrophotometer.

2. 10 µl of DNA sample was taken in a quartz cuvette and the volume was made up to 1ml with TE buffer. Blank values were already recorded using 1 ml TE buffer.

3. The absorbance of DNA solution was measured at 260 and 280 nm.

4. The ratios A260 nm/A280 nm were calculated. A good DNA exhibited the spectral properties of the ratios =1.8 to 2.0. Higher and lower ratios indicated the presence of RNA and protein respectively (Sambrook *et al*., 1989)

5. DNA concentration was calculated using the relationship for double stranded DNA, O.D at 260 nm = 50 µg ml^{-1}

$$\text{Total quantity of DNA (µg/µl)} = \frac{\text{O.D.at } 260\text{nm} \times 50 \times \text{Dilution factor}}{1000}$$

$$\text{Dilution factor} = \frac{\text{Total volume of extract}}{\text{Volume of aliquot}}$$

Hence, quantity of DNA (µg $µl^{-1}$) = OD_{260} ×10

3.8.6 DNA quality check by Agarose gel electrophoresis

The DNA degradation and contamination with other substances were checked by electrophoresis of an aliquot of sample using agarose gel (0.8 %) stain with ethidium bromide (EtBr) and visualized under the gel documentation system (Alpha Innotech geldoc USA).

Procedure:

1. Gel preparation: Agarose gel (0.8 %) was prepared by melting 0.8 gm of agarose in 100 ml of 0.5× TE buffer in a microwave for approximately 2 minutes and allowed to cool for a couple of minutes then 5 µl of ethidium bromide (10 mg/ml) was added, stirred well to mix.

2. Casting of Gel: Gel was casted using gel casting tray. The melted gel was poured into gel casting tray after placing comb. While pouring, sufficient care was taken for not allowing the air bubbles to trap in gel. Then gel was allowed to solidify for a minimum of 20 minutes at room temperature on a flat surface. After the gel solidifies the comb was removed and solidified gel was transferred to electrophoresis tank containing buffer (1X TAE) so as to cover the wells completely.

3. Loading of samples: 5 µl of DNA sample + 2 µl of 1X loading dye were mixed and loaded into separate wells using micropipette. The voltage of 60 V was applied to run the gel.

4. After the dye has reached 3/4th of the gel, the power supply was switched off and gel was photographed using gel documentation system.

5. Then DNA quality was confirmed by the presence of a highly resolved high molecular weight band which indicates good quality of DNA, presence of a smeared band indicates DNA degradation. A sharp single band indicated intact plant genomic DNA.

3.8.7 DNA and primer dilution

30 ng /µl genomic DNA is preferable for PCR reaction so DNA was diluted depends on OD value. Similarly, 0.2 pM/µl forward and 0.8 pM/µl reverse primers are preferable and dilution was done accordingly.

3.8.8 Polymerase chain reaction and amplification of DNA

The DNA extracted from 15 genotypes of *Centella asiatica* were amplified using 20 SSR primers. The amplification was performed by using Corbett Palm Cycler. The master mixed was prepared for 15 samples by adding the following components and 15 µl of the master mix was transferred to sterile PCR tubes including 2.5 µl of respective DNA samples.

1	Reaction buffer (10 X)	1.5 µl
	Primers (Bioserve)	
2	Primer – 10 pM	1.5 µl
3	dNTP´S (10 mM)	0.5 µl
4	Taq DNA polymerase (3U)	0.4 µl
5	Template DNA (30 ngµl^{-1})	3.0 µl
6	$MgCl_2$ (25 mM) (Bengaluru Genei)	0.9 µl
7	Double-Distilled Water	7.2 µl
	Total reaction volume	15 µl

The contents were mixed by repeated pipetting and were spinned down for 15 seconds at 5000 rpm. The tubes were placed firmly in the wells of the Corbett Palm Cycler and the following temperature regime was followed.

Step 1 94°C for 1 min.

Step 2 94°C for 1 min.

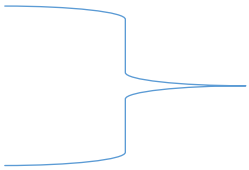
53- 59°C for 1 min. } 30 cycles

72°C for 1 min.

Step 3 94°C for 1 min.

53°C for 1 min.

72°C for 1 min



8 cycles

Step 3 72°C for 10 min. (Final extension)

At the end of the run, the tubes were taken out and stored at 4°C till electrophoresis.

3.8.8.1 Electrophoresis and visualization of amplified products

About 15 µl of the amplified products of PCR were mixed with 2 µl bromophenol blue then mixed it and loaded on 1.5 % agarose gel. Gel casting and electrophoresis was performed as follows.

The agarose (1.5 g) was dissolved in 100 ml of 1X TAE buffer. It was heated and allowed to dissolve in the buffer completely and then cooled to 40°C. 6 µl of Ethidium bromide solution was added per 100 ml of gel and poured solution into gel casting plate on level surface with comb in place, avoiding air bubbles and was allowed to set for 20-30 minutes. After it was set, the comb was removed from gel then this gel was kept inside the gel electrophoresis unit. The gel electrophoresis tank was filled with 1X TAE running buffer and the PCR samples were loaded in the gel. 100 bp ladder (range 100 to 1000 bp) was also loaded in the well of same gel to identify the targeted band size. Electrophoresis was carried out at 70 – 80 volts current for 90 to 120 minutes until it run 2/3 of the gel and viewed the bands under UV light of the gel documentation system Alpha Innotech geldoc and photographed for confirmation.

3.8.9 Analysis of SSR profiles

Amplification profiles of twenty primers (Table 3) in fifteen germplasm of *Centella asiatica* were scored for presence (1) or absence (0) manually and the binary data was used for further analysis.

Table 3: List of SSR primers used in the diversity analysis

	Primer Name	Primer sequence (5 ′ – 3 ′)	T(°C) Annealing temperature
1	mCaCIR002	F: CCACAGGTAACACCGAAT R: GCACTTGCACTATCTGGAA	55
2	mCaCIR004	F: GGGTGGTCTGCCTAAAGA R: TGGAGATCAAGTTTCATGC	59
3	mCaCIR005	F: GGCCTTCAATGTATGCTG R: TTTGATTTGTTGGGTCTTG	55
4	mCaCIR006	F: ACGGGCATTTATTCCATT R: GCAAACCACCACAACTTC	59
5	mCaCIR007	F: TGGAGGTGGTGTAACTGG R: AGGGGATCAAACCTCATC	55
6	mCaCIR009	F: TGCCTATCCTTTGAATGC R: CAAACATGACATTCTTAAAACA	55
7	mCaCIR010	F: AATGTAAAATTCCCGGTGT R: TAAACAGGCGTTCCAAGT	55
8	mCaCIR011	F: TTCATAAAAGTCCTTCCACA R: TAGGTTGATGTGGCCTCT	55
9	mCaCIR012	F: CACGAAAATTGGAAACAA R: CATGTGAGTTTATGAGTTTCTATG	55
10	mCaCIR013	F: CAAGTTCCTCCCACGAAT R:GCCGAAATAATCGAAATATAAG	55
11	mCaCIR018	F: TTGAGTTTAAGAAGTCCCAAAT R: AATCCTTCACACTCCTAAAGC	55
12	mCaCIR019	F: TTTCTTGTTAAATGCGATGA R: AATGACATCACTGCTATGGA	55
13	mCaCIR020	F: TTTAGGAAGTTGGATTTTGC R: GGTTTAATTCAGGACGCTTA	55
14	mCaCIR021	F: TGCCTAGATTTTGGGTTTT R: TCTTACAATGCAATCAACCT	55
15	mCaCIR022	F: AGGAGTATTGACAAGAGGTGA R: GGATGGCAGTCCATTTTA	55
16	mCaCIR024	F: TCTTTCGTTGATACATGCAC R:AAAACTTAAAGAAGATACAAACTCC	53
17	mCaCIR027	F: ACCCCAAGACCTTCAGTT R: CCTTCTGCTTTCCCTTTT	55
18	mCaCIR028	F: CAGAGTTTGGGCAGAAAA R: GACGAGTGGAGGATAAGAAA	55
19	mCaCIR029	F: GGTCTGAGGTCTGTTGAGG R: CGCATTGACAGAACAAAA	55
20	mCaCIR030	F: GGCAAATCGAGAGCAATA R: ACGGAAAAGCCTAACAGC	55

3.8.9.1 Data analysis and estimation of genetic similarity

Binary characters scored as 1 for presence and 0 for absence are converted to binary matrix for analysis using Numerical Taxonomy and Multivariate Analysis System (NTSYS pc, ver. 2.02). The data was used to generate genetic similarity coefficient similarity matrix on the basis of Jaccard's coefficient with SIMQUAL option. The Dendrogram (cluster diagram) generated by Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm using sequential Agglomerative Hierarchial and Nested (SAHN).

3.8.10 Band statistics

3.8.10.1 Polymorphic information content (PIC)

Polymorphic information content (PIC) or average heterozygosity was calculated as per the formula Roldan- Ruiz *et al.*, (2000).

PIC = 2fi (1-fi).

Where fi is the frequency of the amplified allele.

3.8.10.2 Observed heterozygosity (H_O)

Observed heterozygosity (Ho) was calculated by average of the (S) sum of bands present for each allele divided by (NC) number of germplasm under study.

$$Ho = \sum S/NC$$

IV. EXPERIMENTAL RESULTS

The results of the present study entitled “Characterization and evaluation of Mandukaparni (*Centella asiatica* L.) germplasm” are presented in this chapter under the following headings.

4.1 Evaluation of Centella asiatica germplasm for growth, yield and quality attributes

4.2 Assessment for biochemical Variability in fifteen germplasm of Centella asiatica

4.3 Genetic diversity assessment using SSR molecular markers in germplasm of Centella asiatica

4.1 Evaluation of *Centella asiatica* germplasm for growth, yield and quality attributes

4.1.1 Quantitative characters

4.1.1.1 Growth parameters at 30 days after planting (DAP)

Significant differences were recorded with respect to the growth parameters namely shoot length, primary branches per plant, number of leaves per plant, leaf length and leaf width at 30 DAP and comparison among the germplasm were carried out using the best performing check (Table 4; Fig. 2).

The shoot length recorded at 30 DAP ranged from 2.35 cm (IIHR CA-10) to 6.69 cm (IIHR CA-1) whereas check variety Vallabh Medha recorded 4.23 cm. The performance of six germplasm was superior for shoot length compared to check variety.

The number of primary branches ranged from 2.99 (IIHR CA-14) to 4.20 (IIHR CA-9) whereas check variety recorded 3.60 and eight germplasm registered higher values over check variety.

At this stage, none of the germplasm exhibited any nodes. The number of leaves ranged from 3.11 (IIHR CA-14) to 4.28 (IIHR CA-9) whereas check variety recorded

Table 4: Performance of *Centella asisatica* L. germplasm for growth parameters at 30 days after planting

Germplasm	Shoot length (cm)	No. of Primary branches plant^{-1}	No. of nodes	No. of leaves Plant^{-1}	Leaf length (cm)	Leaf width (cm)
IIHR CA-1	6.69	3.50	-	3.92	2.89	4.29
IIHR CA-2	5.35	3.63	-	3.89	2.17	3.20
IIHR CA-4	4.25	3.93	-	4.00	1.98	2.91
IIHR CA-5	4.12	3.97	-	4.26	1.71	2.58
IIHR CA-6	3.75	3.67	-	3.75	1.44	2.47
IIHR CA-7	6.46	3.58	-	3.52	2.35	3.73
IIHR CA-8	2.96	3.58	-	3.65	1.25	1.97
IIHR CA-9	2.93	4.20	-	4.28	1.32	2.07
IIHR CA-10	2.35	3.73	-	3.85	1.43	1.85
IIHR CA-11	2.94	3.43	-	3.45	1.68	2.89
IIHR CA-12	3.06	3.63	-	3.69	1.44	2.30
IIHR CA-13	4.67	3.47	-	3.61	2.03	3.33
IIHR CA-14	4.94	2.99	-	3.11	1.95	3.17
IIHR CA-15	3.71	3.23	-	3.27	1.96	3.05
Vallabh Medha*	4.23	3.60	-	3.93	1.81	2.83
S.Em. ±	0.16	0.17	-	0.12	0.10	0.09
CD at 5%	0.47	0.51	-	0.34	0.29	0.25

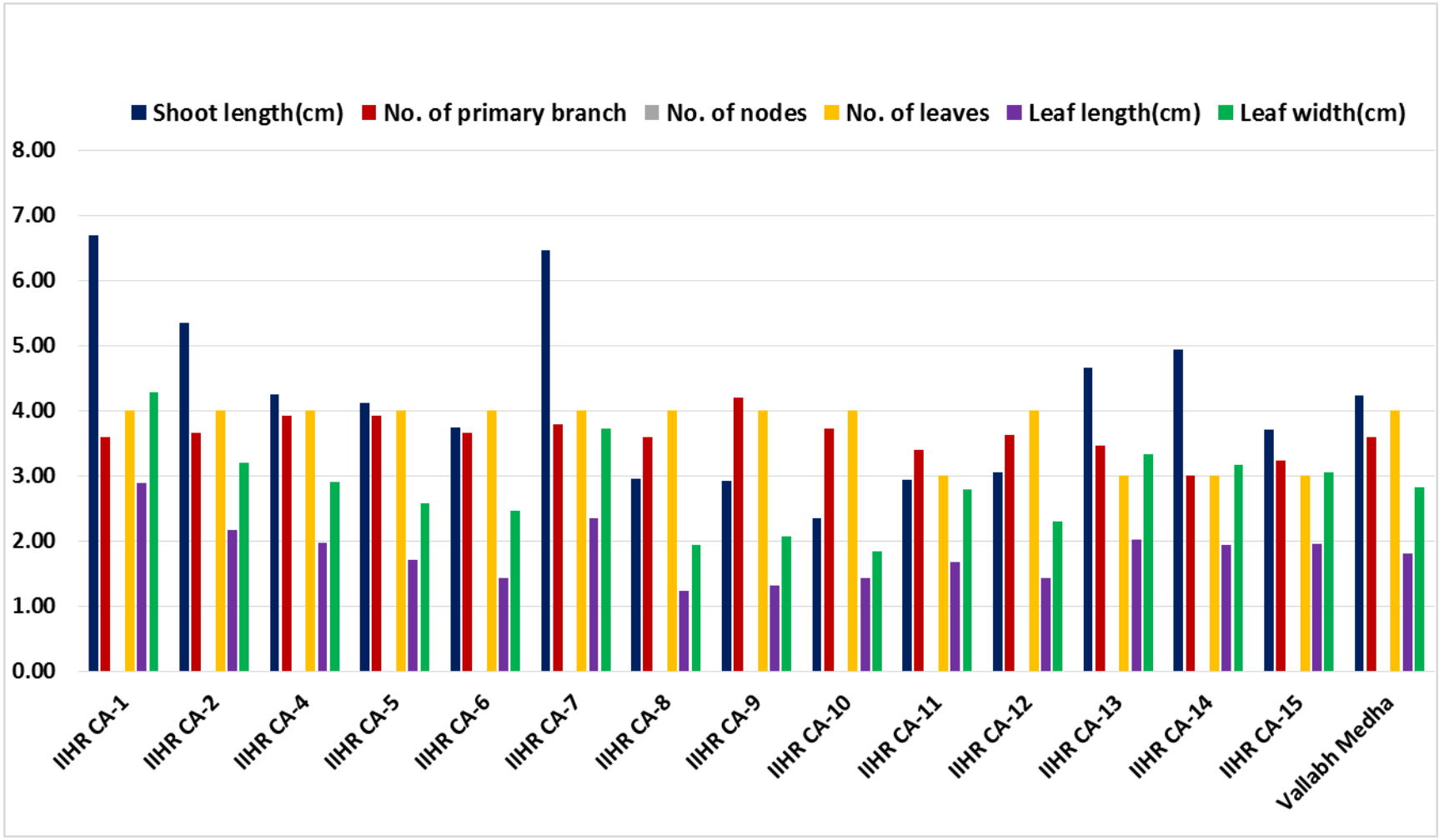


Fig. 2: Growth parameters of *Centellaasiatica* L. germplasm at 30 days after planting (DAP)

3.93 and three germplasm had higher number of leaves over check variety. The Leaf length ranged from 1.25 (IIHR CA-8) to 2.89 cm (IIHR CA-1) whereas check variety recorded 1.81 cm and seven germplasm had more leaf length over check variety.

The Leaf width ranged from 1.85 (IIHR CA-10) to 4.29 cm. (IIHR CA-1) as compared with check variety 2.83 cm and eight germplasm recorded more leaf width over check variety.

4.1.1.2. Growth parameters at 60 days after planting (DAP)

The plant growth parameters were recorded at 60 DAP for shoot length, number of primary branches per plant, number of nodes per plant, number of leaves per plant, leaf length and leaf width were found to be vary significant among the germplasm (Table 5; Fig. 3).

The shoot length recorded at 60 days ranged from 3.24 cm (IIHR CA-9) to 10.09 cm (IIHR CA-1) whereas check variety Vallabh Medha recorded 4.82 cm. Among the germplasm eight germplasm performed better than check variety.

The number of primary branches ranged from 4.08 (IIHR CA-1) to 6.10 (IIHR CA-2) whereas check variety recorded 4.73 and eight germplasm showed better performance over check variety.

The number of nodes ranged from 2.05 (IIHR CA-10) to 5.85 (IIHR CA-11) whereas check variety recorded 4.99 and only two germplasm *viz*., IIHR CA-11 and IIHR CA-12 recorded higher number of nodes over the check variety.

The number of leaves ranged from 10.80 (IIHR CA-10) to 20.13 (IIHR CA-11) whereas the check variety recorded 15.33 and as many as eleven germplasm recorded higher number of leaves over the check variety.

The leaf length ranged from 1.69 (IIHR CA-12) to 3.48 cm (IIHR CA-1) whereas the check variety recorded 2.16 cm and seven germplasm were found to be superior in leaf length over the check variety.

Table 5: Performance of *Centellaasisatica*L. germplasm for growth

Germplasm	Shoot length(cm)	No. of Primary branches plant^{-1}	No. of nodes plant^{-1}	No. of leaves plant^{-1}	Leaf length (cm)	Leaf width (cm)
IIHR CA-1	10.09	4.08	4.06	15.40	3.48	5.04
IIHR CA-2	6.97	6.10	3.68	15.67	2.40	4.01
IIHR CA-4	5.92	5.17	3.75	16.77	2.33	3.45
IIHR CA-5	4.71	5.23	3.57	15.87	2.21	3.16
IIHR CA-6	4.68	5.07	2.89	16.17	1.95	2.99
IIHR CA-7	7.76	5.50	3.77	16.34	2.79	4.55
IIHR CA-8	4.36	5.12	2.84	14.97	1.75	2.52
IIHR CA-9	3.24	5.47	4.94	18.01	1.71	2.51
IIHR CA-10	3.37	4.43	2.05	10.80	1.81	2.22
IIHR CA-11	4.99	4.67	5.85	20.13	1.90	3.09
IIHR CA-12	4.13	4.70	5.33	17.48	1.69	2.70
IIHR CA-13	6.00	4.43	3.94	16.33	2.39	3.91
IIHR CA-14	6.16	4.82	3.44	15.23	2.19	3.55
IIHR CA-15	6.29	5.60	3.02	15.83	2.16	3.62
VallabhMedha*	4.82	4.73	4.99	15.33	2.16	3.32
S.Em. ±	0.11	0.22	0.18	0.79	0.11	0.12
CD at 5%	0.32	0.63	0.53	2.29	0.31	0.35

Parameters at 60 days after planting

*Check variety-Vallabh Medha

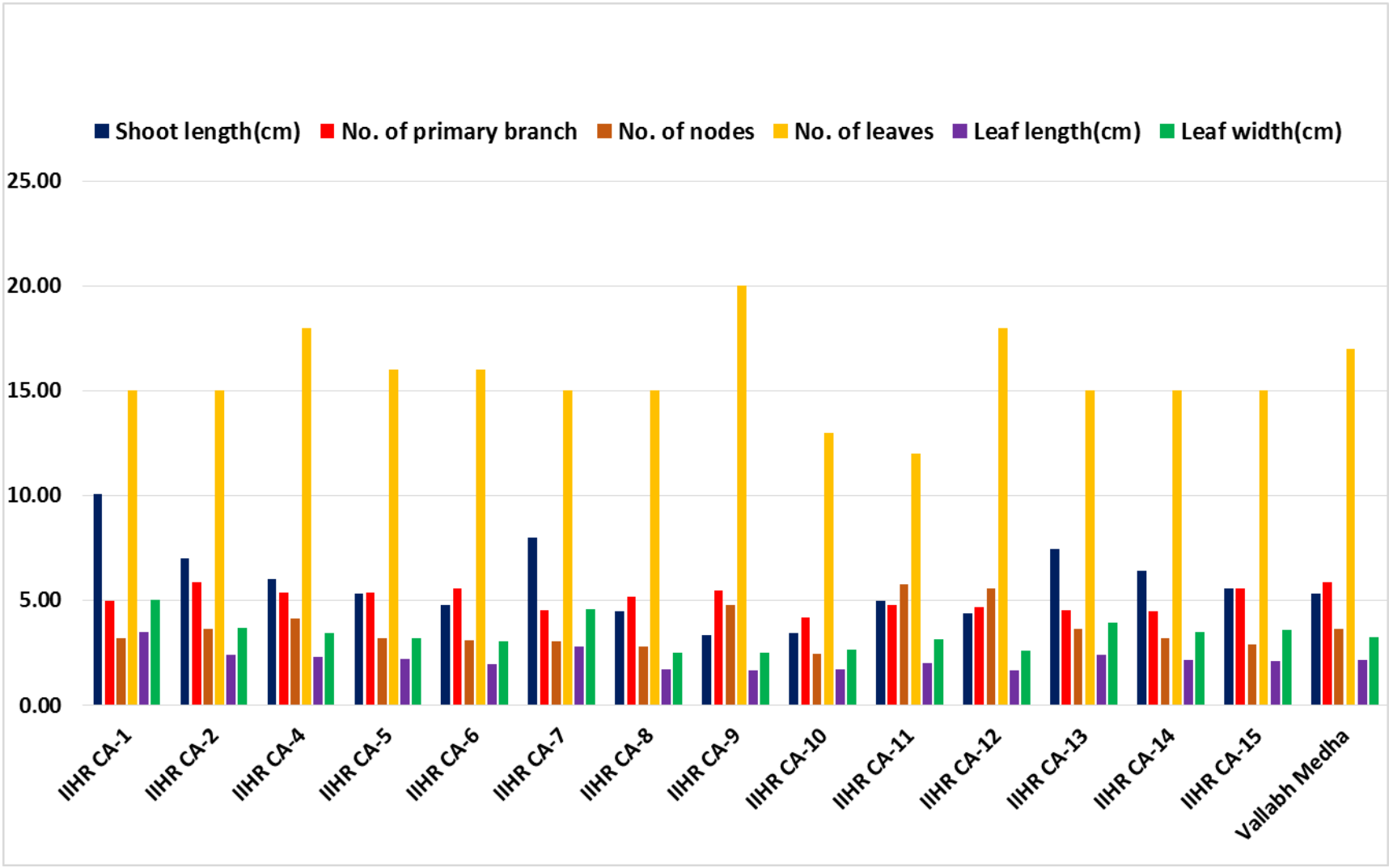


Fig. 3: Growth parameters of *Centellaasiatica* L. germplasm at 60 days after planting (DAP)

The leaf width ranged from 2.22 (IIHR CA-10) to 5.04 cm (IIHR CA-1) whereas the check variety recorded 3.32 cm and seven germplasm exhibited more leaf width than check variety.

4.1.1.3. Growth parameters at 90 days after planting (DAP)

The data recorded on growth parameters *viz*., shoot length, number of primary branches per plant, number of nodes per plant, number of leaves per plant, leaves length and leaves width at 90 days after planting showed significant variation among the germplasm (Table 6; Fig. 4).

The shoot length at 90 days ranged from 3.82 cm (IIHR CA-9) to 10.92 cm (IIHR CA-1) whereas check variety Vallabh Medha was 6.71 cm and seven germplasm recorded greater shoot length over the check variety.

The number of primary branches ranged from 5.00 (IIHR CA-1) to 7.27 (IIHR CA-2) whereas check variety recorded 6.47 and only two germplasm registered higher mean values over the check variety.

The number of nodes ranged from 5.43 (IIHR CA-2) to 9.10 (IIHR CA-12) whereas the check variety recorded 7.90 and five germplasm exceeded the check variety.

The number of leaves ranged from 21.72 (IIHR CA-10) to 34.00 (IIHR CA-12) whereas the check variety recorded 33.07 and only one germplasm recorded higher leaf number than check variety.

The leaf length ranged from 1.85 cm (IIHR CA-9, IIHR CA-12) to 3.79cm (IIHR CA-1) whereas the check variety recorded 2.45 cm and six germplasm were found superior over the check variety.

The leaf width ranged from 2.64 (IIHR CA-10) to 5.54 cm (IIHR CA-1) whereas the check variety recorded 3.79 cm and six germplasm were to exhibit higher leaf width over the check variety.

Table 6: Performance of *Centella asisatica* L. germplasm for growth parameters at 90 days after planting

Germplasm	Shoot length (cm)	No. of Primary branches plant^{-1}	No. of nodes plant^{-1}	No. of leaves plant^{-1}	Leaf length (cm)	Leaf width (cm)
IIHR CA-1	10.92	5.00	6.07	25.13	3.79	5.54
IIHR CA-2	7.35	7.27	5.43	25.67	2.79	4.33
IIHR CA-4	7.06	6.03	7.97	30.23	2.58	3.74
IIHR CA-5	6.47	6.10	6.93	27.60	2.36	3.41
IIHR CA-6	5.64	5.80	6.57	23.87	2.21	3.26
IIHR CA-7	9.89	6.00	7.07	27.93	3.15	5.33
IIHR CA-8	4.74	6.05	6.22	25.15	1.88	2.83
IIHR CA-9	3.82	6.17	8.23	30.73	1.85	2.78
IIHR CA-10	4.02	5.40	6.63	21.72	1.98	2.64
IIHR CA-11	5.18	5.20	6.38	23.63	2.23	3.46
IIHR CA-12	5.23	5.22	9.10	34.00	1.85	2.93
IIHR CA-13	8.52	5.20	8.07	30.02	2.65	4.03
IIHR CA-14	9.16	5.93	8.83	30.88	2.44	4.21
IIHR CA-15	8.84	6.67	7.43	30.28	2.59	4.19
Vallabh Medha*	6.71	6.47	7.90	33.07	2.45	3.79
S.Em. ±	0.22	0.25	0.17	1.23	0.09	0.13
CD at 5%	0.64	0.72	0.50	3.58	0.26	0.38

*Check variety- Vallabh Medha

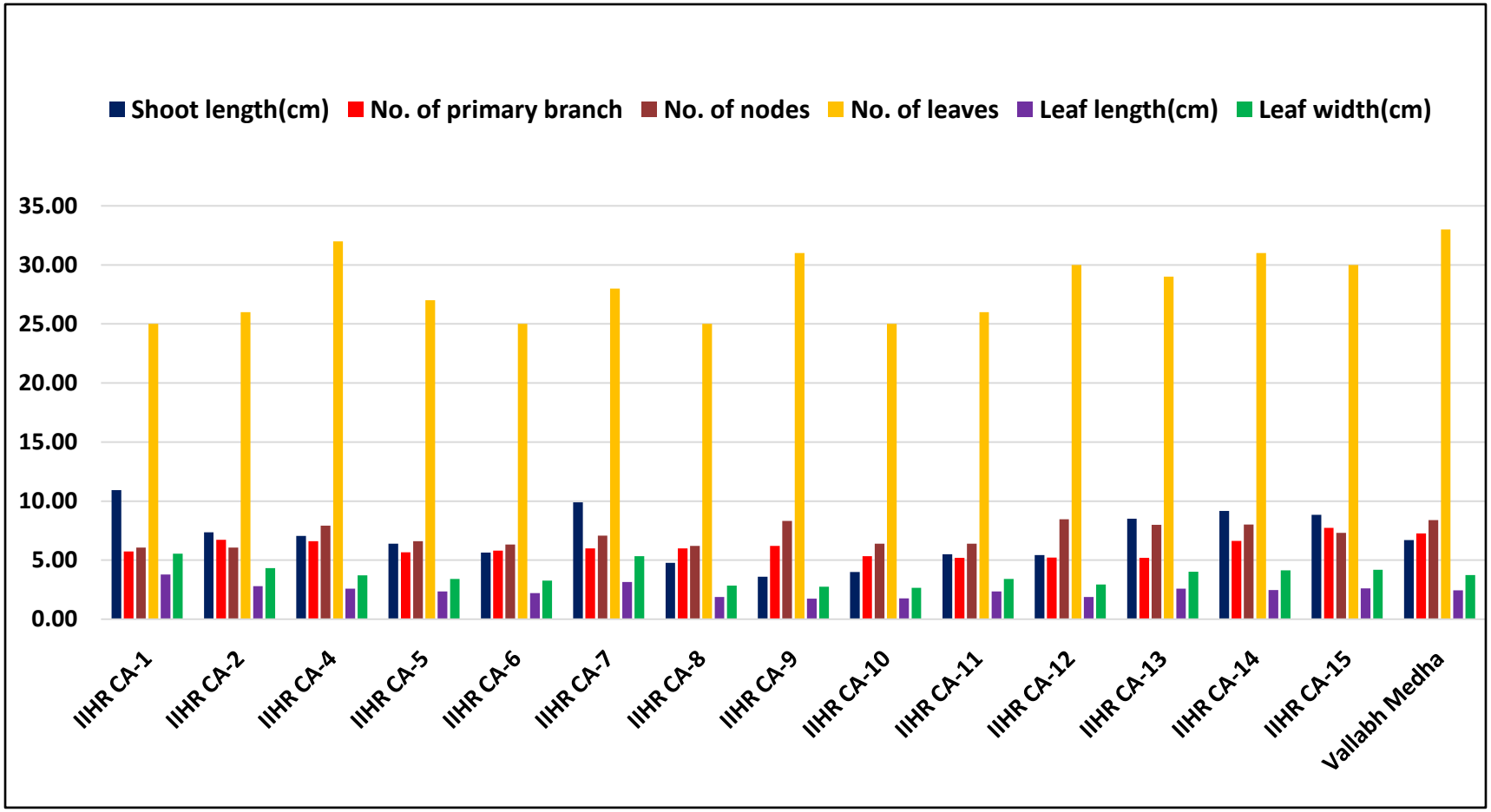


Fig. 4: Growth parameters of *Centellaasiatica*L. germplasm at 90 days after planting (DAP)

4.1.1.4. Growth parameters at 120 days after planting (DAP)

The observations recorded on growth parameters *viz*., shoot length, number of primary branches per plant, number of nodes per plant, number of leaves per plant, leaf length and leaf width exhibited significant variation among the germplasm (Table 7; Fig. 5).

The shoot length at 120 days ranged from 5.30 cm (IIHR CA-9) to 16.42 (IIHR CA-1) whereas check variety recorded 9.85 cm and three germplasm exceeded the check variety Vallabh Medha.

The number of primary branches ranged from 11.66 (IIHR CA-1) to 18.78 (IIHR CA-2) whereas check variety recorded 17.44 and only two germplasm registered higher mean values over the check variety.

The number of nodes ranged from 10.66 (IIHR CA-1) to 18.00 (IIHR CA-7) whereas the check variety recorded 17.33 and three germplasm exceeded check variety.

The number of leaves ranged from 51.10 (IIHR CA-1) to 81.33 (IIHR CA-7) whereas the check variety recorded 73.22 and three germplasm recorded higher leaf number than the check variety.

The leaf length ranged from 1.92 (IIHR CA-12) to cm 4.09 cm (IIHR CA-1) whereas the check variety recorded 3.00 cm and four germplasm were found to exhibit more leaf length over the check variety.

The leaf width ranged from 2.80 (IIHR CA-10) to 7.24 cm (IIHR CA-1) whereas the check variety recorded 4.27 cm and five germplasm were found superior to the check variety.

4.1.1.5. Estimated leaf yield at 90, 120 and 150 days after panting (DAP)

Germplasm significantly differed with respect to leaf yield at all stages of harvest viz., 90, 120 and 150 days after planting (Table 8; Fig. 6; Fig. 7).

Table 7*: Performance of Centella asisatica L. germplasm for growth*

Germplasm	*Shoot length (cm)*	*No. of Primary branches plant^{-1}*	*No. of nodes plant^{-1}*	*No. of leaves plant^{-1}*	*Leaf length (cm)*	*Leaf width (cm)*
IIHR CA-1	*16.42*	*11.66*	*10.66*	*51.10*	*4.09*	*7.24*
IIHR CA-2	*9.73*	*18.78*	*15.22*	*64.44*	*3.23*	*5.19*
IIHR CA-4	*8.03*	*17.89*	*15.44*	*67.77*	*2.62*	*4.10*
IIHR CA-5	*7.99*	*14.33*	*14.55*	*65.66*	*2.73*	*4.07*
IIHR CA-6	*6.52*	*16.78*	*17.88*	*72.22*	*2.58*	*4.06*
IIHR CA-7	*11.35*	*16.89*	*18.00*	*81.33*	*3.86*	*6.65*
IIHR CA-8	*5.75*	*13.33*	*11.50*	*53.85*	*1.97*	*3.22*
IIHR CA-9	*5.30*	*15.66*	*12.10*	*68.78*	*1.99*	*3.04*
IIHR CA-10	*5.92*	*14.77*	*13.33*	*62.10*	*2.07*	*2.80*
IIHR CA-11	*7.05*	*12.34*	*12.94*	*59.59*	*2.33*	*3.77*
IIHR CA-12	*5.81*	*14.55*	*13.22*	*62.91*	*1.92*	*2.99*
IIHR CA-13	*8.71*	*13.66*	*15.77*	*67.22*	*2.86*	*4.19*
IIHR CA-14	*11.18*	*17.33*	*17.11*	*78.11*	*2.95*	*4.82*
IIHR CA-15	*9.02*	*15.50*	*17.78*	*77.92*	*3.28*	*5.03*
*Vallabh Medha**	*9.85*	*17.44*	*17.33*	*73.22*	*3.00*	*4.27*
S.Em. ±	*0.49*	*0.73*	*0.78*	*2.67*	*0.08*	*0.12*
CD at 5%	*1.42*	*2.14*	*2.28*	*7.78*	*0.24*	*0.34*

parameters at 120 days after planting

*Check variety- Vallabh Medha

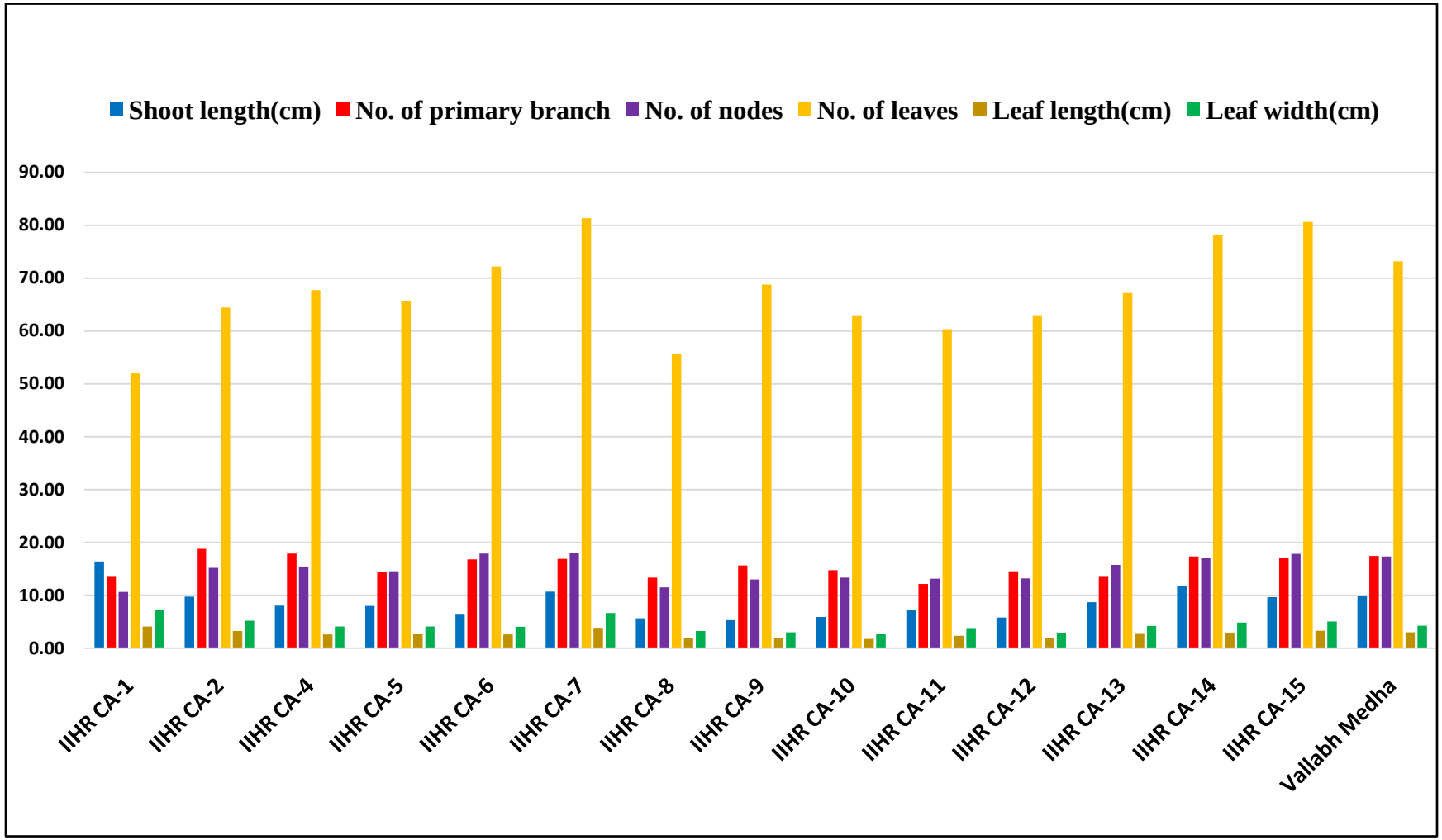


Fig. 5: Growth parameters of *Centellaasiatica* L. germplasm at 120 days after planting (DAP)

Table 8: Estimated leaf yield of *Centella asiatica* germplasm at different stages of growth

Germplasm	90 DAP				120 DAP				150 DAP			
	Fresh leaf yield/plot (g)	Fresh leaf yeild (q ha^{-1})	Dry leaf yield/plot (g)	Dry leaf yield (q ha^{-1})	Fresh leaf yield/plot (g)	Fresh leaf yield (q ha^{-1})	Dry leaf yield/plot (g)	Dry leaf yield (q ha^{-1})	Fresh leaf yield/plot (g)	Fresh leaf yeild (q ha^{-1})	Dry leaf yield/plot (g)	Dry leaf yield (q ha^{-1})
IIHR CA-1	1386.03	19.25	317.21	4.37	1,485.79	20.64	325.28	4.52	1523.21	21.16	332.733	4.62
IIHR CA-2	1064.67	14.78	196.39	2.73	1,260.67	17.50	202.67	2.82	1280.90	17.79	213.63	2.97
IIHR CA-4	783.34	10.88	130.19	1.81	1,062.80	14.76	178.88	2.48	1456.40	20.23	233.00	3.24
IIHR CA-5	692.26	9.62	139	1.93	1,065.51	15.26	164.60	2.29	1254.33	17.42	283.30	3.62
IIHR CA-6	710.19	9.86	135.8	1.88	865.997	12.03	137.35	1.91	1271.47	17.66	220.80	3.07
IIHR CA-7	1531.95	21.27	316.77	4.40	1,557.78	21.64	324.56	4.53	1568.13	21.78	333.20	4.63
IIHR CA-8	293.54	4.07	79.3	1.10	479.513	6.68	136.66	1.89	810.667	11.26	151.92	2.11
IIHR CA-9	177.39	2.46	47.15	0.65	251.61	3.54	57.83	0.80	620.867	8.62	150.43	2.09
IIHR CA-10	270.70	3.76	70.2	0.98	429.987	5.97	80.14	1.11	1294.17	17.97	234.43	3.26
IIHR CA-11	504.30	7.00	89.18	1.24	1,044.33	14.46	169.16	2.35	1046.03	14.53	207.15	2.88
IIHR CA-12	398.23	5.55	120.44	1.67	612.353	8.50	157.66	2.18	1334.80	18.54	219.90	3.06
IIHR CA-13	1241.07	17.24	207.09	2.88	1,379.54	19.05	239.71	3.33	1454.57	20.20	250.43	3.73
IIHR CA-14	1032.16	14.34	186.66	2.59	1,119.54	15.55	216.66	3.01	1177.83	16.36	220.16	3.06
IIHR CA-15	924.43	12.84	124.42	1.73	1,029.07	14.00	325.28	2.17	1100.60	15.29	161.36	2.24
Vallabh Medha*	1196.86	16.62	206.88	2.87	1,350.01	18.79	221.56	3.07	1370.92	19.04	231.21	3.21
S.Em. ±	31.75	0.44	15.58	0.22	43.63	0.61	9.46	0.131	29.97	0.42	9.58	0.13
C.D at 5%	92.45	1.28	45.37	0.63	127.04	1.76	27.56	0.38	87.26	1.21	27.91	0.38

*Check variety- Vallabh Medha

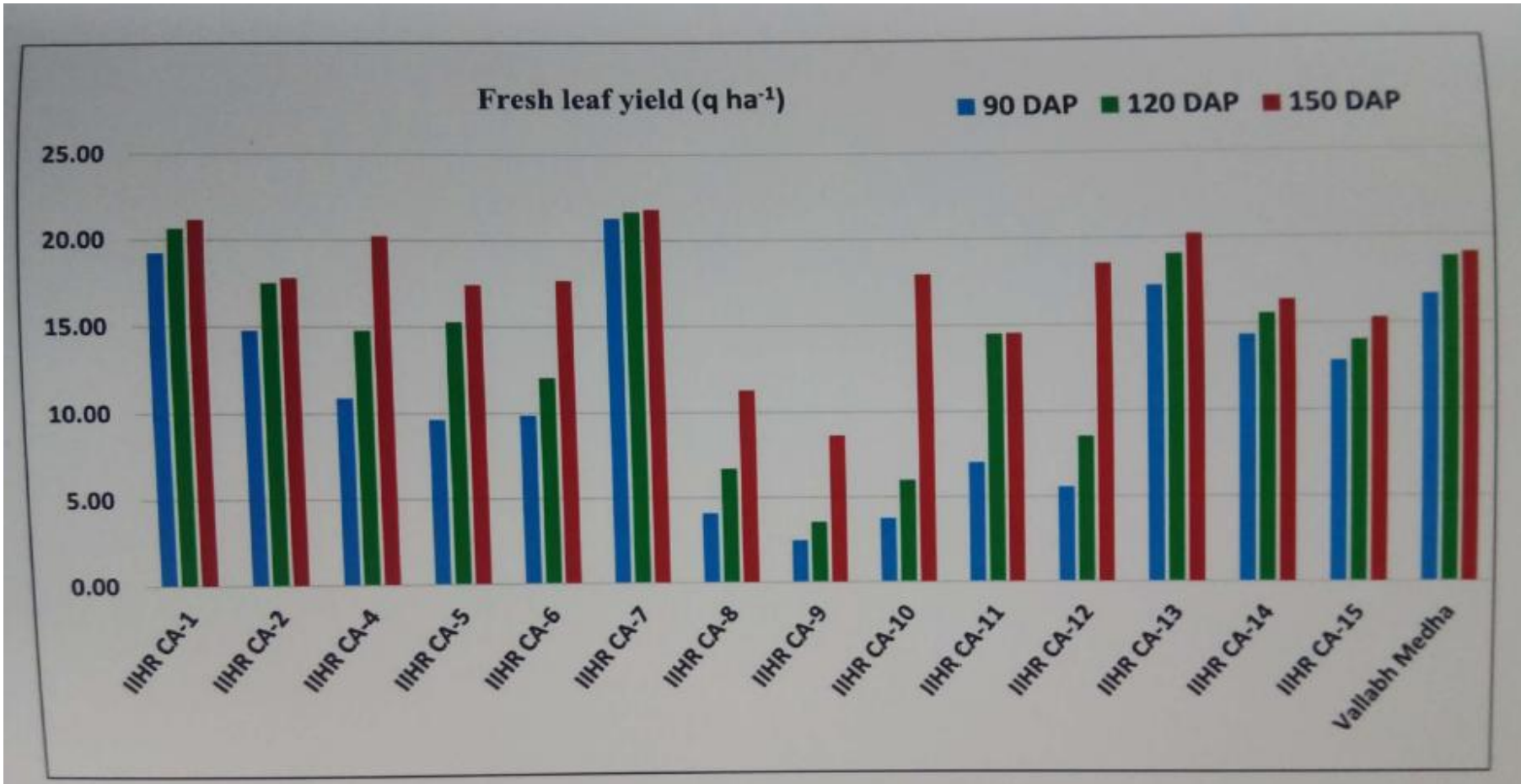


Fig. 6: Fresh leaf yield of *Centella asiatica* L. germplasm at different stages of harvest.

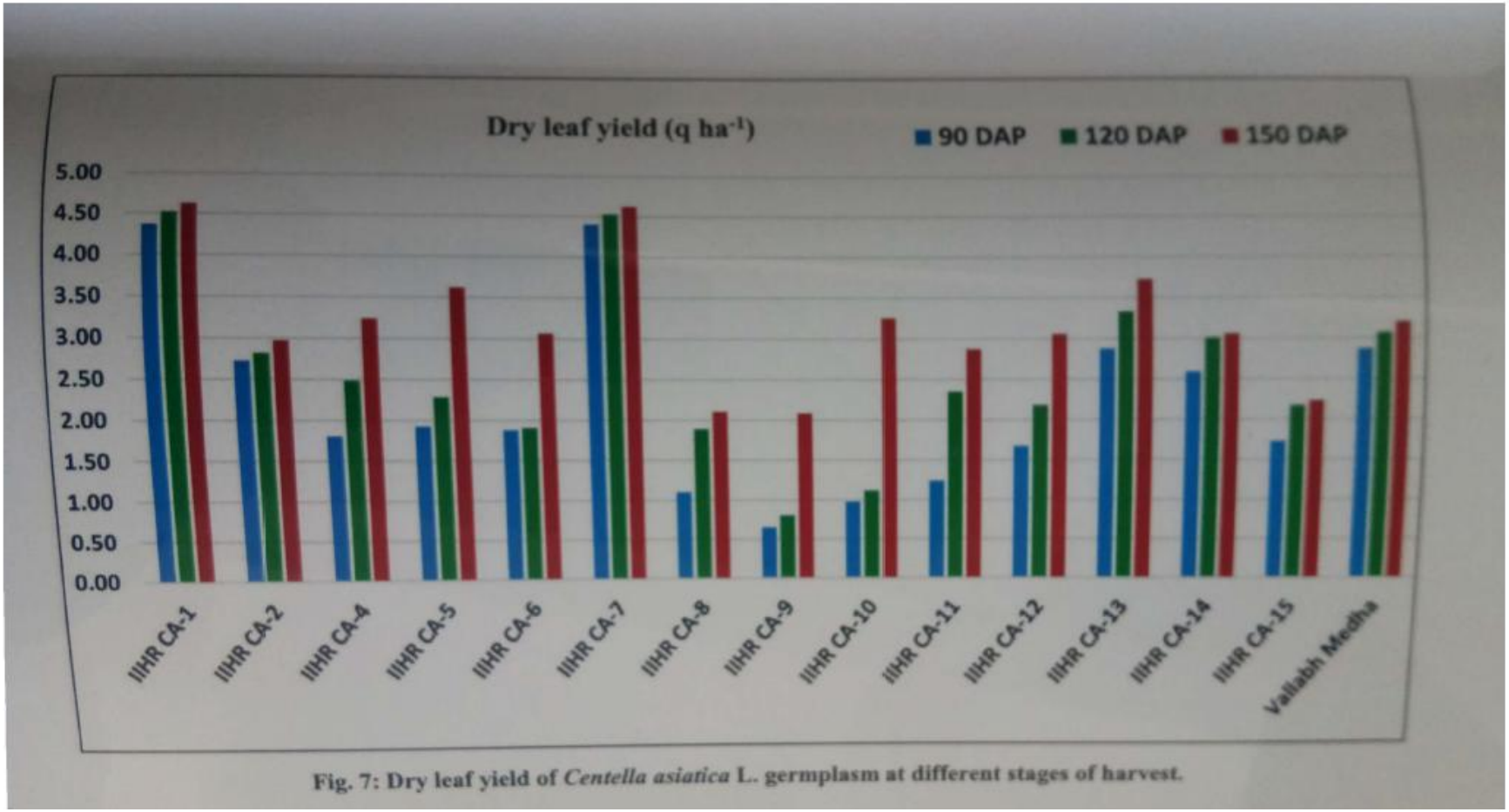


Fig. 7: Dry leaf yield of *Centella asiatica* L. germplasm at different stages of harvest.

Total fresh leaf weight at 90 days after planting ranged from 177.39 g/plot (IIHR CA-9) to 1531.95 g/plot (IIHR CA-7) and 2.46 (IIHR CA-9) to 21.27 q ha^{-1}(IIHR CA-7) whereas check variety Vallabh Medha recorded 1196.86 g/plot (16.62 q ha^{-1}). Three germplasm (IIHR CA-7, IIHR CA-1 and IIHR CA-13) exceeded the check variety. The Total leaf dry weight at 90 days after planting ranged from 47.15 g/plot (IIHR CA-9) to 316.77 g/plot (IIHR CA-9) and 0.65 q ha^{-1} (IIHR CA-9) to 4.40 q ha^{-1} (IIHR CA-7) whereas check variety recorded 206.88 g/plot (2.87 q ha^{-1})and three germplasm (IIHR CA-7, IIHR CA-1 and IIHR CA-13) recorded higher dry leaf yield than the check variety.

Total fresh leaf weight at 120 days after planting ranged from 251.61g/plot (IIHR CA-9) to 1,557.78 g/plot (IIHR CA-7) and3.54 (IIHR CA-9) to 21.64 q ha^{-1} (IIHR CA-7) whereas check variety Vallabh Medha recorded 1,350.01 g/plot (18.79 q ha^{-1}). Three germplasm (IIHR CA-7, IIHR CA-1 and IIHR CA-13) exceeded the check variety. The total leaf dry weight at 120 days after planting among the genotypes ranged from 57.83g/plot (IIHR CA-9) to 324.56gm/plot (IIHR CA-7) and 0.80 q ha^{-1} (IIHR CA-9) to 4.53 q ha^{-1} (IIHR CA-7) whereas check variety recorded 221.56 g/plot (3.07 q ha^{-1}) and three germplasm (IIHR CA-7, IIHR CA-1 and IIHR CA-13) recorded higher dry leaf weight than the check variety.

Among the germplasm total fresh leaf weight at 150 days after planting ranged from 620.86 g/plot (IIHR CA-9) to 1,568.13 (IIHR CA-7) and8.62 (IIHR CA-9) to 21.78 q ha^{-1} (IIHR CA-7) whereas check variety Vallabh Medha recorded 1,370.92 g/plot (19.04 q ha^{-1}). Four germplasm (IIHR CA-1, IIHR CA-7, IIHR CA-4 and IIHR CA-13) exceeded the check variety. The total leaf dry weight at 150 days after planting ranged from 150.43 g/plot (IIHR CA-9) to 333.20 g/plot (IIHR CA-7) and 2.09 q ha^{-1} (IIHR CA-9) to 4.63 q ha^{-1} (IIHR CA-7). Whereas, check variety recorded 231.21 g/plot (3.21 q ha^{-1})and six germplasm (IIHR CA-7, IIHR CA-1, IIHR CA-5, IIHR CA-13, IIHR CA-10 and IIHR CA-4) recorded higher dry leaf weight than the check variety.

4.1.2 Qualitative characters

Various qualitative traits *viz.,* leaf shape, leaf margin, leaf colour, petiole colour, flower colour was found to have variation among the germplasm and are listed in Table 9 and depicted in Plate 2, 3, 4, 5 and 6.

Leaf shape of germplasm showed very minute differences, the germplasm IIHR CA-14 and Vallabh Medha was found orbicular-reniform leaf shape whereas IIHR CA-11 has reniform-deltoid and rest of the germplasm showed reniform leaf shape. Leaf margin was found dentate for IIHR CA-5 and IIHR CA-15 germplasm whereas IIHR CA-8 and IIHR CA-12 which showed narrow crenate-dentate undulated margin and rest of them showed crenate-dentate. Leaf colour was varied between green (143-A), green (143-B) and green (143-C) colour among germplasm. The five germplasm IIHR CA-8, IIHR CA-9, IIHR CA-10, IIHR CA-13 and IIHR CA-15 showed green (143-A) colour. The germplasm IIHR CA-1, IIHR CA-2, IIHR CA-6, IIHR CA-11 and Vallabh Medha showed green (143-B) colour and germplasm IIHR CA-4, IIHR CA-5, IIHR CA-7, IIHR CA-12 and IIHR CA-15 showed green (143-C) colour. The yellow green (144-A, B, C) petiole colour were recorded in four germplasm IIHR CA-1, IIHR CA-4, IIHR CA-14 and IIHR CA-7 whereas remaining eleven germplasm showed green (A, B, C) in colour. Flower colour of germplasm were recorded where white colour (NN155-D) was found in IIHR CA-5 and remaining germplasm flowers were found to have different group of red purple colour.

4.1.3. Variability, heritability and genetic advance for quantitative and yield traits

Estimates of Genotypic Co-efficient of variation (GCV), Phenotypic Co-efficient of variation (PCV), heritability (h^2) and genetic advance as per cent of mean (GA as % mean) for 12 different traits (Table 10; Fig. 8).

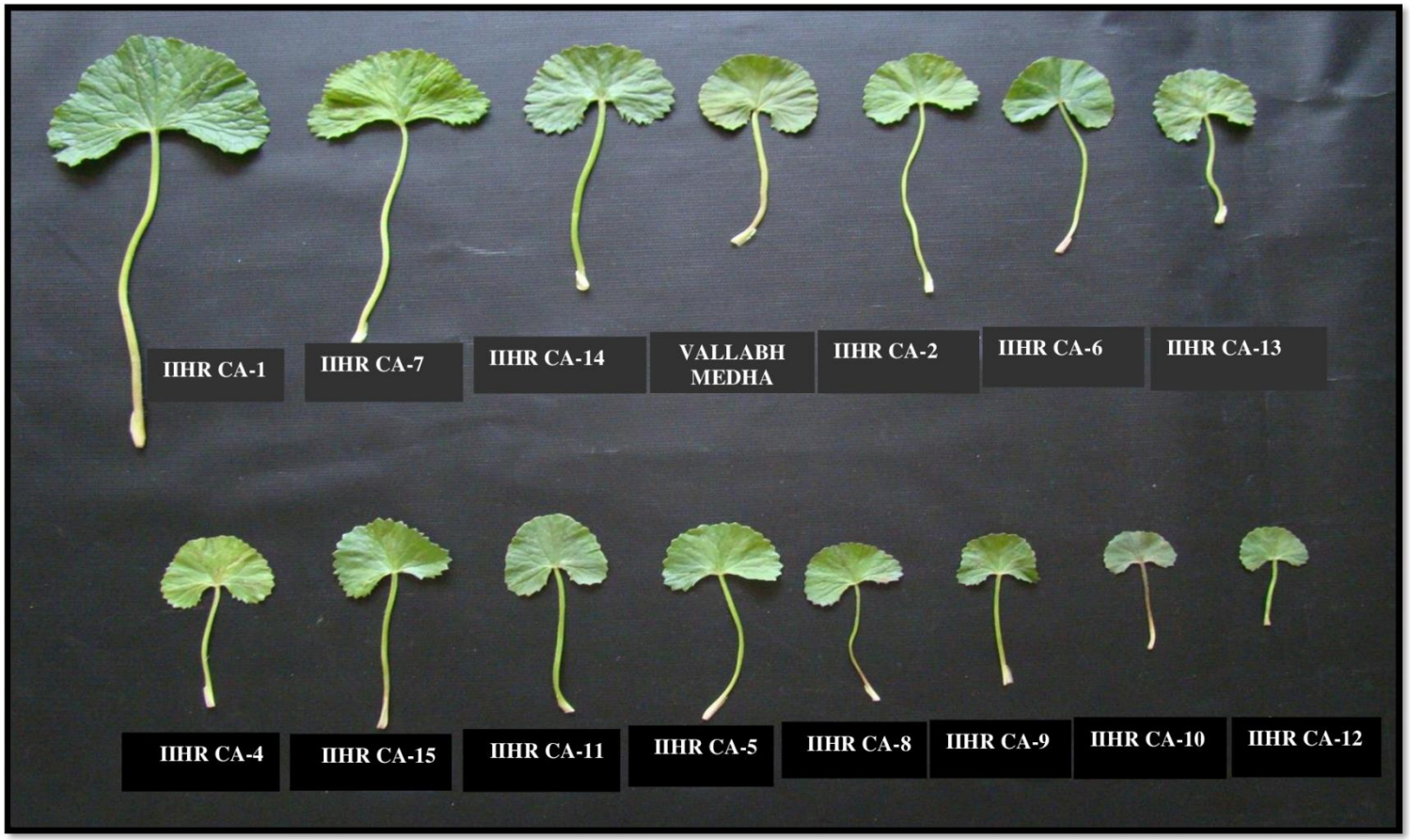


Plate 2: Variability of leaf in fifteen germplasm of Centella asiatica

Plate 3: Total fifteen germplasm of Centella asiatica

Plate 4: Variability for leaf shape in Centella asiatica germplasm

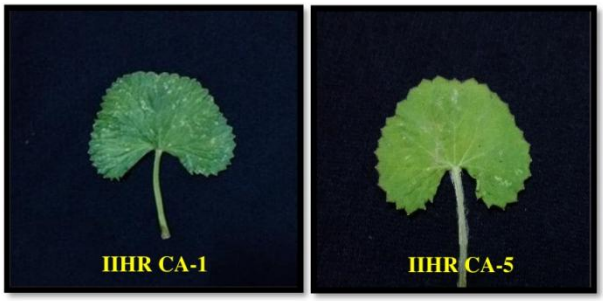
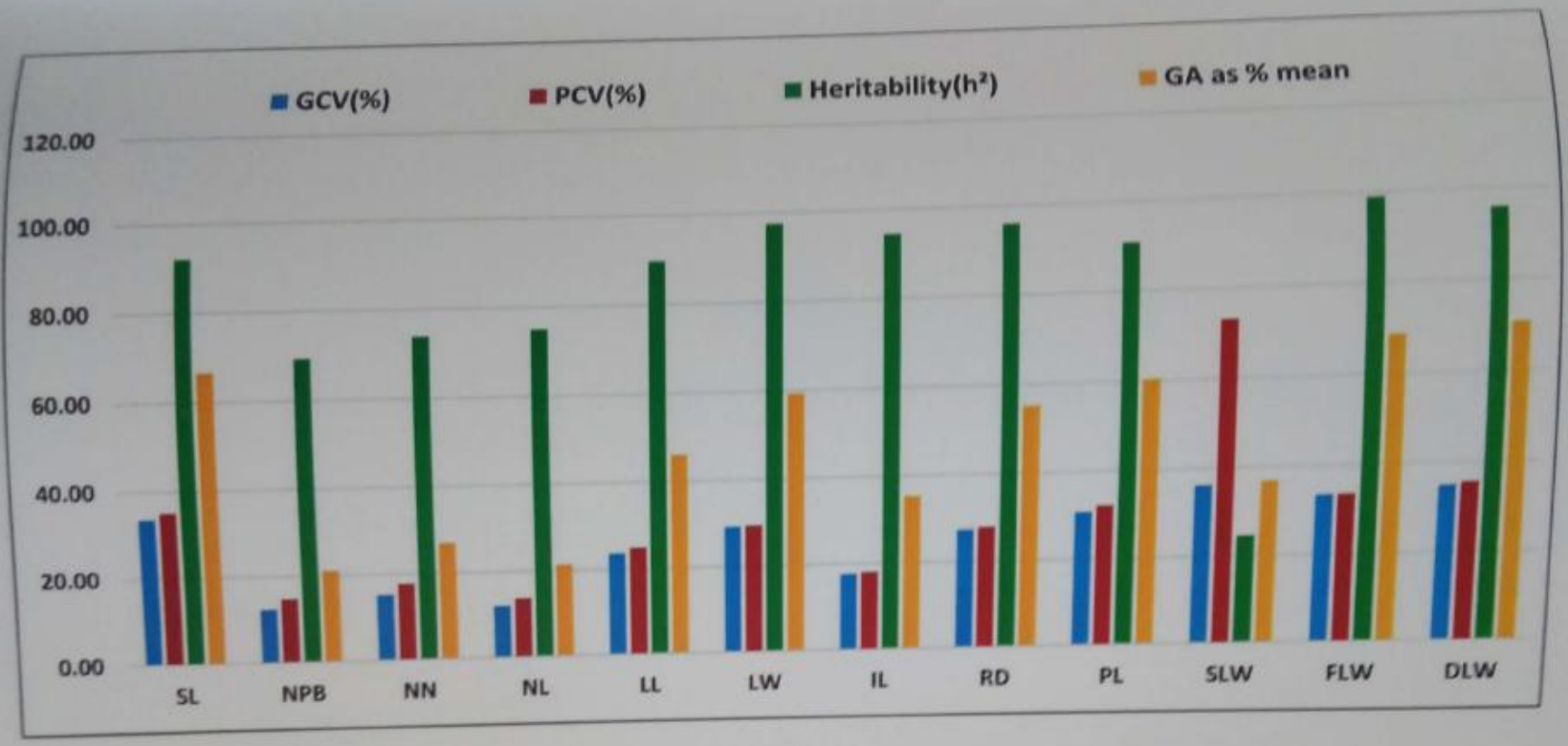


Plate 5: Variability for leaf margin in Centella asiatica germplasm

Plate 6: Variability of flower colour in Centella asiatica germplasm



SL - Shoot length, **NPB** - Number of primary branches, **NN** - Number of nodes, **NL** - Number of leaves, **LL** - Leaf length, **LW** - Leaf weight, **IL** - Internodal length, **RD** - Rosette diameter, **PL** - Petiole length, **SLW** - Specific leaf weight, **FLW** - Fresh leaf weight, **DLW** - Dry leaf weight.

Fig. 8: Genetic variability parameters for twelve morphological traits in *Centella asiatica* L. germplasm

Table 9: Qualitative traits of *Centellaasiatica* L. germplasm

Germplasm	Leaf shape	Leaf margin	Leaf colour	Petiole colour	Flower colour
IIHR CA-1	Reniform	Crenate-Dentate	Green (143-B)	Yellow green (144-A)	Red purple (67-A)
IIHR CA-2	Reniform	Crenate-Dentate	Green (143-B)	Green (143-C)	Red purple (61-A)
IIHR CA-4	Reniform	Crenate-Dentate	Green (143-C)	Yellow Green (144- B)	Red purple (61-B)
IIHR CA-5	Reniform	Dentate	Green (143-C)	Green (141-C)	White (NN155-D)
IIHR CA-6	Reniform	Crenate-Dentate	Green (143-B)	Green (143-C)	Red purple (64-B)
IIHR CA-7	Reniform	Crenate-Dentate	Green (143-C)	Yellow-green (144- C)	Red purple (63-A)
IIHR CA-8	Reniform	Narrow crenate-undulated margin	Green (143-A)	Green (143-B)	Red purple (61-C)
IIHR CA-9	Reniform	Crenate-Dentate	Green (143-A)	Green (143-A)	Red purple (63-A)
IIHR CA-10	Reniform	Crenate-Dentate	Green (143-A)	Green (143-C)	Red purple (67-B)
IIHR CA-11	Reniform deltoid	Crenate-Dentate	Green (143-B)	Green (143-C)	Red purple (64-B)
IIHR CA-12	Reniform	Narrow crenate-undulated margin	Green (143-C)	Green (143-B)	Red purple (64-B)
IIHR CA-13	Reniform	Crenate-dentate	Green (143-A)	Green (143-C)	Red purple (64-A)
IIHR CA-14	Orbicular reniform	Crenate- Dentate	Green (143-C)	Yellow green (144- B)	Red purple (64-D)
IIHR CA-15	Reniform	Dentate	Green (143-A)	Green (143-C)	Red purple (68-A)
VallabhMedha	Orbicular reniform	Crenate-Dentate	Green (143-B)	Green (143-C)	Red purple (59-C)

Table 10: Genetic variability parameters for quantitative and yield traits in twelve *Centella asiatica* L. germplasm

Sl. No.	Characters	GCV (%)	PCV (%)	Heritability (h^2)	GA as % mean
1	SL	33.66	35.09	92.02	66.52
2	NPB	12.36	14.84	69.37	21.21
3	NN	15.26	17.76	73.84	27.02
4	NL	11.99	13.85	75.04	21.40
5	LL	23.63	24.96	89.65	46.10
6	LW	29.15	29.52	97.47	59.28
7	IL	17.71	18.23	94.40	35.45
8	RD	27.36	27.95	95.90	55.21
9	PL	30.73	32.25	90.79	60.31
10	SLW	36.22	73.15	24.52	36.94
11	FLW	33.48	33.66	98.88	68.57
12	DLW	35.03	35.78	95.84	70.64

Where

SL Shoot length

NPB Number of primary branches

NN Number of nodes

NL Number of leaves

LL Leaf length

LW **Leaf** width

IL **Internodal length**

RD **Rosette diameter**

PL **Petiole length**

SLW **Specific leaf weight**

FLW **Fresh leaf weight**

DLW **Dry leaf weight**

Shoot length (SL) recorded higher genotypic coefficient of variation (33.66 %), phenotypic coefficient of variation (35.09 %), heritability (92.02 %) and genetic advance as per cent of mean (66.52 %).

Number of primary branches (NPB) recorded moderate genotypic coefficient of variation (12.36 %), phenotypic coefficient of variation (14.84 %), high heritability (69.37 %) and genetic advance as per cent of mean (21.21 %).

Number of nodes (NN) recorded moderate genotypic coefficient of variation (15.26 %), phenotypic coefficient of variation (17.76 %), high heritability (73.84 %) and genetic advance as per cent of mean (27.02 %).

Number of leaves (NL) recorded moderate genotypic coefficient of variation (11.99 %), phenotypic coefficient of variation (13.85 %), high heritability (75.04 %) and genetic advance as per cent of mean (21.40 %).

Leaf length (LL) recorded higher genotypic coefficient of variation (23.63 %), phenotypic coefficient of variation (24.96 %), heritability (89.65 %) and genetic advance as per cent of mean (46.10 %).

Leaf width (LW) recorded higher genotypic coefficient of variation (29.15 %), phenotypic coefficient of variation (29.52 %), heritability (97.47 %) and genetic advance as per cent of mean (59.28 %).

Internode length (IL) recorded moderate genotypic coefficient of variation (17.71 %), phenotypic coefficient of variation (18.23 %), high heritability (94.40 %) and genetic advance as per cent of mean (35.45 %).

Rosette diameter (RD) recorded higher genotypic coefficient of variation (27.36 %), phenotypic coefficient of variation (27.95 %), heritability (95.90 %) and genetic advance as per cent of mean (55.21 %).

Petiole length (PL) recorded higher genotypic coefficient of variation (30.73%), phenotypic coefficient of variation (32.25 %), heritability (90.79 %) and genetic advance as per cent of mean (60.31 %).

Specific leaf weight (SLW) recorded higher genotypic coefficient of variation (36.22 %), phenotypic coefficient of variation (73.15 %), lower heritability (24.52 %) and genetic advance as per cent of mean (36.94 %).

Fresh leaf weight (FLW) higher genotypic coefficient of variation (33.48 %), phenotypic coefficient of variation (33.66 %), heritability (98.88 %) and genetic advance as per cent of mean (68.57 %).

Dry leaf weight (DLW) recorded higher genotypic coefficient of variation (35.03 %), phenotypic coefficient of variation (35.78 %), heritability (95.84 %) and genetic advance as per cent of mean (70.64 %). Therefore, overall higher PCV and GCV were recorded by specific leaf weight (73.15 % and 36.22 %) followed by dry leaf weight (35.78 %, 35.03 %) and shoot length (35.09 %, 33.66 %) (Table.10) and lowest value was found in number of leaves (13.85 %,11.99 %).

Highest heritability (h^2) value was recorded of fresh leaf weight (98.88 %) followed byleaf width (97.47 %) and lowest was recorded by Specific leaf weight (24.52 %).

Highest genetic advance as per cent of mean value was recorded of dry leaf weight (70.64 %) and lowest value by number of primary branches (21.21 %).

4.1.4 Character association

Correlation provides the degree and direction of a relationship between variables at phenotypic, genotypic and environmental levels. The association between traits that can be directly observed is called phenotypic correlation. Genotypic correlation is the inherent association between variables. This type of correlation is more stable and is of paramount importance for a plant breeder to bring about genetic improvement in one character by selecting the other character of a pair that is genetically correlated. Genotypic correlations were higher than phenotypic correlations for sixteen morphological traits(Table11and12).

Table 11: Phenotypic correlations between dry leaf weight and its contributing traits of *Centella asiatica* L. germplasm

Char.	SL	NPB	NN	NL	LL	LW	IL	RD	PL	SLW	FLW	MD	AT	MA	A A	DLW
SL	1.000	0.111^{NS}	0.074^{NS}	0.030^{NS}	0.873**	0.904**	0.886**	0.892**	0.744**	0.185^{NS}	0.784**	0.620**	0.376*	-0.545**	-0.462**	0.842**
NPB		1.000	0.489**	0.510**	0.246^{NS}	0.181^{NS}	0.131^{NS}	0.099^{NS}	0.110^{NS}	0.257^{NS}	0.269^{NS}	0.226^{NS}	0.144^{NS}	-0.384**	-0.313*	0.127^{NS}
NN			1.000	0.869**	0.245^{NS}	0.180^{NS}	0.132^{NS}	0.075^{NS}	0.329*	0.344*	0.397**	-0.079^{NS}	0.103^{NS}	-0.306*	-0.124^{NS}	0.166^{NS}
NL				1.000	0.182^{NS}	0.142^{NS}	0.098^{NS}	-0.013^{NS}	0.282^{NS}	0.430**	0.265^{NS}	-0.196^{NS}	0.006^{NS}	-0.232^{NS}	-0.068^{NS}	0.095^{NS}
LL					1.000	0.924**	0.899**	0.886**	0.768**	0.234^{NS}	0.857**	0.580**	0.430**	-0.599**	-0.467**	0.897**
LW						1.000	0.877**	0.837**	0.767**	0.282^{NS}	0.813**	0.499**	0.335*	-0.597**	-0.492**	0.859**
IL							1.000	0.914**	0.777**	0.208^{NS}	0.814**	0.517**	0.333*	-0.463**	-0.345*	0.892**
RD								1.000	0.778**	0.106^{NS}	0.834**	0.526**	0.352*	-0.501**	-0.382**	0.861**
PL									1.000	0.269^{NS}	0.782**	0.139^{NS}	0.178^{NS}	-0.449**	-0.302*	0.813**
SLW										1.000	0.217^{NS}	-0.119^{NS}	-0.115^{NS}	-0.135^{NS}	-0.153^{NS}	0.245^{NS}
FLW											1.000	0.498**	0.371*	-0.583**	-0.520**	0.914**
MD												1.000	0.609**	-0.420**	-0.507**	0.537**
AT													1.000	-0.506**	-0.112^{NS}	0.338*
MA														1.000	0.717**	-0.533**
AA															1.000	-0.533**
DLW																1.000

***Significant at 5%** **Significant at 1% NS- Non significant

SL- Shoot length, **NPB** - Number of primary branches, **NN** - Number of nodes, **NL** - Number of leaves, **LL** - Leaf length, **LW** - Leaf weight, **IL** - Internodal length, **RD** - Rosette diameter, **PL** - Petiole length, **SLW** - Specific leaf weight, **FLW** - Fresh leaf weight, **MD** - Madecassoside, **AT** - Asiaticoside, **MA** - Madecassic acid, **AA** - Asiatic acid, **TT** - Total terpenoids, **DLW** - Dry leaf weight.

Table 12: Genotypic correlations between dry leaf weight and its contributing traits of *Centella asiatica* L. germplasm

Char.	SL	NPB	NN	NL	LL	LW	IL	RD	PL	SLW	FLW	MD	AT	MA	A A	DLW
SL	1.00	0.196^{NS}	0.077^{NS}	0.050^{NS}	0.955**	0.941**	0.920**	0.954**	0.802**	0.411**	0.829**	0.632**	0.427**	-0.683**	-0.564**	0.883**
NPB		1.000	0.735**	0.671**	0.314*	0.246^{NS}	0.166^{NS}	0.094^{NS}	0.158^{NS}	0.456**	0.303*	0.285^{NS}	0.125^{NS}	-0.573**	-0.474**	0.157^{NS}
NN			1.000	0.956**	0.230^{NS}	0.232^{NS}	0.113^{NS}	0.047^{NS}	0.376*	0.618**	0.466**	-0.120^{NS}	0.142^{NS}	-0.430**	-0.209^{NS}	0.203^{NS}
NL				1.000	0.154^{NS}	0.196^{NS}	0.084^{NS}	-0.059^{NS}	0.332*	1.027**	0.306*	-0.293^{NS}	-0.050^{NS}	-0.362*	-0.093^{NS}	0.129^{NS}
LL					1.000	0.967**	0.945**	0.934**	0.805**	0.510**	0.919**	0.655**	0.568**	-0.829**	-0.633**	0.963**
LW						1.000	0.911**	0.872**	0.808**	0.562**	0.831**	0.544**	0.421**	-0.786**	-0.630**	0.884**
IL							1.000	0.940**	0.829**	0.568**	0.840**	0.508**	0.363*	-0.560**	-0.420**	0.917**
RD								1.000	0.837**	0.239^{NS}	0.855**	0.574**	0.442**	-0.619**	-0.451**	0.897**
PL									1.000	0.587**	0.829**	0.136^{NS}	0.193^{NS}	-0.635**	-0.423**	0.841**
SLW										1.000	0.446**	-0.173^{NS}	-0.211^{NS}	-0.316*	-0.337*	0.577**
FLW											1.000	0.528**	0.453**	-0.745**	-0.645**	0.928**
MD												1.000	0.634**	-0.496**	-0.611**	0.552**
AT													1.000	-0.565**	-0.125^{NS}	0.383**
MA														1.000	0.690**	-0.654**
AA															1.000	-0.645**
DLW																1.000

*****Significant at 5% **Significant at 1% NS- Non significant

SL - Shoot length, **NPB** - Number of primary branches, **NN** - Number of nodes, **NL** - Number of leaves, **LL** - Leaf length, **LW** - Leaf weight, **IL** - Internodal length, **RD** - Rosette diameter, **PL** - Petiole length, **SLW** - Specific leaf weight, **FLW** - Fresh leaf weight, **MD** - Madecassoside, **AT** - Asiaticoside, **MA** - Madecassic acid, **AA** - Asiatic acid, **DLW** - Dry leaf weight

4.1.4.1 Phenotypic and Genotypic correlation between dry leaf weight and its components

The dry leaf weight showed significant positive phenotypic correlation with shoot length (0.842), leaf length (0.897), leaf width (0.859), Internodal length (0.892), rosette diameter (0.861), petiole length (0.813), fresh leaf weight (0.914), Madecassoside (0.537) and Asiaticoside (0.338) whereas, fresh leaf weight found significant positive correlation with plant height (0.784), number of nodes (0.397), leaf length (0.857), leaf width (0.813), Internodal length (0.814), rosette diameter (0.834), petiole length (0.782), Madecassoside (0.498) and Asiaticoside (0.371). Madecassoside and Asiaticoside were significant negative correlation with Madeccasic acid (-0.583 and -0.533) and Asiatic acid (-0.520 and -0.533) with respect to fresh leaf weight and dry leaf weight respectively.

Genotypic correlations are more important as it explains magnitude and direction of association at genotypic level. Fresh leaf weight was found to have significant positive genotypic correlation with shoot length (0.829), number of primary branches (0.303), number of nodes (0.466), number of leaf (0.306), leaf length (0.919), leaf width (0.831), internode length (0.840), rosette diameter (0.855), petiole length (0.829), specific leaf weight (0.446), Madecassoside (0.528) and Asiaticoside (0.453) whereas, Madeccasic acid (-0.745) and Asiatic acid (-0.645) showed significant negative correlation.

Dry leaf weight showed significant positive correlation with shoot length (0.883), leaf length (0.963), leaf width (0.884), internode length (0.917), rosette diameter (0.897), petiole length (0.841), specific leaf weight (0.577), fresh leaf weight (0.928), Madecassoside (0.552) and Asiaticoside (0.383) whereas non-significant correlation with number of primary branch, number of nodes and number of leaves. However significantly negative correlation was observed with Madeccasic acid (-0.654) and Asiatic acid (-0.645).

4.1.5. Genetic divergence using Mahalanobis D^2 analysis

4.1.5.1. Clustering pattern of fifteen *Centella asiatica* L. germplasm

The quantitative assessment of genetic divergence among fifteen germplasm of *Centella asiatica* L. was made by adopting Mahalanobis D^2 statistics for sixteen characters as suggested Tocher (Rao 1952).

The fifteen germplasm of *Centella asiatica* were grouped into five clusters. The distribution of different germplasm for sixteen characters into V different clusters and their mean values are presented in Table 13. Out of the five clusters formed, cluster I was the largest comprising of six germplasm (Vallabh Medha, IIHR CA-1, IIHR CA-2, IIHR CA-4, IIHR CA-5, IIHR CA-6) followed by cluster III (IIHR CA-7, IIHR CA-14, IIHR CA-15) and Cluster IV (IIHR CA-8, IIHR CA-9, IIHR CA-11) with three germplasm in each clusters respectively, Cluster II with two germplasm (IIHR CA-10, IIHR CA-12) and cluster V was solitary with only one germplasm (IIHR CA-13).

4.1.5.2. Average inter and intra cluster distance

The inter and intra cluster distance (D^2 and D) values among five clusters involving fifteen germplasm of *Centella asiatica* are presented in Table 14.

Among the five different clusters, the intracluster distance (diagonal values) was maximum in cluster IV (24.45), followed by cluster I (22.13) and cluster III (21.67) while, minimum in cluster II (9.26). However, the intracluster distance of solitary clusters V was zero.

The inter cluster distance (off diagonal values) was maximum between cluster IV and III (40.45) followed by cluster V and IV (36.81) and between cluster III and II (34.77) while minimum between clusters IV and II (16.41).

Table 13: Cluster members of D^2 analysis of *Centella asiatica* L. germplasm.

Cluster No.	No. of germplasm	Cluster members
I	6	IIHR CA-1, IIHR CA-2, IIHR CA- 4, IIHR CA- 5, IIHR CA- 6, Vallabh Medha
II	2	IIHR CA-10, IIHR CA-12
III	3	IIHR CA-7, IIHR CA-14, IIHR CA-15
IV	3	IIHR CA- 8, IIHR CA- 9, IIHR CA-11
V	1	IIHR CA-13

Table 14: Inter and intra cluster divergence (D^2) and D values among five clusters involving fifteen germplasm of *Centella asiatica* L.

Clusters	I	II	III	IV	V
I	489.69 (22.13)	796.20 (28.22)	615.01 (24.80)	1125.02 (33.54)	352.04 (18.76)
II		85.82 (9.26)	1208.90 (34.77)	269.13 (16.41)	930.12 (30.50)
III			469.67 (21.67)	1636.49 (40.45)	433.38 (20.82)
IV				597.58 (24.45)	1355.21 (36.81)
V					0.00 (0.00)

4.1.5.3. Mean performance of five clusters for sixteen different quantitative characters

The cluster means values for different characters were evaluated for all the sixteen characters are presented in Table 15. It indicated that differences among the clusters and also differences exist for all the traits studied. The data indicated that cluster mean for shoot length ranged from 5.86 cm (cluster II) to 10.52 cm (cluster III). Highest shoot length was recorded in cluster III (10.52 cm) followed by cluster I (9.76 cm) and cluster V (8.71 cm), while lowest was recorded in cluster II (5.86 cm). Number of primary branches per plant was highest in cluster III (16.57) followed by cluster I (16.48), while lowest in cluster IV (13.44) followed by cluster V (13.66).

Number of nodes was highest in cluster III (17.63) followed by cluster V (15.77), while lowest in cluster IV (12.48) followed by cluster II (13.27).

The germplasm of cluster III recorded maximum number of leaves (79.19) followed by cluster V (67.22) and cluster I (65.89), while the germplasm of cluster IV (60.79) recorded minimum number of leaves. The germplasm of cluster III (3.10 cm) produced maximum leaf length followed by cluster I (3.04 cm) and minimum in II (1.99 cm).The germplasm of cluster III (5.50 cm) produced maximum leaf width followed by cluster I (4.82 cm) and minimum was recorded in II (2.90 cm). Internodal length was maximum in germplasm of cluster III (7.72 cm) followed by cluster I (7.57 cm) and cluster V (6.89 cm), while minimum in cluster IV (5.84 cm).

The germplasm of cluster I was recorded maximum rosette diameter (13.57 cm) followed by cluster V (13.04), while minimum was in cluster II (8.65 cm) followed by IV (8.78 cm).The germplasm of cluster III recorded highest petiole length (10.61 cm) followed by cluster V (9.63 cm) and I (8.57 cm), while lowest in cluster IV (5.54 cm) followed by cluster II (5.92 cm). Cluster III germplasm recorded maximum specific leaf weight (0.47) followed by cluster V (0.26) while minimum was recorded in germplasm of cluster I (0.21) followed by IV (0.24).

Table 15: Cluster mean value for sixteen characters in *Centella asiatica* L. germplasm

Clusters	SL	NPB	NN	NL	LL	LW	IL	RD	PL	SLW	FLW	DLW	MD	AT	MA	A A
I	9.76	16.48	15.18	65.89	3.04	4.82	7.57	13.57	8.57	0.21	16.26	2.98	3.15	1.38	0.326	0.035
II	5.86	14.66	13.27	63.00	1.99	2.90	6.22	8.65	5.92	0.24	10.04	2.04	3.13	1.04	0.056	0.078
III	10.52	16.57	17.63	79.19	3.10	5.50	7.72	12.94	10.61	0.47	17.07	3.15	3.82	1.83	0.030	0.034
IV	6.02	13.44	12.48	60.79	2.10	3.34	5.84	8.78	5.54	0.22	8.06	1.68	2.99	1.62	0.037	0.046
V	8.71	13.66	15.77	67.22	2.85	4.20	6.89	13.04	9.63	0.26	18.00	3.20	3.70	2.22	0.022	0.029

Where,

SL - Shoot length, **NPB** - Number of primary branches, **NN** - Number of nodes, **NL** - Number of leaves, **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), **MD** - Madecassoside, **AT** - Asiaticoside, **MA** - Madecassic acid, **AA** - Asiatic acid.

Cluster V germplasm recorded highest fresh leaf weight (18.00 q ha^{-1}) and dry leaf weight (3.20 q ha^{-1}) followed by cluster III and I (17.07, 3.15 and 16.26, 2.98 q ha^{-1}) respectively. While, lowest in cluster IV (8.06, 1.68 q ha^{-1}) followed by II (10.04, 2.04 q ha^{-1}). The maximum Madecassoside (MD) content was recorded in germplasm of cluster III (3.82 %) followed by cluster V (3.70 %) and I (3.15 %), while minimum in cluster IV (2.99 %). Highest Asiaticoside (AT) content was recorded in cluster V (2.22 %) followed by cluster III (1.83 %) and lowest content in cluster II (1.04 %) followed by cluster I (1.38 %). Maximum content of Madecassic acid (MA) were recorded in cluster I (0.326 %) followed by II (0.056 %), while minimum was recorded in cluster V (0.022%) followed by cluster III (0.03) and I (0.326 %). Asiatic acid (AA) content recorded highest in germplasm of cluster II (0.078 %) followed by cluster IV (0.046 %), while lowest was recorded in cluster V (0.029 %) followed by cluster III (0.034 %) and I (0.035 %).

4.2. Biochemical variability among fifteen germplasm of *Centella asiatica*.

Leaves of *Centella asiatica* were analysed for their terpenoids content by using Nexera X_2 HPLC for the simultaneous determination of Madecassoside, Asiaticoside, Madecassic and Asiatic acid content. Data pertaining to various terpenoid compounds present in fifteen germplasm of *Centella asiatica* leaf revealed, that there is wide variation in contents of various terpenoids and are shown in Table 16 to 18.

4.2.1. Terpenoids content (% w/w) among the fifteen germplasm of *Centella asiatica* L. at 90 days after planting (DAP)

Madecassoside content in leaf varied from 1.172 (IIHR CA-11) to 2.751 % (IIHR CA-1) whereas check variety Vallabh Medha recorded 2.556 %. Highest Madecassoside was found in IIHR CA-1(2.751 %) followed by IIHR CA-2 (2.611 %). Out of fifteen germplasm two germplasm recorded higher content over check variety (Table 16).

Asiaticoside content varied from 0.543 (IIHR CA-12) to 1.221 % (IIHR CA-13) whereas check recorded 1.147 %. Highest content of Asiaticoside was found in IIHR CA-

Table 16: Terpenoids content (%w/w) among the fifteen germplasm of *Centella asiatica* L. leaf at 90 days after planting (DAP)

Germplasm	Terpenoids (%)				
	Madecassoside	Asiaticoside	Madecassic Acid	Asiatic acid	Total
IIHR CA-1	2.751	1.105	0.024	0.023	3.904
IIHR CA-2	2.611	1.165	0.035	0.033	3.844
IIHR CA-4	1.235	0.702	0.028	0.036	2.002
IIHR CA-5	1.597	0.771	0.054	0.058	2.479
IIHR CA-6	1.600	1.122	0.046	0.066	2.834
IIHR CA-7	1.548	0.924	0.023	0.026	2.520
IIHR CA-8	1.510	0.810	0.061	0.043	2.423
IIHR CA-9	1.347	0.999	0.049	0.078	2.461
IIHR CA-10	1.311	0.842	0.079	0.112	2.344
IIHR CA-11	1.172	0.895	0.075	0.108	2.251
IIHR CA-12	1.552	0.543	0.078	0.041	2.214
IIHR CA-13	1.607	1.221	0.032	0.046	2.907
IIHR CA-14	1.375	0.837	0.055	0.073	2.342
IIHR CA-15	1.314	0.796	0.038	0.045	2.194
Vallabh Medha*	2.556	1.147	0.037	0.033	3.773
S.Em. ±	0.112	0.072	0.008	0.010	0.161
CD at 5%	0.326	0.210	0.022	0.030	0.469

*Check variety- Vallabh Medha

13 (1.221 %) followed by IIHR CA-2 (1.165 %) and two germplasm recorded higher contents compared to check variety.

Madecassic acid content varied from 0.023 (IIHR CA-7) to 0.079 % (IIHR CA-10) whereas check variety recorded 0.037 % and nine germplasm showed higher content over check variety. Asiatic acid content varied from 0.023 (IIHR CA-1) to 0.112 % (IIHR CA-10) whereas check variety recorded 0.033 %. Eleven germplasm showed higher content over check variety.

The total terpenoids content among the germplasm varied from 2.002 (IIHR CA-4) to 3.903 % (IIHR CA-1) whereas check variety recorded 3.772 %. Only two germplasm had higher content over check variety.

4.2.2. Terpenoids content of leaf (% w/w) among the fifteen germplasm of *Centella asiatica* L. at 120 days after planting (DAP)

Madecassoside content in leaf varied from 2.351 (IIHR CA-9) to 3.809 % (IIHR CA-7) whereas check variety Vallabh Medha recorded 3.021 %. Out of fifteen germplasm nine germplasm recorded higher Madecassoside content over check variety (Table 17).

Asiaticoside content varied from 0.401 (IIHR CA-12) to 1.795 % (IIHR CA-13) whereas check recorded 1.391 % and seven germplasm recorded higher Asiaticoside content compared to check variety.

Madecassic acid content varied from 0.014 (IIHR CA-13) to 0.063 % (IIHR CA-12) whereas check variety recorded 0.033 %. Only three germplasm showed higher content over the check variety.

Asiatic acid content varied from 0.011 (IIHR CA-7) to 0.043 % (IIHR CA-6) whereas check variety recorded 0.032 %. Only two germplasm showed higher content over mean value of check variety.

Table 17: Terpenoids content (%w/w) among the fifteen germplasm of *Centella asiatica* L. leaf at 120 days after planting (DAP)

Germplasm	Terpenoids (%)				
	Madecassoside	Asiaticoside	Madecassic acid	Asiatic acid	Total
IIHR CA-1	3.588	1.493	0.036	0.030	5.147
IIHR CA-2	3.057	1.291	0.021	0.019	4.389
IIHR CA-4	2.517	1.052	0.022	0.021	3.611
IIHR CA-5	3.122	1.194	0.022	0.015	4.353
IIHR CA-6	3.289	1.761	0.039	0.043	5.134
IIHR CA-7	3.809	1.630	0.016	0.011	5.466
IIHR CA-8	2.933	1.621	0.019	0.023	4.597
IIHR CA-9	2.351	1.274	0.033	0.036	3.695
IIHR CA-10	2.698	1.362	0.022	0.023	4.106
IIHR CA-11	2.593	1.187	0.016	0.015	3.811
IIHR CA-12	3.552	0.401	0.063	0.012	4.028
IIHR CA-13	3.122	1.795	0.014	0.014	4.944
IIHR CA-14	3.489	1.708	0.020	0.018	5.236
IIHR CA-15	3.329	1.592	0.022	0.020	4.963
Vallabh Medha*	3.021	1.391	0.033	0.032	4.482
S.Em. ±	0.190	0.117	0.008	0.005	0.290
CD at 5%	0.553	0.342	0.022	0.015	0.845

*Check variety- Vallabh Medha

The total terpenoids content among the germplasm varied from 3.611 (IIHR CA-4) to 5.466 % (IIHR CA-7) whereas check variety recorded 4.482 %. Seven germplasm were found to contain higher total terpenoids content compared to check variety.

4.2.3. Terpenoids content of leaf (% w/w) among the fifteen germplasm of *Centella asiatica* L. at 150 days after planting (DAP)

Madecassoside content in leaf varied from 3.379 (IIHR CA-6) to 6.839 % (IIHR CA-14) whereas check variety Vallabh Medha recorded 3.527 %.

Out of fifteen germplasm thirteen germplasm recorded higher content over check variety (Table 18). Asiaticoside content varied from 0.801 (IIHR CA-12) to 3.670 % (IIHR CA-13) whereas check recorded 1.449 % and thirteen germplasm recorded higher content compared to check variety.

Madecassic acid content varied from 0.018 (IIHR CA-13) to 0.070 % (IIHR CA-12) whereas check variety recorded 0.028 %. Eight germplasm showed higher content over the check variety.

Asiatic acid content varied from 0.021 (IIHR CA-2) to 0.063 % (IIHR CA-4) whereas check variety recorded 0.029 %. Ten germplasm showed higher content over check variety.

4.2.4. Comparision of total terpenoids content (% w/w) among the fifteen germplasm of *Centella asiatica* L. at different days after planting (DAP)

The total terpenoids content of all the germplasm exhibited more at 150 days after planting than 120 and 90 days after planting. Highest content was found in IIHR CA-14 (10.435%) followed by IIHR CA-13 (10.075%), IIHR CA-7 (9.199%), IIHR CA-15 (8.942%) and IIHR CA-8 (8.480%) whereas check variety recorded lowest content 5.033 % and all fourteen germplasm were found with higher content compared to check variety (Table 19; Fig. 9a-9e).

Table 18: Terpenoids content (%w/w) among the fifteen germplasm of *centella asiatica* L. leaf at 150 days after planting (DAP)

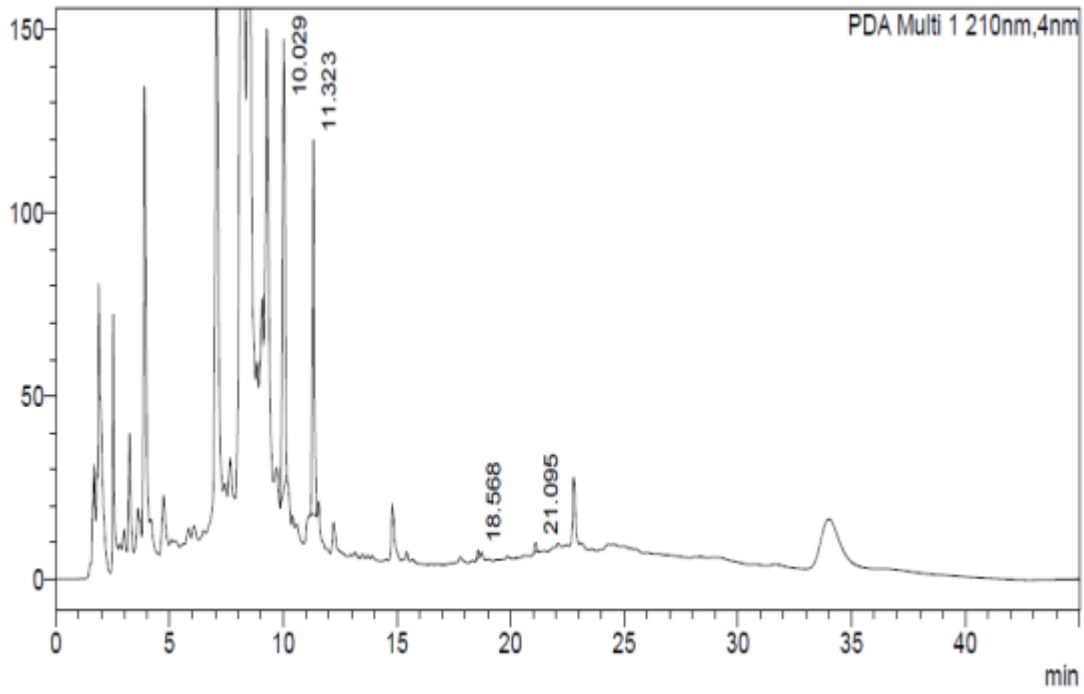
Germplasm	Terpenoids (%)				
	Madeca-ssoside	Asiaticoside	Madecassic Acid	Asiatic acid	Total
IIHR CA-1	5.357	2.006	0.022	0.024	7.411
IIHR CA-2	4.027	1.756	0.022	0.021	5.825
IIHR CA-4	4.354	1.738	0.055	0.063	6.210
IIHR CA-5	4.861	1.882	0.046	0.045	6.835
IIHR CA-6	3.379	1.934	0.023	0.033	5.368
IIHR CA-7	6.417	2.610	0.026	0.025	9.199
IIHR CA-8	5.403	3.020	0.024	0.034	8.480
IIHR CA-9	4.668	2.347	0.033	0.050	7.096
IIHR CA-10	4.647	2.268	0.031	0.045	6.991
IIHR CA-11	4.995	2.528	0.028	0.031	7.582
IIHR CA-12	4.988	0.801	0.070	0.030	5.889
IIHR CA-13	6.359	3.670	0.018	0.028	10.075
IIHR CA-14	6.839	3.518	0.040	0.048	10.435
IIHR CA-15	5.964	2.911	0.032	0.036	8.942
Vallabh Medha*	3.527	1.449	0.028	0.029	5.033
S.Em. ±	0.056	0.058	0.002	0.001	0.092
CD at 5%	0.164	0.169	0.005	0.004	0.268

*Check variety- Vallabh Medha

Table 19: Total terpenoids content (% w/w) among the fifteen germplasm of *Centella asiatica* L. at different days after planting (DAP)

Germplasm	Total terpenoids (%)		
	90 DAP	120 DAP	150 DAP
IIHR CA-1	3.904	5.147	7.411
IIHR CA-2	3.844	4.389	5.825
IIHR CA-4	2.002	3.611	6.210
IIHR CA-5	2.479	4.353	6.835
IIHR CA-6	2.834	5.134	5.368
IIHR CA-7	2.520	5.466	9.199
IIHR CA-8	2.423	4.597	8.480
IIHR CA-9	2.461	3.695	7.096
IIHR CA-10	2.344	4.106	6.991
IIHR CA-11	2.251	3.811	7.582
IIHR CA-12	2.214	4.028	5.889
IIHR CA-13	2.907	4.944	10.075
IIHR CA-14	2.342	5.236	10.435
IIHR CA-15	2.194	4.963	8.942
Vallabh Medha*	3.773	4.482	5.033
S.Em. ±	0.161	0.290	0.092
CD at 5%	0.469	0.845	0.268

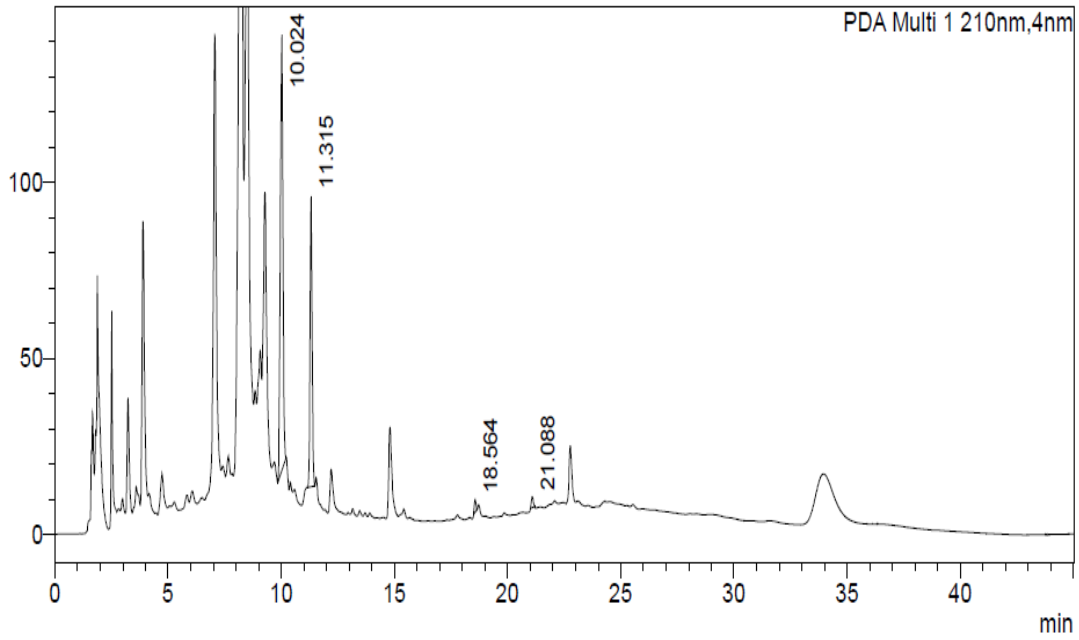
*Check variety- Vallabh Medha



PDA Ch1 210nm

Peak	Ret. Time	Name
1	10.029	Madecassoside
2	11.323	Asiaticoside
3	18.568	Madecassic acid
4	21.095	Asiatic acid

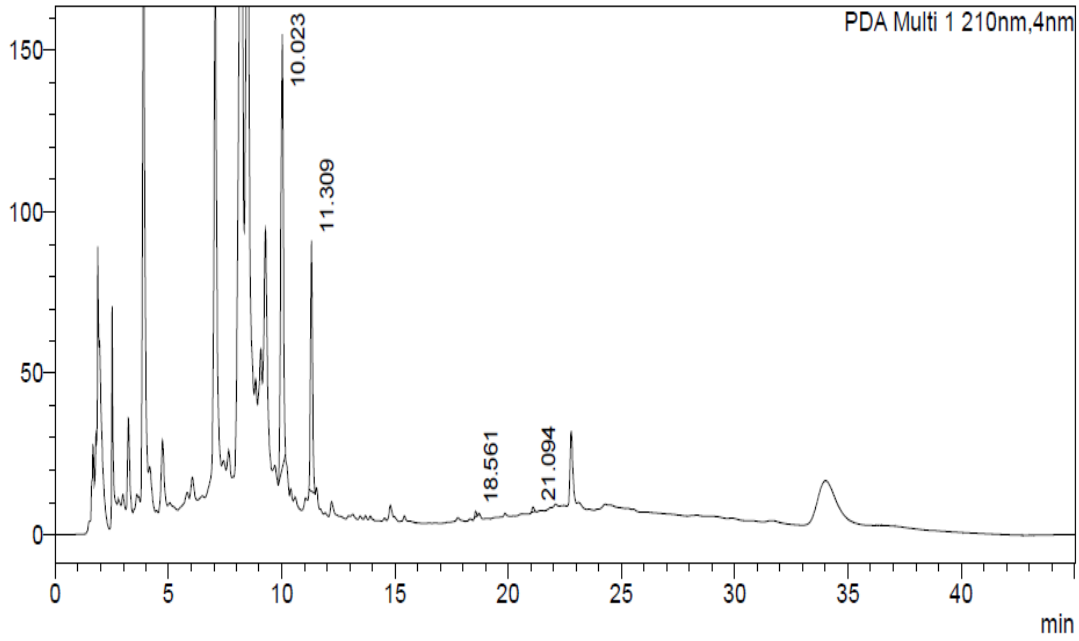
Fig. 9a: HPLC Chromatogram of leaf terpenoids in IIHR CA-13



PDA Ch1 210nm

Peak	Ret. Time	Name
1	10.024	Madecassoside
2	11.315	Asiaticoside
3	18.564	Madecassic acid
4	21.088	Asiatic acid

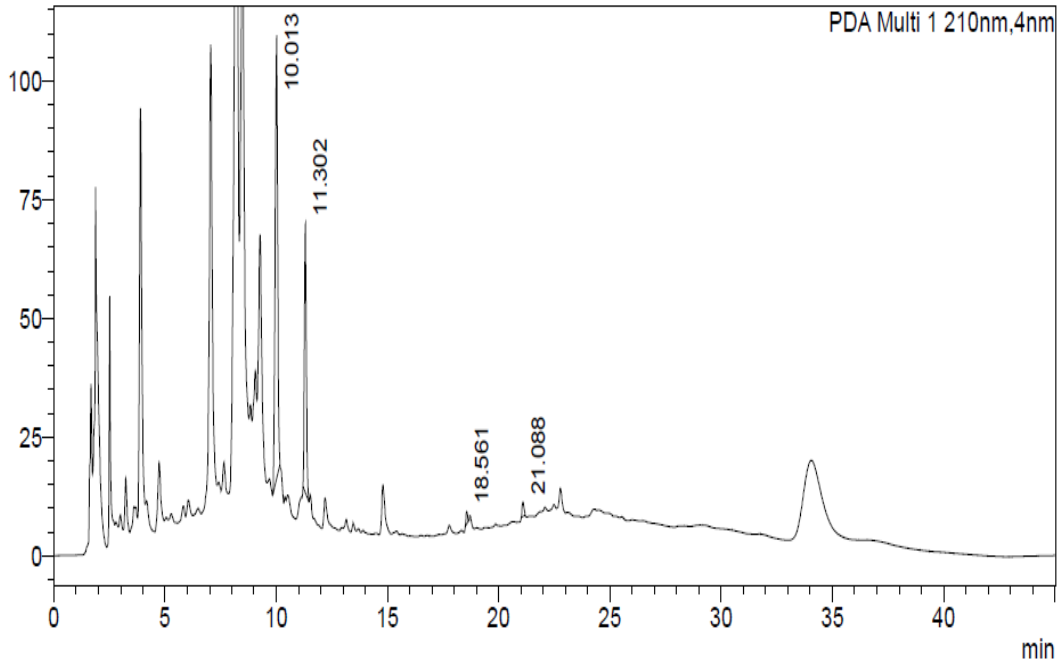
Fig. 9b: HPLC Chromatogram of leaf terpenoids in IIHR CA-14



PDA Ch1 210nm

Peak	Ret. Time	Name
1	10.023	Madecassoside
2	11.309	Asiaticoside
3	18.561	Madecassic acid
4	21.094	Asiatic acid

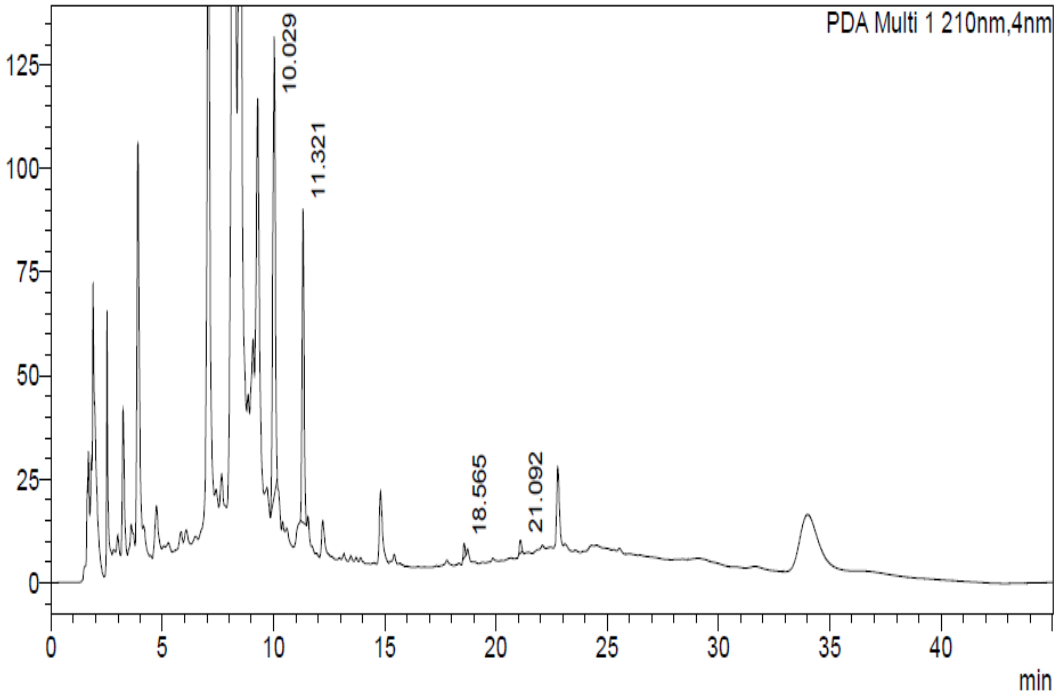
Fig. 9c: HPLC Chromatogram of leaf terpenoids in IIHR CA-7



PDA Ch1 210nm

Peak	Ret. Time	Name
1	10.013	Madecassoside
2	11.302	Asiaticoside
3	18.561	Madecassic acid
4	21.088	Asiatic acid

Fig. 9d: HPLC Chromatogram of leaf terpenoids in IIHR CA-8



PDA Ch1 210nm

Peak	Ret. Time	Name
1	10.029	Madecassoside
2	11.321	Asiaticoside
3	18.565	Madecassic acid
4	21.092	Asiatic acid

Fig. 9e: HPLC Chromatogram of leaf terpenoids in IIHR CA-15

When compared to 120 DAP and 150 DAP terpenoids content increased two folds in case of Madecassoside and Asiaticoside. These two terpenoids only contributing for higher content over time but other two terpenoids *viz*., Madecassic acid and Asiatic acid did not contribute for the significant difference.

4.2.4. Total terpenoids yield (kgha^{-1})

The results for total terpenoid yield analyzed at 90, 120 and 150 days after planting in fifteen germplasm revealed significance among them (Table 20).

At 90 days after planting among the germplasm IIHR CA-1 recorded high total terpenoids content of 17.043 kg ha^{-1} when compared to check variety Vallabh Medha (10.828 kg ha^{-1}). Least content of 1.599 kg ha^{-1} was recorded in IIHR CA-9. Two germplasm were found to have higher content compared to check variety.

At 120 days after planting, among the germplasm IIHR CA-7 recorded high total terpenoids content of 24.760 kg ha^{-1} when compared to check variety Vallabh Medha (13.759 kg ha^{-1}). Least content of 2.956 kg ha^{-1} was recorded in IIHR CA-9. Four germplasm were found to have higher content compared to check variety.

At 150 days after planting, among the germplasm IIHR CA-7 recorded high total terpenoids content of 42.591 kg ha^{-1} when compared to check variety Vallabh Medha (16.155 kg ha^{-1}). Least content of 18.020 kg ha^{-1} was recorded in IIHR CA-12. All fourteen germplasm were found to have higher content compared to check variety.

The total terpenoids content of all the germplasm exhibited more at 150 days after planting than 120 and 90 days after planting. Highest content was found in IIHR CA-7 (42.591 kg ha^{-1}) followed by IIHR CA-13 (37.590 kg ha^{-1}), IIHR CA-1 (34.238 kg ha^{-1}), IIHR CA-14 (31.931 kg ha^{-1}) and IIHR CA-5 (24.742 kg ha^{-1}) (Table 20).

Two folds increase of total terpenoids content over the period of time 90, 120 and 150 days after planting was observed in most of the germplasm.

Table 20: Total terpenoids content (kg ha^{-1}) among the fifteen germplasm of *Centella asiatica* L. at different days after planting (DAP)

Germplasm	Total terpenoids (kg ha^{-1})		
	90 DAP	120 DAP	150 DAP
IIHR CA-1	17.043	23.264	34.238
IIHR CA-2	10.494	12.376	17.300
IIHR CA-4	3.623	8.955	20.120
IIHR CA-5	4.784	9.968	24.742
IIHR CA-6	5.327	9.805	16.479
IIHR CA-7	11.088	24.760	42.591
IIHR CA-8	2.665	8.688	17.892
IIHR CA-9	1.599	2.956	14.830
IIHR CA-10	2.297	4.557	22.790
IIHR CA-11	2.791	8.955	21.836
IIHR CA-12	3.697	8.781	18.020
IIHR CA-13	8.372	16.463	37.590
IIHR CA-14	6.065	15.760	31.931
IIHR CA-15	3.795	10.769	20.030
Vallabh Medha*	10.828	13.759	16.155
S.Em. ±	0.65	0.82	1.32
CD at 5%	2.55	3.21	4.25

*Check variety- Vallabh Medha

4.3. Genetic diversity assessment using SSR molecular marker of *Centella asiatica* L. germplasm

4.3.1 Extraction of genomic DNA

Total genomic DNA was isolated from all fifteen germplasm. The extraction was done using CTAB method described by Doyle and Doyle (1990). RNase treatment was also given to degrade RNA in isolated DNA for further PCR amplification work. The quality and quantity of DNA was tested by agarose gel electrophoresis and UV- spectrophotometer respectively. Spectrophotometer ensures the use of good quality DNA for SSR primer analysis.

4.3.2 DNA quality testing

To assess the DNA quality test, gel electrophoresis was done with 1 µl of stock DNA sample on agarose gel (0.8 %) stained with ethidium bromide and bands appeared in the gel were documented by Alpha Imager 1200 (*Alpha Innotech Inc., USA*).

4.3.3 DNA quantification

Quantification of DNA by spectrophotometer base on the absorbance at 260 nm and 280nm. The A260/A280 ratio of DNA samples ranged from 1.5 to 2.0 µg/ml indicating sufficient concentration of isolated DNA for further PCR amplification work.

4.3.4 PCR amplification

All the twenty SSR primers were screened and used for PCR amplification. The PCR parameters were optimized *viz.,* 30 ng/µl concentration of genomic DNA, 1U Taq DNA polymerase (Bangalore Genei Pvt. Ltd. India) 0.8 pM Reverse and 0.2 pM Forward, $MgCl_2$ at 1.5 mM, dNTPs at 0.15 mM. The annealing temperature of each primer was standardized, by observing high stringency for generating clear and reproducible bands. The amplified products were separated on 1.5 % agarose gel having 6 µl/100 ml of ethidium bromide. The gels were documented under UV light source in a gel documentation unit Alpha Imager 1200 (*Alpha Innotech Inc., USA*) which shows good resolution to score the bands (Plate 7a – 7t).

4.3.5 SSR profile analysis

SSR analysis of fifteen germplasm of *Centella asiatica* was carried out using twenty tested SSR primers developed for *Centella asiatica* (Rakotondralambo *et al*., 2012). Out of twenty primers screened eighteen primers gave maximum number of total bands which showed 100 % polymorphism and two primers gave monomorphism. They were polymorphic and generated a total of 67 bands, out of which 65 were polymorphic (97.40 % polymorphism) across the germplasm The number of bands generated by each primer varied from 1 (mCaCIR013, mCaCIR021) to 6 (mCaCIR028) with an average of 3.45 bands per primer.

4.3.6 Comparison of different efficiency parameters of SSR primers

SSR primers exhibited polymorphism in germplasm of *Centella asiatica* (Table 21). The genetic analysis was carried out by using Power Marker software 3.25 (Liu and Muse, 2005). Expected heterozygosity (*H*e), 0bserved heterozygosity (*H*o) and Polymorphic information content (PIC) were calculated according to the formula of Nei (1973).

a) Gene diversity or Expected Heterozygosity (H *Exp*):

Gene diversity is often referred to as expected heterozygosity and is defined as the probability that two randomly chosen alleles from a population are different. It is considered as an indication of genetic diversity among the population studied. The values of gene diversity ranged from 0.24 to 0.44 among fifteen germplasm studied. The mean gene diversity from all the markers was 0.30 (Table 21). The markers revealed an average of 3.35 bands per marker at a given SSR locus and it ranged between 1 and 6. Major allele frequency was quite high with a mean of 0.68 ranging between 0.67 and 0.85.

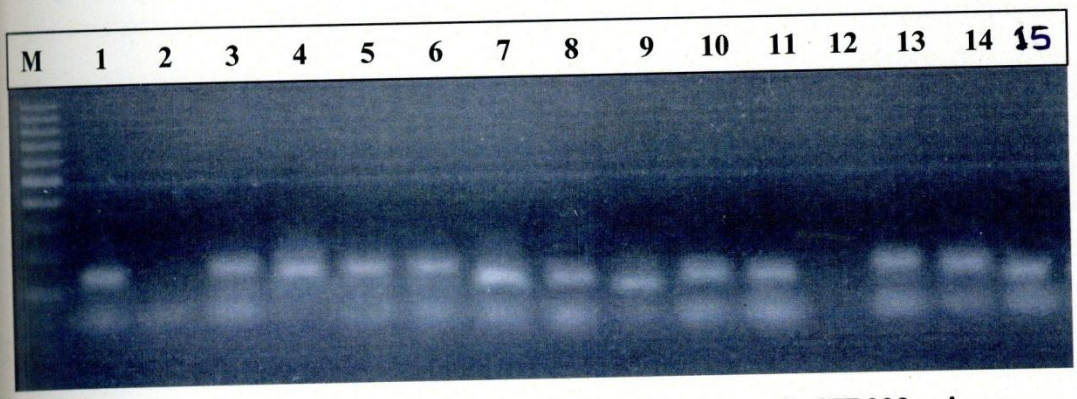


Plate. 5a: SSR profile of *Centella asiatica* germplasm using mCaCIR002 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

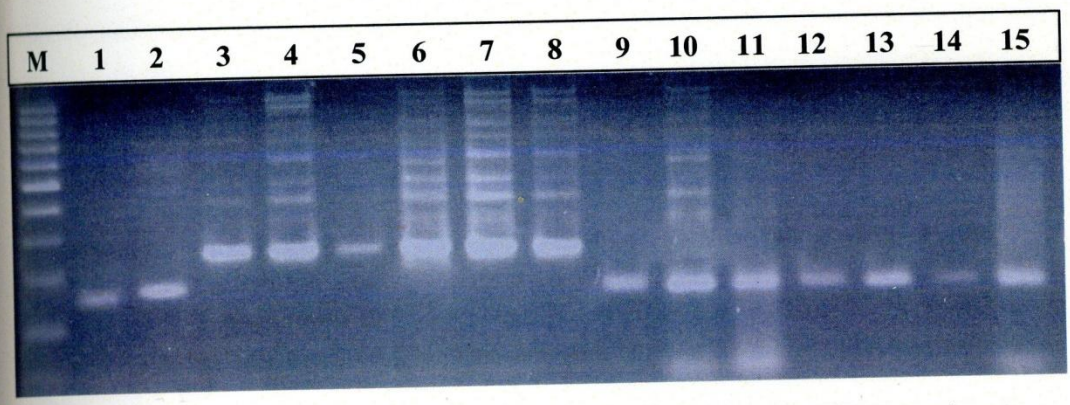


Plate. 5b: SSR profile of *Centella asiatica* germplasm using mCaCIR004 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

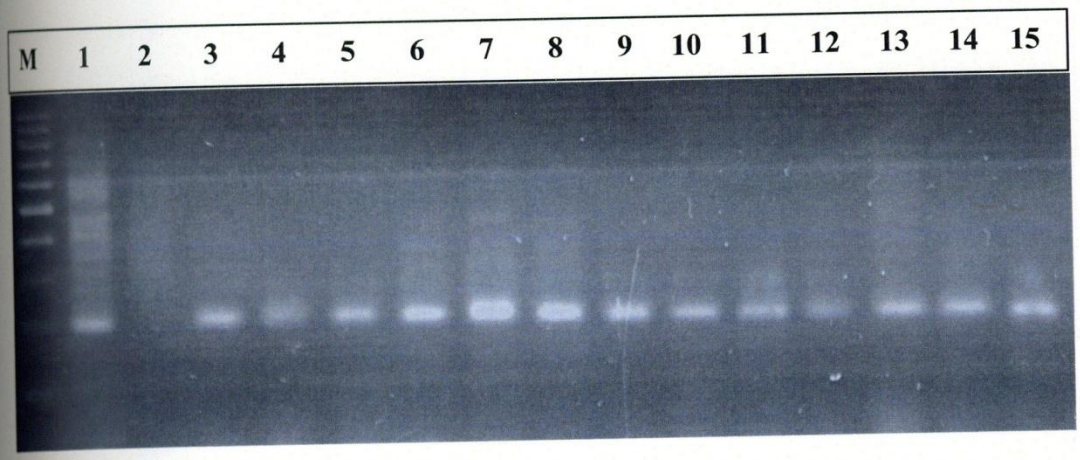


Plate. 5c: SSR profile of *Centella asiatica* germplasm using mCaCIR005 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

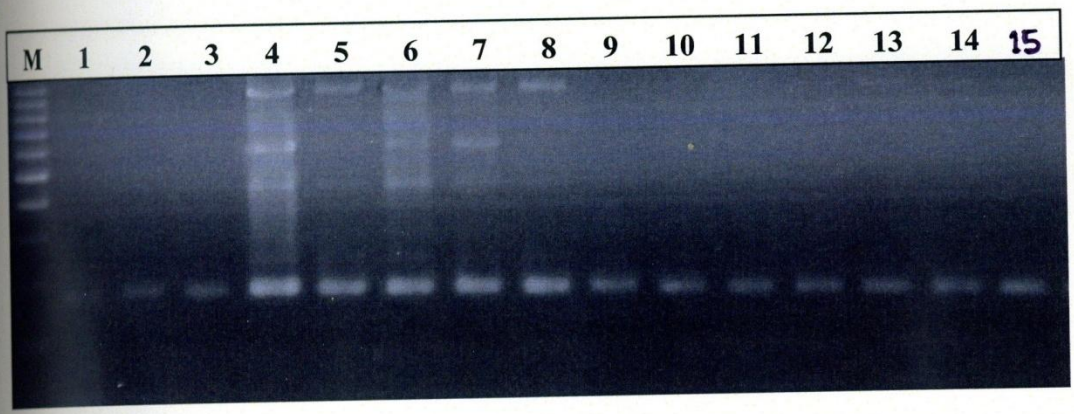


Plate. 5d: SSR profile of *Centella asiatica* germplasm using mCaCIR006 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

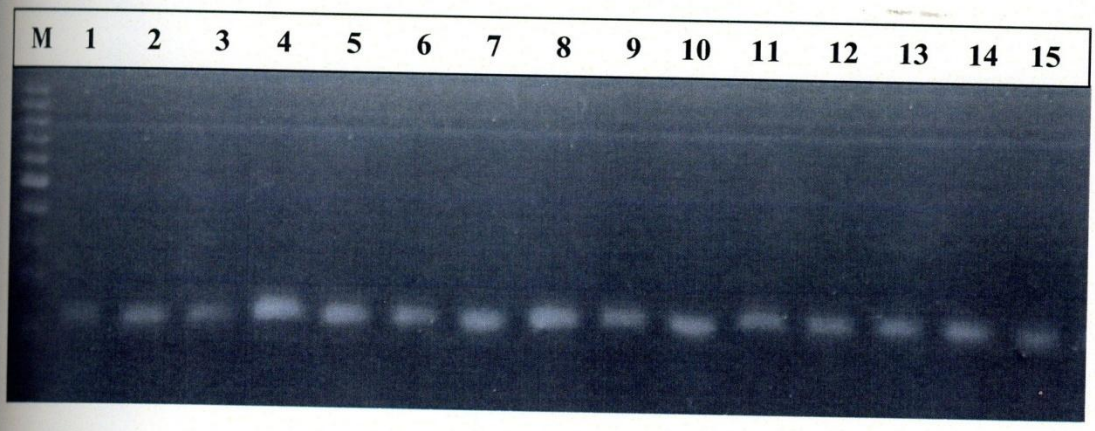


Plate. 5e: SSR profile of *Centella asiatica* germplasm using mCaCIR007 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

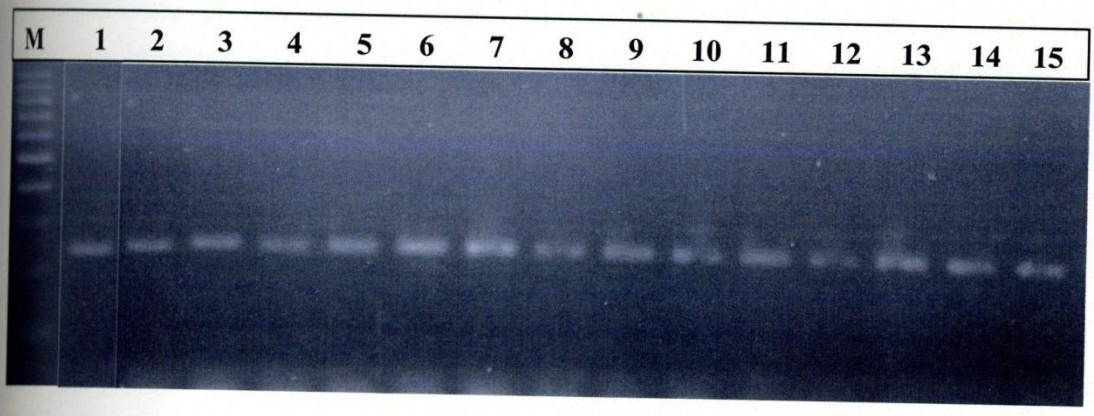


Plate. 5f: SSR profile of *Centella asiatica* germplasm using mCaCIR009 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

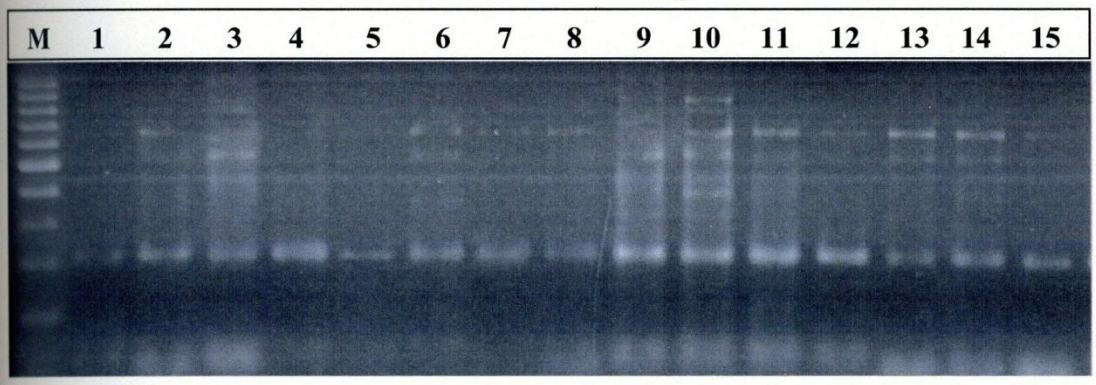


Plate. 5g: SSR profile of *Centella asiatica* germplasm using mCaCIR010 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

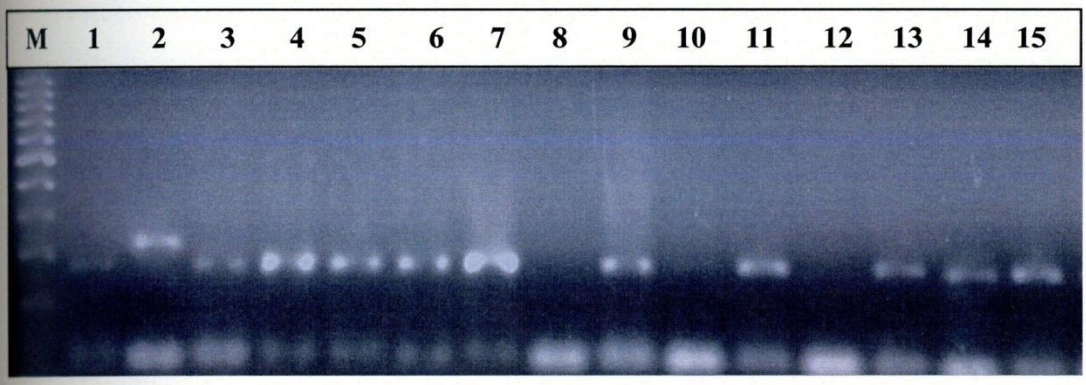


Plate. 5h: SSR profile of *Centella asiatica* germplasm using mCaCIR011 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

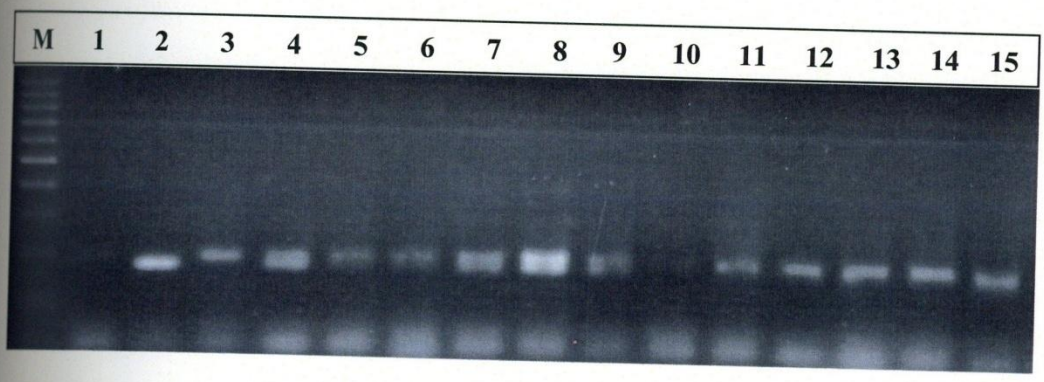


Plate. 5i: SSR profile of *Centella asiatica* germplasm using mCaCIR012 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

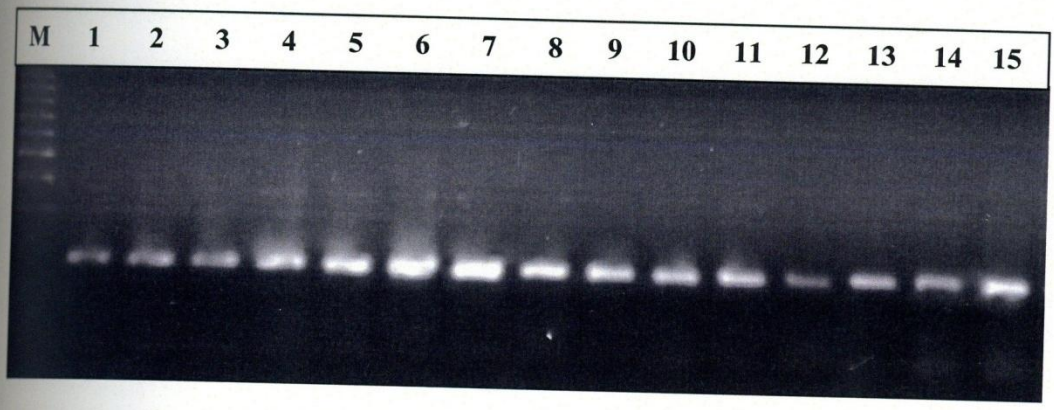


Plate. 5j: SSR profile of *Centella asiatica* germplasm using mCaCIR013 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

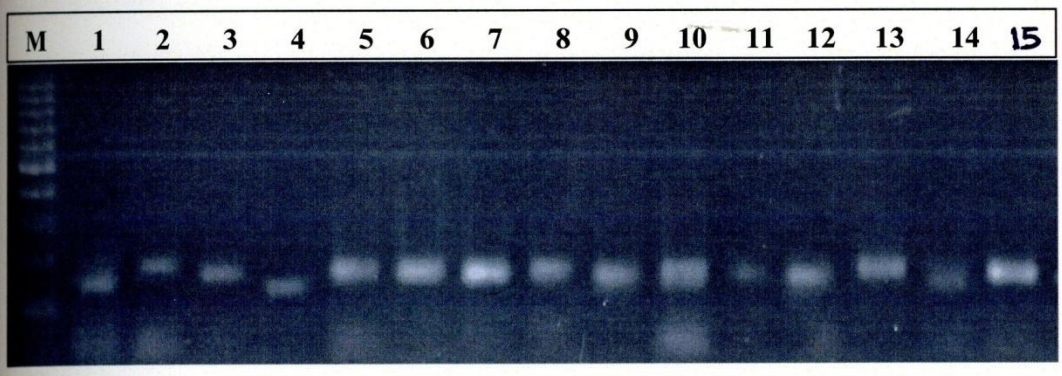


Plate. 5k: SSR profile of *Centella asiatica* germplasm using mCaCIR018 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

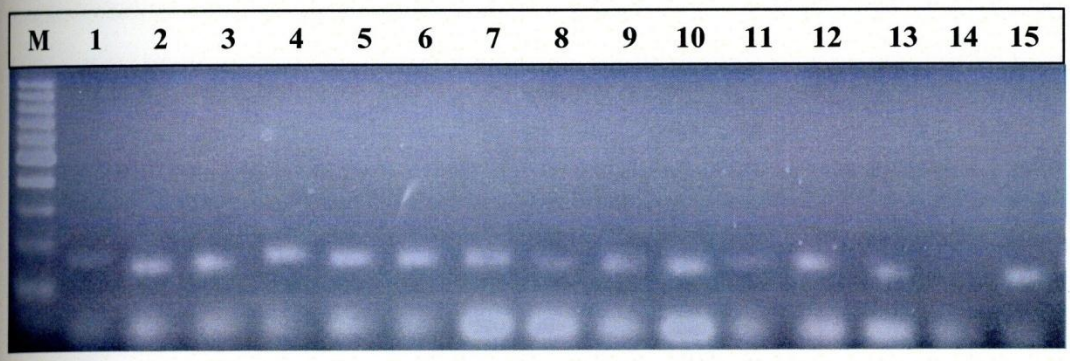


Plate. 5l: SSR profile of *Centella asiatica* germplasm using mCaCIR019 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

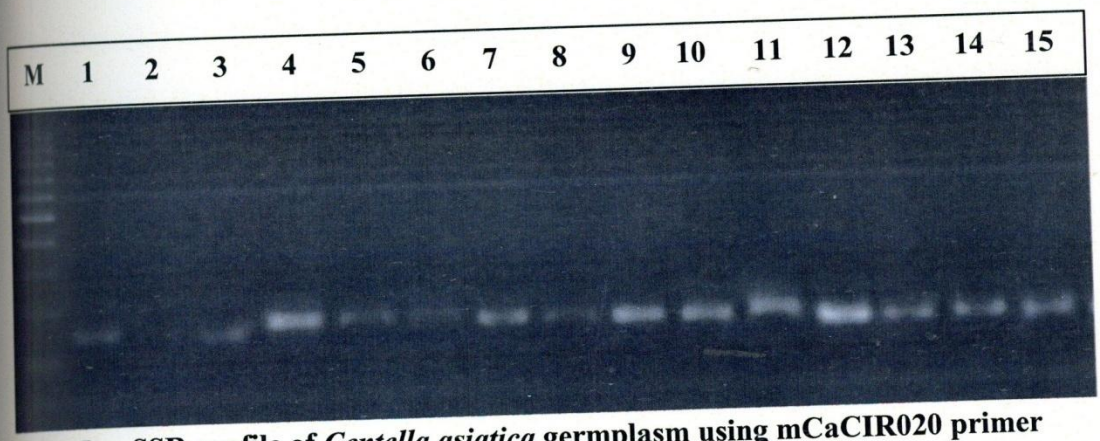


Plate. 5m: SSR profile of *Centella asiatica* germplasm using mCaCIR020 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

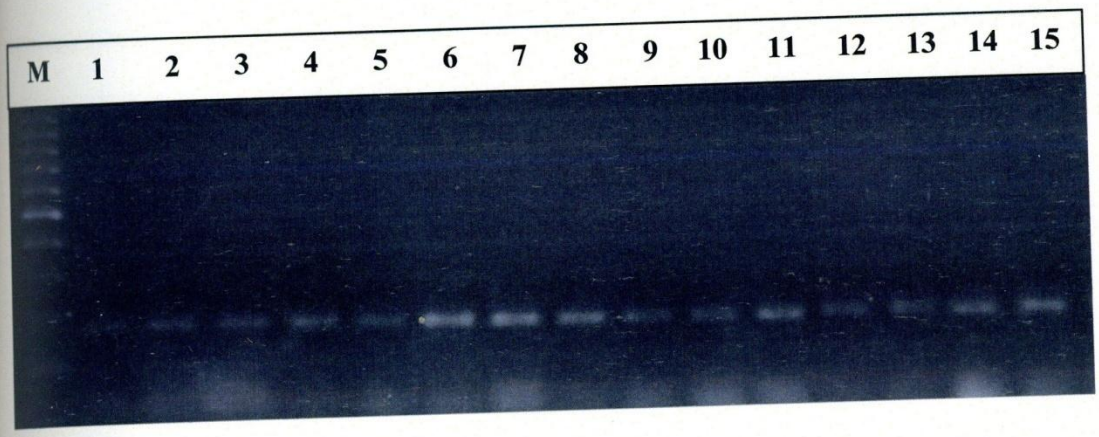


Plate. 5n: SSR profile of *Centella asiatica* germplasm using mCaCIR021 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

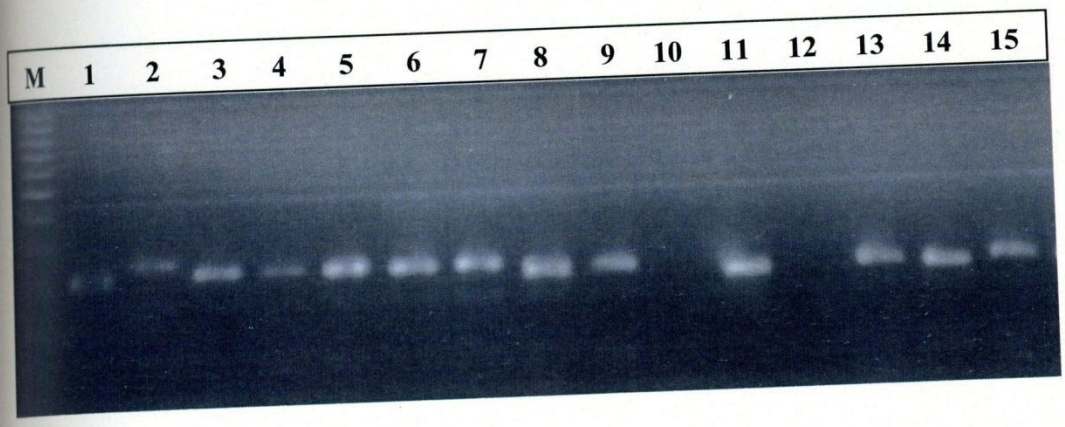


Plate. 5o: SSR profile of *Centella asiatica* germplasm using mCaCIR022 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

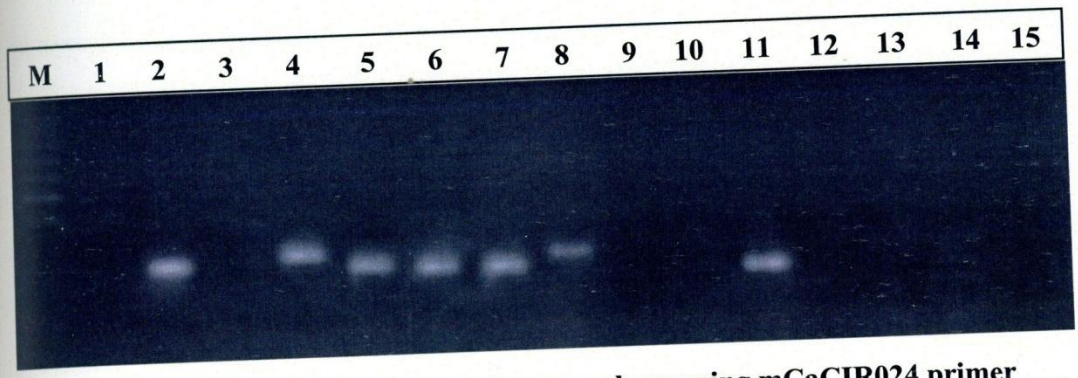


Plate. 5p: SSR profile of *Centella asiatica* germplasm using mCaCIR024 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

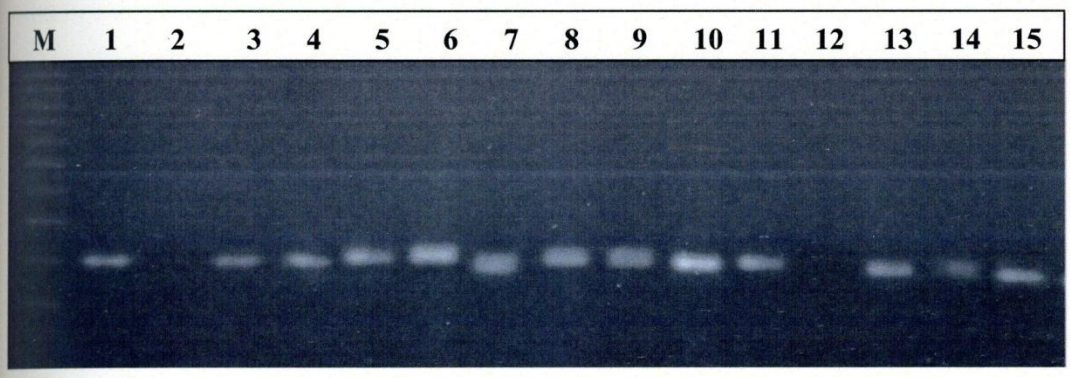


Plate. 5q: SSR profile of *Centella asiatica* germplasm using mCaCIR027 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

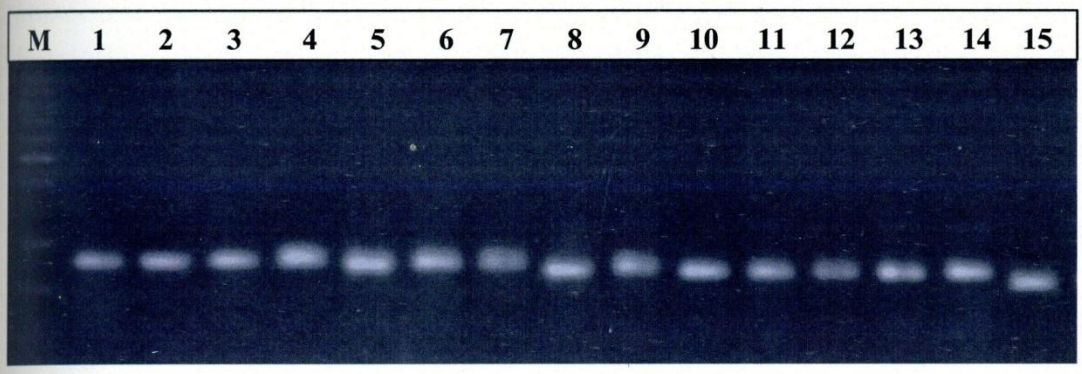


Plate. 5r: SSR profile of *Centella asiatica* germplasm using mCaCIR028 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

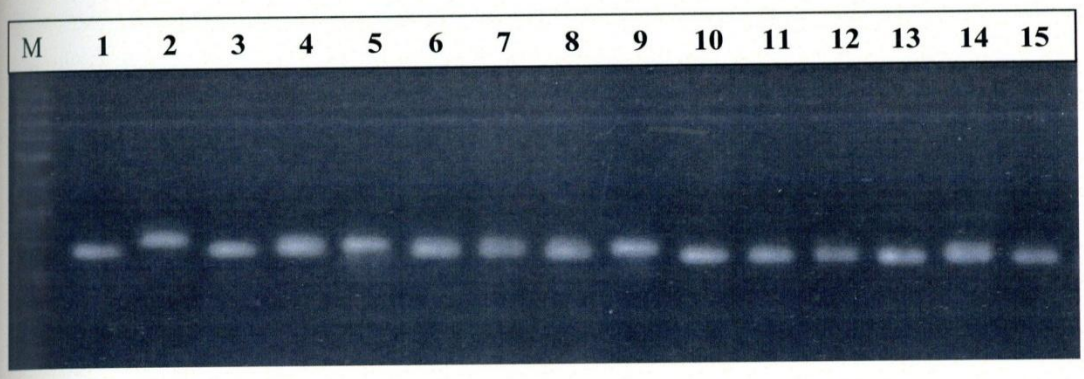


Plate. 5s: SSR profile of *Centella asiatica* germplasm using mCaCIR029 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

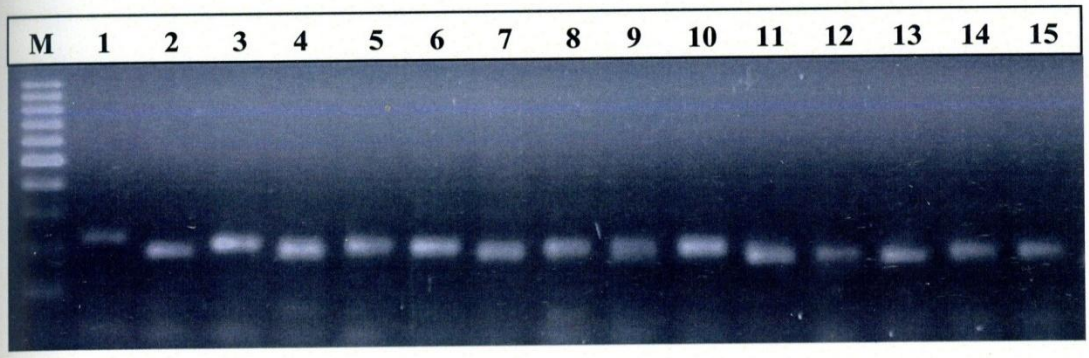


Plate. 5t: SSR profile of *Centella asiatica* germplasm using mCaCIR030 primer

M - 100bp ladder, **1** - IIHR CA-1, **2** - IIHR CA-2, **3** - Vallabh Medha, **4** - IIHR CA-7, **5** - IIHR CA-4, **6** - IIHR CA-5, **7** - IIHR CA-6, **8** - IIHR CA-8, **9** - IIHR CA-9, **10** - IIHR CA-10, **11** - IIHR CA-13, **12** - IIHR CA-11, **13** - IIHR CA-12, **14** - IIHR CA-14, **15** - IIHR CA-15.

Table 21: SSR primers generated band statistics in *Centella asiatica* L germplasm.

Primer Name	Sequence (5 ′ – 3 ′)	Total no. of bands	Polymorphic bands	Major allele frequency	Gene diversity	PIC	Heterozygosity
mCaCIR002	F: CCACAGGTAACACCGAAT R: GCACTTGCACTATCTGGAA	3	3	0.67	0.44	0.34	0.66
mCaCIR004	F: GGGTGGTCTGCCTAAAGA R: TGGAGATCAAGTTTCATGC	3	3	0.69	0.39	0.30	0.58
mCaCIR005	F: GGCCTTCAATGTATGCTG R: TTTGATTTGTTGGGTCTTG	2	2	0.71	0.42	0.28	0.41
mCaCIR006	F: ACGGGCATTTATTCCATT R: GCAAACCACCACAACTTC	3	3	0.71	0.40	0.31	0.60
mCaCIR007	F: TGGAGGTGGTGTAACTGG R: AGGGGATCAAACCTCATC	3	3	0.71	0.38	0.30	0.57
mCaCIR009	F: TGCCTATCCTTTGAATGC R: CAAACATGACATTCTTAAAACA	5	5	0.82	0.27	0.23	0.68
mCaCIR010	F: AATGTAAAATTCCCGGTGT R: TAAACAGGCGTTCCAAGT	3	3	0.67	0.42	0.32	0.62
mCaCIR011	F: TTCATAAAAGTCCTTCCACA R: TAGGTTGATGTGGCCTCT	4	4	0.82	0.26	0.22	0.55
mCaCIR012	F: CACGAAAATTGGAAACAA R: CATGTGAGTTTATGAGTTTCTATG	3	3	0.73	0.32	0.28	0.55
mCaCIR013	F: CAAGTTCCTCCCACGAAT R:GCCGAAATAATCGAAATATAAG	1	-	-	-	-	-
mCaCIR018	F: TTGAGTTTAAGAAGTCCCAAAT R: AATCCTTCACACTCCTAAAGC	4	4	0.74	0.33	0.26	0.65
mCaCIR019	F: TTTCTTGTTAAATGCGATGA R: AATGACATCACTGCTATGGA	4	4	0.76	0.32	0.26	0.65
mCaCIR020	F: TTTAGGAAGTTGGATTTTGC R: GGTTTAATTCAGGACGCTTA	4	4	0.78	0.30	0.25	0.61
mCaCIR021	F: TGCCTAGATTTTGGGTTTT R: TCTTACAATGCAATCAACCT	1	-	-	-	-	-
mCaCIR022	F: AGGAGTATTGACAAGAGGTGA R: GGATGGCAGTCCATTTTA	5	5	0.82	0.28	0.24	0.72
mCaCIR024	F: TCTTTCGTTGATACATGCAC R:AAAACTTAAAGAAGATACAAACTCC	2	2	0.79	0.31	0.25	0.31
mCaCIR027	F: ACCCCAAGACCTTCAGTT R: CCTTCTGCTTTCCCTTTT	5	5	0.85	0.24	0.20	0.59
mCaCIR028	F: CAGAGTTTGGGCAGAAAA R: GACGAGTGGAGGATAAGAAA	6	6	0.83	0.27	0.22	0.80
mCaCIR029	F: GGTCTGAGGTCTGTTGAGG R: CGCATTGACAGAACAAAA	3	3	0.71	0.36	0.28	0.54

mCaCIR030	F: GGCAAATCGAGAGCAATA R: ACGGAAAAGCCTAACAGC	5	5	0.82	0.27	0.22	0.67
Mean		3.45	3.45	0.68	0.30	0.24	0.54

b) Observed heterozygosity (Ho)

Observed heterozygosity (Ho) ranged from 0.31 to 0.80 with a mean of 0.54. The maximum (Ho) value was found for primer mCaCIR028 (0.80), followed by mCaCIR022 (0.72). The (Ho) value was low for the primers mCaCIR024 (0.31) followed by mCaCIR005 (0.41).

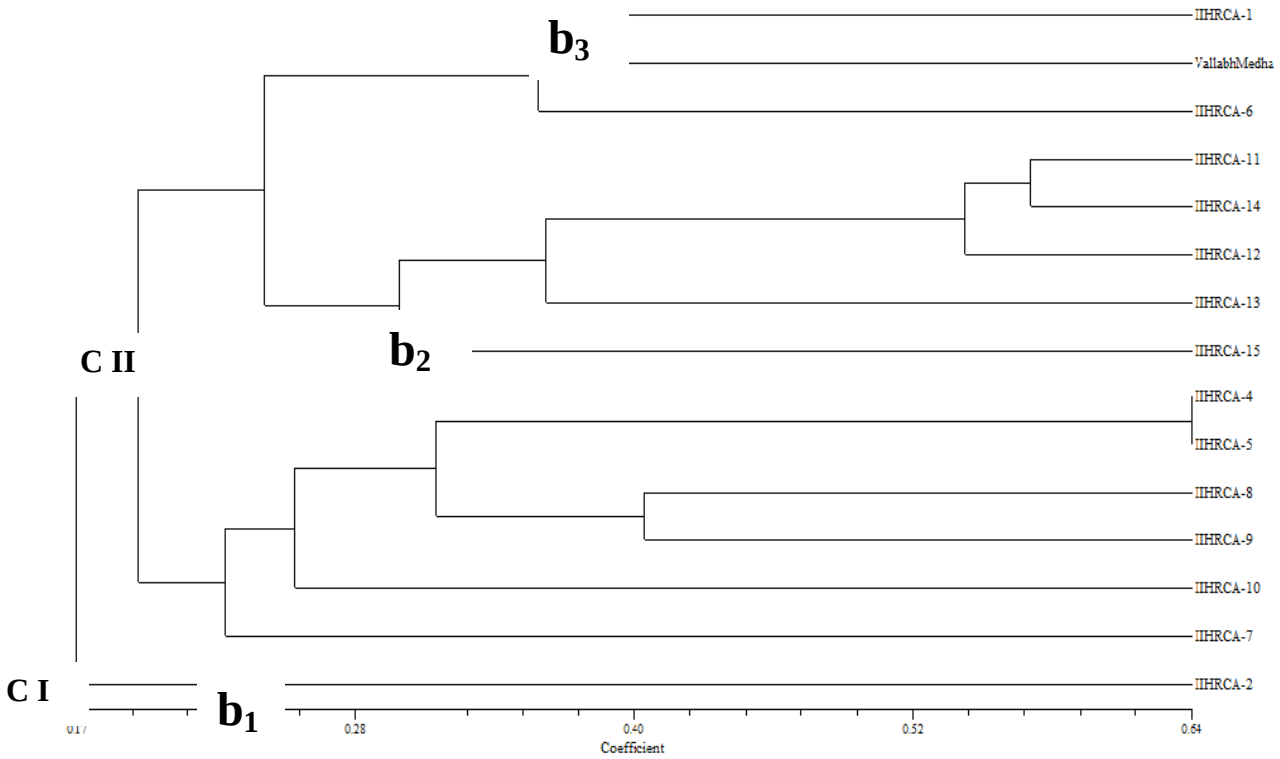
c) Polymorphic information content (PIC)

Polymorphic information content of each locus was analyzed to determine the extent of diversity revealed by these markers. The PIC values ranged from 0.20 to 0.34 with mean of 0.24. The maximum PIC value was found in mCaCIR002 (0.34) and least for primer mCaCIR027 (0.20) among the fifteen germplasm of *Centella asiatica*.

4.3.7 Construction of dendrogram

The binary data were analyzed using Numerical Taxonomy and Multivariate Analysis System, (NTSYS-pc version 2.02) computer software package (Rohlf, 1998) to generate pair wise band similarities for fifteen *Centella asiatica* germplasm. The simple matching coefficient between each pair of germplasm were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) and Sequential agglomerative Hierarchial Nested clustering (SHAN) by using NTSYS pc 2.02 computer software. The SSR amplified bands were scored as 1 and 0 based on presence and absence of the amplified sequence.

The cluster analysis grouped the germplasm into two major Cluster *viz.,* I and II (Fig. 10). The major Cluster I with one germplasm IIHR CA-2 entirely diverged from all other germplasm. The major cluster II has three sub clusters b_1, b_2 and b_3 where b1 sub clusters includes IIHR CA-7, IIHR CA-10, IIHR CA-9, IIHR CA-8 and IIHR CA-5, IIHR CA-4 The b_2 includes IIHR CA-15, IIHR CA-13, IIHR CA-12, IIHR CA-14 and IIHR CA-11. The b_3 sub cluster includes IIHR CA-6, Vallabh Medha and IIHR CA-1. Among these germplasm IIHR CA-5 and IIHR CA-4 showed highest similarity co-efficient under sub cluster b_1 with the value of 0.64 followed by IIHR CA-14 and IIHR



Jaccard's similarity

Fig. 10: UPGMA Dendrogram of *Centella asiatica* L. germplasm based on SSR primers

CA-11 with the similarity co-efficient of 0.57 under sub cluster b_2. The lowest similarity co-efficient of 0.10 was observed between IIHR CA-2 and IIHR CA-14.

The genetic similarity matrix generated by Jaccard's coefficient shown the extent of relatednes s in *Centella asiatica* germplasm and ranged from 10 to 64 % (Table 22). The highest genetic similarity of 64 % was observed between IIHR CA-5 and IIHR CA-4. Lowest genetic similarity of (10 %) was observed between IIHR CA-2 and IIHR CA-14.

Table. 22: Genetic similarity matrix (Jaccard's) of *Centella asiatica* L. germplasm

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.21	1.00													
3	0.39	0.13	1.00												
4	0.14	0.16	0.24	1.00											
5	0.22	0.20	0.33	0.64	1.00										
6	0.34	0.16	0.38	0.17	0.25	1.00									
7	0.11	0.20	0.29	0.37	0.25	0.25	1.00								
8	0.26	0.13	0.21	0.28	0.33	0.33	0.14	1.00							
9	0.23	0.13	0.19	0.39	0.27	0.31	0.19	0.41	1.00						
10	0.17	0.19	0.20	0.19	0.24	0.24	0.20	0.38	0.21	1.00					
11	0.26	0.19	0.21	0.13	0.09	0.30	0.21	0.17	0.22	0.29	1.00				
12	0.27	0.21	0.22	0.14	0.11	0.18	0.15	0.11	0.19	0.25	0.55	1.00			
13	0.34	0.20	0.33	0.14	0.18	0.33	0.18	0.18	0.23	0.29	0.35	0.39	1.00		
14	0.37	0.10	0.23	0.15	0.12	0.23	0.15	0.12	0.16	0.17	0.57	0.54	0.36	1.00	
15	0.12	0.13	0.19	0.18	0.12	0.12	0.23	0.12	0.13	0.17	0.32	0.37	0.19	0.33	1.00

Where,

1-IIHR CA-1 2- IIHR CA-2 3- Vallabh Medha 4- IIHR CA-4 5- IIHR CA-5 6- IIHR CA-6 7- IIHR CA-7 8- IIHR CA-8

9- IIHR CA-9 10- IIHR CA-10 11- IIHR CA-11 12- IIHR CA-12 13- IIHR CA-13 14- IIHR CA-14 15- IIHR CA-15

V. DISCUSSION

Centella asiatica L. is an important medicinal herb used in both traditional Chinese medicine and Indian Ayurvedic medicine since thousands of years. It is indigenous to tropical and sub-tropical regions of the world including parts of India, Pakistan, Sri Lanka, Madagascar, Thailand, Vietnam, Australia, South Africa, South Pacific and Eastern Europe. It is one of the important medicinal plants in the International market of medicinal plant trades. However, large scale collection from wild habitat increases the threat of genetic erosion and does not result in quality of the raw material. Therefore there is need to evolve high yielding cultivars with higher chemical content for commercial cultivation. Variability present in the natural wild populations of *Centella asiatica* offer ample scope for a breeder to select and identify new superior accessions in terms of more yield, quality along with higher chemical content for industries and commercial cultivation.

Selection based on only phenotypic differences will often mislead the breeder as it is influence by environment on expression of traits. Hence, the knowledge of genetic parameters such as genotypic coefficient of variation (GCV), heritability (h^2), genetic advance as per cent of mean (GAM) is vital to judge the best genotype. The best genotype should possess high heritability of desired characters coupled with high genetic advance. For 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.

It is essential to study the extent of genetic diversity in order to maintain, evaluate and utilize germplasm fruitfully. Morphological characterization is considered to be an important first step in description and classification of germplasm since the breeding programme in any crop is mainly based on the magnitude of genetic variability (Smith and Smith, 1989). Only morphological variability is unable to sufficiently categorize the available genotypes for breeders therefore, there is necessary to involve molecular characterization by using DNA marker for effective crop improvement program.

In the present study, “Characterization and evaluation of Mandukaparni (*Centella asiatica* L.) germplasm” the following results are discussed below with reference to earlier findings.

5.1. Evaluation for growth and yield parameters of Mandukaparni (*Centella asiatica* L.) germplasm based on performance

In any selection breeding programme, the mean performance of collected germplasms for different characters provide important pattern for removing the undesirable types. The results of present study revealed that significant difference for growth and yield parameters among fifteen Mandukaparni germplasm.

In the present study, maximum shoot length was found in the accession IIHR CA-1 (10.92 and 16.42 cm) at 90 and 120 DAP followed by IIHR CA-7 (Fig. 2). The increased shoot length may be due to the plant growth habit, where this germplasm is growing erect and coupled with genetic makeup of the germplasm.

The highest number of primary branches was recorded for IIHR CA-2 (7.27 and 18.78) at 90 and 120 DAP, respectively followed by IIHR CA-15 (Fig. 3). The plants that reveal higher number of primary branches might be due to the accessions had originated from different geographical locations with different genetic makeup (Bhan *et al*. 2005). The lowest number of primary branches was recorded in IIHR CA-1 (4.08, 5.00 and 11.66 at 60, 90 and 120 DAP respectively).

The number of nodes was recorded highest in IIHR CA-11 (5.85), IIHR CA-12 (9.10) and IIHR CA-7 (18.00) at 60, 90 and 120 DAP respectively (Fig. 4). The increase in number of nodes in different germplasm might be due to the different growth habit of stem.

The number of leaves was recorded highest in IIHR CA-9 (4.28), IIHR CA-11 (20.13), IIHR CA-12 (30.00) and IIHR CA-7 (81.33) at 30, 60, 90 and 120 DAP respectively (Fig. 5). These might be the result of population from diverse phytogeographical regions varying in climate, habit and morphological traits as similar results were found in *Ocimum tenuiflorum* (Pavan *et al*., 2015).

Among the germplasm the maximum leaf length was recorded in IIHR CA-1 (2.89, 3.48, 3.79 and 4.09 cm at 30, 60, 90 and 120 DAP followed by IIHR CA-7. However lowest leaf length were recorded in IIHR CA-12 (1.44, 1.69, 1.85 and 1.92 cm at 30, 60, 90 and 120 DAP) (Fig. 6). The differences in leaf length among germplasm might be due to growth habit of the germplasm as they are collected from different geographical origins with different environmental conditions.

The maximum leaf width was recorded in IIHR CA-1 (4.29, 5.04, 5.54 and 7.24 cm at 30, 60, 90 and 120 DAP, whereas lowest leaf width was found in IIHR CA-10 (1.85, 2.22, 2.64 and 2.80 cm at 30, 60, 90 and 120 DAP respectively (Fig. 7). The differences of maximum and minimum of leaf width among germplasm might be due to different geographical origins with different environmental conditions. Similar variation was reported in description of variation in the Indian accessions of the medicinal plant *Centella asiatica* (Mathur *et al*., 2003).

The maximum fresh leaf weight was observed in IIHR CA-7 *i.e* 1531.95, 1557.78 and 1568.13 gm/plot (21.27, 21.64 and 21.78 q ha^{-1}) at 90, 120 and 150 days respectively after planting and followed by IIHR CA-1 and IIHR CA-13 (Fig. 8). The yield variation might be due to germplasm growth habit as from different geographical origins with different seasons. The result were in conformance with studies of Rahajanirina *et al.* (2016), Prasad *et al*. (2014) and Mathur *et al.* (2003).

The dry leaf weight was found highest in the germplasm IIHR CA-7 *i.e*, 316.77, 324.56 and 333.20 gm/plot (4.40, 4.53 and 4.63 q ha^{-1}) at 90, 120 and 150 DAP, respectively followed by IIHR CA-1 and IIHR CA-13. Whereas, lowest value was found in IIHR CA-9 (Fig. 9). The results are positively correlated with fresh leaf weight as more fresh leaf weight corresponds to higher dry leaf weight.

The highest fresh and dry leaf weight was recorded due to increase in overall performance such as shoot length, primary branches, number of nodes, number of leaves and leaf length ultimately increased to the fresh and dry leaf weight. The promising germplasm *viz*., IIHR CA-7, IIHR CA-1 and IIHR CA-13 was found to have higher leaf weight.

5.2 Variability, heritability and genetic advance for quantitative and yield traits

The knowledge of genetic variation is important for selection in crop improvement programmes and 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

The evaluation of genetic variation parameters, *viz*., genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance as per cent of mean for different characters are given in (Fig. 12).

In the present study, the germplasm revealed significant amount of variability for all the twelve traits studied. Statistically, the genotypic coefficient of variation (GCV) for all characters studied were lesser than phenotypic coefficient of variation (PCV) suggesting that the variability is due to an interaction with the environment up to some extent (Pushpa *et al.,* 2013). The difference between genotypic and phenotypic coefficient of variation was observed to be highest in specific leaf weight as it is strongly influenced by environment. Low GCV and PCV was 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 was recorded high genotypic and phenotypic coefficient of variation. It was indicated that these traits were governed by additive gene effects with low environmental effects. Similar results was obtained for shoot length in *Andrographis paniculata* (Misra *et al*., 2001).

Moderate GCV and PCV was recorded for number of primary branches, number of nodes, number of leaves and internodes length. The similar results were found for primary branches in Sweet basil (*Ocimum basilicum*) (Ibrahim *et al.,* 2013).

The coefficient of variation indicates only an extent of variability present for different characters and do not take into account for the heritable portion. To obtain the knowledge about heritable portion of variability, it is essential to know the heritability estimates for different quantitative characters. The heritability estimates devoid environmental influence from the total variability, indicates the accuracy with which a superior segregants in a population can be selected by its 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 which helps plant breeder in selection of elite germplasm from genetically diverse population. High genetic advance coupled with high heritability estimates offers the most suitable condition for selection. It also indicates the presence of additive genes in the trait and further suggest reliable crop improvement through selection of such traits. Estimates of heritability with genetic advance are more reliable and meaningful than individual consideration of the parameters (Nwangburuka and Denton, 2012). Therefore, Heritability estimates along with high genetic advance is more useful criterion in predicting the resultant effects for selecting the best individual. This is due to the fact that a character may have very high heritability but very less phenotypic variation gives rise to very low genetic gain.

Most of all the characters recorded high heritability along with genetic advance as per cent of mean except specific leaf weight with low heritability. 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)

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 per cent of mean. Similar report was found in stevia for leaf yield per plant (Gaurav *et al.,* 2008). Low heritability was observed for specific leaf weight (24.52 %) 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 environment on the genotypic effects.

In this present study, 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 per cent of mean and these traits are agreeable for the selection and can be effectively used for genetic improvement of breeding programs.

5.3 Association analysis

5.3.1 Correlation studies

In the present investigation, correlation coefficient were estimated between yield and its components at genotypic and phenotypic levels to identify the interrelationship among different traits. It was found that genotypic correlation coefficients were higher than phenotypic correlation in seventeen morphological traits because of the masking effect of germplasm for the expression of these characters.

5.3.1.1. Correlation studies between dry leaf weight and its component traits

The genotypic and phenotypic correlation coefficient were worked out among different characters including dry leaf weight as a dependent variable in correlation analysis. In the present study, dry leaf weight exhibited highly positive significant correlation both at phenotypic correlation coefficients and genotypic levels for all ten traits. The remaining five characters where, number of primary branches, number of nodes and number of leaf showed positive non-significant association with dry leaf weight whereas, Madecassic acid and Asiatic acid showed negative significant association with dry leaf weight.

The positive and highly significant association of traits *viz*., leaf length, leaf width, internode length, rosette diameter, petiole length, specific leaf weight, fresh leaf weight, Madecssoside and Asiaticosside with dry leaf weight. Hence, it may be concluded that these traits may be considered as the most important yield contributing attributes in *Centella asiatica.* These results coincide with the findings in potato (Dayal *et al.*, 1983), fenugreek (Datta *et al*., 2005), and in hyacinth bean (Golani *et al.,* 2007). However, the information about the association with dry leaf weight and its attributes alone is not sufficient. The interrelationship between these component characters themselves may affect the overall

influence of the characters on yield. Hence, it was suggested that selection based on the yield components would be effective in improving yield, provided the components are highly heritable and genotypic correlations among them are not negative (Doku, 1970).

Regarding interrelation of the yield components, most of the traits had highly significant positive correlation with each other. Number of primary branches (NPB) was positively significant with number of nodes (NN), number of leaf (NL), leaf length (LL), specific leaf weight (SLW) and fresh leaf weight (FLW) however NN and NL were non- significant to dry (DLW) but non-significant to leaf width (LW), internode length (IL), rosette diameter (RD), petiole length (PL), Medicassoside (MD) and DLW while, negatively significant to Madeccassic acid (MA) and Asiatic acid (AA). NN were positively significant with NL, PL, SLW and FLW. However, positive non-significant with LL, LW, IL, RD, AT and DLW and negatively non-significant to MD and AA whereas, MA were negatively significant to all the characters. The NL were positively significant with PL, SLW and FLW and positively non-significant with LL, LW, IL and DLW while negative non-significant with RD, MD, AT and AA.

The present study revealed that the traits like shoot length, leaf length, leaf width, internode length, rosette diameter, petiole length, specific leaf weight, fresh leaf weight, Madecssoside and Asiaticosside exhibited high positive association with dry leaf weight at both genotypic and phenotypic level. Hence these traits should be taken into consideration at the time of selection for realizing the highest dry leaf weight. Similar result were reported for leaf length and leaf width in *Mentha arvensis* (Singh *et al*., 2000).

5.3.2 Genetic diversity analysis

To study genetic divergence among the germplasm Mahalanobis D^2 statistics was used which enables to discriminate among different cultivars according to the diversity present (Mahaanobis, 1936). It gives clear idea about the diverse nature of the populations. The clusters formed according to Tocher's method (Rao, 1952) were used to know the distances between and within the cluster. These results could give an insight about diverse nature of the germplasm in a cluster. Cluster means also give the degree of differences between germplasm which belong to different clusters.

Genetic diversity among germplasm plays a major role in genetic improvement of any crop, since it provides the way to determine the most divergent parents based on the contribution of different qualitative and quantitative traits, for further utilization in any hybridization programme (Shukla *et al*, 2009). The results obtained on genetic divergence of *Centella asiatica* are discussed hereunder.

In the present study, D^2 analysis was carried out on fifteen germplasm using sixteen characters showed significant variability for all characters evaluated and were further confirmed through the pattern of distribution of fifteen *Centella asiatica* germplasm into V clusters based on genetic divergence D^2 statistics. Among five cluster, 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 indicating wider divergence among the germplasm.

The estimates of intra- and inter-cluster distances for the five clusters are presented in Table 14. The intra-cluster distance was minimum in cluster II (9.26) and maximum in cluster IV (24.45). The magnitude of inter cluster distance measures the extent of genetic distance between two clusters. The inter cluster distance in most of the cases were higher than the intra cluster distance indicating wider genetic diversity among the germplasm of different groups. The maximum inter-cluster distance was observed between cluster IV and III (40.45) indicating that the germplasm of these two clusters are highly divergence which may be used for initiating hybridization programme for genetic improvement of *Centella asiatica*. While, the minimum inter-cluster distance was found between clusters IV and II (16.41) followed by V and II (18.76). The germplasm belonging to these clusters were relatively closer to each other, in comparison to lines grouped in other clusters (Kumar *et al*., 2008). Therefore, clustering patterns showed that germplasm which include in clusters III (IIHR CA-7, IIHR CA-14, IIHR CA-15) and IV (IIHR CA-8, IIHR CA-9, IIHR CA-11) can be used in hybridization programme to generate wide range of transgressive segregants in population for developing new varieties.

Based on the cluster means (Table 14) the important cluster was III for shoot length, number of primary branches, number of nodes, number of leaf, leaf length, leaf width, internode length, petiole

length, specific leaf weight, fresh leaf weight, dry leaf weight, Madecassoside and Asiaticoside content. Cluster V had highest mean for number of nodes, number of leaf, rosette diameter, petiole length, fresh leaf weight, dry leaf weight, Madeccasoside and Asiaticoside content. Cluster I had highest mean for shoot length, number of primary branches, leaf length, leaf width, internode length, rosette diameter, Madecassic acid content. Cluster II had highest mean for Asiatic acid. Cluster IV had highest mean for Madeccasic acid and Asiatic acid content.

It could be concluded that highest shoot length, number of primary branches, number of nodes, number of leaf, leaf length, leaf width, internode length, petiole length, specific leaf weight, fresh leaf weight and dry leaf weight from cluster III, germplasm for maximum rosette diameter, Madecassoside and Asiaticoside from cluster I and highest Madeccasic acid and Asiatic acid content from cluster II could be suggested and selected as parents for the exploitation of hybridization program for both yield and vegetative traits along with biochemical content of *Centella asiatica.*

5.4 Biochemical variability among fifteen germplasm of Mandukaparni (*Centella asiatica* L.)

The variation in content of terpenoids such as Madecassoside, Asiaticoside, Madecassic acid and Asiatic acid in leaf of fifteen germplasm of Mandukaparni are presented in Table 16, 17 and 18.

Madecassoside content in leaves was found highest in germplasm IIHR CA-1 (2.751 %), IIHR CA-7 (3.809 %) and IIHR CA-14 (6.89 %) at 90, 120 and 150 days after planting, followed by IIHR CA-2 (2.611 %), IIHR CA-1 (3.588 %) and IIHR CA-7 (6.417 %). Whereas, lowest content was found in IIHR CA-11 (1.172%), IIHR CA-9 (2.351 %) and IIHR CA-6 (3.379 %) respectively.

Asiaticoside content was highest in IIHR CA-13 (1.221 %), (1.795 %) and (3.670 %) at 90, 120 and 150 days after planting, followed by IIHR CA-2 (1.165 %), IIHR CA-6 (1.761 %) and IIHR CA-14 (3.518 %). Whereas, lowest content was found in IIHR CA-12 (0.543 %), (0.401 %) and (0.801 %) respectively. In these results, the variations of Madecassoside and Asiaticoside content among the germplasm are mainly due to genetic variations make-up and there is no correlation between Madecassoside and Asiaticoside contents with the altitude of their original collection locations. Similar result was reported by Thomas *et al.,* (2010).

Madecassic acid content was found highest in IIHR CA-10 (0.079 %), IIHR CA-12 (0.063 %) and (0.070 %) at 90, 120 and 150 days after planting followed by IIHR CA-12 (0.078 %), IIHR CA-6 (0.039 %) and IIHR CA-4 (0.055 %). While, the lowest content was found in IIHR CA-7, (0.023 %), IIHR CA-13 (0.014 %) and (0.018 %) respectively.

Asiatic acid content was found highest in IIHR CA-10 (0.112 %), IIHR CA-6 (0.043 %) and IIHR CA-4 (0.063 %) at 90, 120 and 150 days after planting, followed by IIHR CA-11 (0.108 %), IIHR CA-9 (0.036 %) and IIHR CA-9 (0.050 %). While, lowest content was found in IIHR CA-1(0.023 %), IIHR CA-7(0.011 %) and IIHR CA-2 (0.021 %) respectively.

Highest total terpenoids was found in IIHR CA-1 (0.050 %), IIHR CA-7 (5.466 %) and IIHR CA-14 (10.435 %) at 90, 120 and 150 days after planting, followed by IIHR CA-2 (3.844 %), IIHR CA-14 (5.236 %) and IIHR CA-13 (10.075 %). While, lowest content was found in IIHR CA-4 (2.002 %), IIHR CA-4 (3.611 %) and Vallabh Medha (5.033 %) respectively.

The variability of terpenoids content in leaf of fifteen germplasm of *Centella asiatica* were presented in Table 16, 17, 18 and 19. The highest Madecassoside content was found in IIHRCA 14, IIHR CA-7, IIHR CA-1 and IIHR CA-13. Asiaticoside content was found higher in IIHR CA-13, IIHR CA-14, IIHR CA-8 and IIHR CA-15. The result was supported by Thomas *et al.,* (2010) in sixty accessions of *Centella asiatica* of south India and the Andaman Islands. Madecassic acid content was found highest in IIHR CA-12, IIHR CA-10, IIHR CA-5 and Asiatic acid content recorded highest in IIHR CA-10, IIHR CA-9, IIHR CA-11 and IIHR CA-6. Among all fifteen germplasm, the total terpenoids yield was found highest in IIHR CA-7 followed by IIHR CA-13, IIHR CA-1, IIHR CA-14 and IIHR CA-5 (Table 20). Where, most of the germplasm is significantly increased two folds of total terpenoids yield over the period of time at 90, 120 and 150 days after planting. However, the two terpenoids *viz*., Madecassoside and Asiaticoside These two terpenoids only contributing for higher total terpenoid content over time but other two terpenoids *viz.,* Madecassic acid and Asiatic acid did not contribute for the significant difference. Therefore, it showed that the ideal time of higher total terpenoids content was found at 150 days after planting with respect to 90 and 120 days after planting.

In these present biochemical study, showed variability of terpenoids content in leaf of different germplasm might be due to the diverse environmental conditions, age of leaves, harvesting time and genetic makeup of the germplasm. The results were in accordance with James and Dubery, (2009).

5.5 Molecular studies of Mandukaparni (*Centella asiatica* L.) germplasm

The morphological characterization alone may not be sufficient in assessing the genetic diversity of germplasm and may not provide accurate information of the genetic divergence. Since, the variability present in morphological traits are limited and influenced by environment there is a need to use of molecular marker at DNA level to study the genetic diversity. Hence, the present research work was carried out to assess molecular diversity using SSR markers in Mandukaparni. The results obtained from this study are discussed hereunder.

5.6 PCR analysis in Mandukaparni (*Centella asiatica* L.)

The twenty SSR primers were utilized to assess the genetic diversity in fifteen Mandukaparni germplasm. SSR markers for Mandukaparni have been developed for genetic and molecular studies (Rakotondralambo *et al.,* 2012).

5.6.1 SSR profiling

The twenty Simple Sequence Repeats (SSR) primers analysis was carried out in fifteen germplasm of Mandukaparni. All the twenty primers showed the good amplification with the clear reproducible bands and eighteen primers were polymorphic and the remaining two were monomorphic. A total of 67 alleles were produced at the 18 polymorphic loci, with an average of 3.45 alleles per locus. Two SSR primers (CaCIR005, and mCaCIR024) generated two alleles per locus, and the rest produced three or more than three alleles per locus. Similar study in *Centella asiatica* was carried out by Rakotondralambo *et al.,* (2012) revealed that total of 73 alleles were produced at the 17 polymorphic loci, with an average of 4.3 alleles per locus, whereas in the present study, more number of primers with 100 % polymorphism showed that SSR markers is usefully applicable for diversity analysis at DNA level. The present findings showed SSR markers found to be appropriate markers for analyzing diversity. Padmalatha and Prasad (2008) reported 87 % polymorphism using RAPD marker in *Centella asiatica* for diversity analysis at DNA level. The present findings showed higher polymorphism compared to the findings of Sujin and Britto (2012) where they reported 63.65 % polymorphism using RAPD marker. 10 ISSR primers that generated a total of 98 bands of which polymorphic bands were 66.33 % was reported by Zhang *et al*., (2012). In vanilla, Verma *et al.,* (2009) used 10 ISSR primers that generated 108 bands of which 93 were polymorphic (86.11 %). Palaniappan and Marappa (2006) recorded 68.2 and 69.7% polymorphism by RAPD and ISSR primers and 70 % polymorphism using ISSR primer in molecular characterization of *Coleus forskohlii* was reported by Niraj *et al.*, (2013).

5.7 Comparison of different efficiency parameters of SSR primers

The SSR primers are evaluated by using estimates of various parameters (Table 20). Expected Heterozygosity (He) ranged from 0.24 (mCaCIR027) to 0.44 (mCaCIR002) and mean was 0.30. Observed heterozygosity (Ho) ranged from the 0.31 (mCaCIR024) to 0.80 (mCaCIR028) with a mean of 0.54. Similar results was reported by Rakotondralambo *et al*., (2012) in *Centella asiatica* and Zhang *et al*., (2013) on *Astragalus camptodontus* by use of SSR markers in genetic divergence.

The polymorphic information content (PIC) of each locus was analysed to determine the extent of diversity revealed by this markers. The PIC value ranged from 0.20 (mCaCIR027) to 0.34 (mCaCIR002) with mean of 0.24. The study was supported by Kumar *et al*., (2014) on *Justica adhatoda* by using of ISSR markers in genetic divergence where, the results revealed that PIC ranged from 0.17 to 0.36. The primers with higher PIC value are more useful. Hence, mCaCIR002 and mCaCIR010 which showed higher PIC content can be used in further studies for distinguishing *Centella asiatica* germplasm. The PIC from present findings were contrast and noticed to be less as compared to the results of Sahu *et al*., (2015) using EST-SSR primers, where PIC was reported to be 0.93 to 0.98. Hence, more number of SSR primers need to be screened for maximum diversity analysis in germplasm of *Centella asiatica.*

5.8 Genetic diversity analysis

In the present study, the genetic similarity matrix was generated by Jaccard's coefficient in *Centella asiatica* germplasm. The highest genetic similarity was observed between IIHR CA-5 and IIHR CA-4 with the value of 0.64. The lowest similarity co-efficient of 0.10 was observed between IIHR CA-2 and IIHR CA-14 germplasm. These similarity coefficients ranged from 0.10 to 0.64 with mean at 0.37 indicating good variability among the studied collection. The results are in accordance with the findings of Sathyanarayana *et al*., (2011) in *Mucuna* accessions.

Cluster analysis based on SSR data grouped fifteen germplasm into two major clusters. The major Cluster I with one germplasm IIHR CA-2 entirely diverged from all other germplasm. The Cluster II was subdivided into three sub clusters b_1, b_2 and b_3 where sub cluster b_1 was the largest with six germplasm *viz.* IIHR CA-7, IIHR CA-10, IIHR CA-9, IIHR CA-8 and IIHR CA-5, IIHR CA-4, sub cluster b_2 with five germplasm *viz*., IIHR CA-15, IIHR CA-13, IIHR CA-12, IIHR CA-14 and IIHR CA-11. The sub cluster b_3 includes three germplasm *viz*., IIHR CA-6, Vallabh Medha and IIHR CA-1.

5.8.1 Comparison of dendrogram generated by molecular markers with morphological, yield and biochemical parameters

The germplasm IIHR CA-4 and IIHR CA-5 are closely related as shown in dendrogram even though they are originated from different locations but in case of yield (Table 8) and terpenoids content (Table 16) were obtained almost similar with each other. There were differences between some morphological and molecular level. The germplasm (IIHR CA-11 and IIHR CA-14) of same cluster at molecular level were present in different cluster at morphological level (Table 7), whereas there is also germplasm of same cluster are present in both molecular and morphological level were seen with IIHR CA-1 and Vallabh Medha, IIHR CA-4 and IIHR CA-5 (Table 13).

The cluster analysis of fifteen germplasm of *Centella asiatica* with molecular approaches revealed that germplasm collected from nearby locations fell into different clusters and those from geographically different locations fell in the same cluster. Occasionally, some of the accessions from nearby geographical locations fell in the same cluster (Table 1). These results imply that multiplicity of factors including the geographical locations were responsible for the selection of genotypes that got naturalized at the sites of collections. Similar result was obtained in *Bacopa monnieri* by Tripathi *et al.,* (2012).

VI. SUMMARY

The present investigation was carried out at ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru during 2015-16 using fifteen germplasm with an aim to identify superior germplasm with higher yield, quality with higher chemical content and assess the information on the nature and magnitude of genetic variability, estimates of heritability and genetic advance, correlation coefficients and nature, extent of genetic diversity among the germplasm and identify diverse germplasm for hybridization programme. The findings of the present investigation are summarized hereunder.

The variation was observed for the characters studied. The analysis of variance revealed the existence of significant differences among the germplasm studied for most of the traits indicating the presence of considerable amount of variability.

6.1Morphological variability in *Centellaasiatica*L. germplasm

Based on the performance on growth parameters of fifteen *Centellaasiatica*germplasm*viz.,*IIHR CA-7 followed by IIHR CA-1 and IIHR CA-13 were identified for growth and yield attributing characters among the germplasm.

The qualitative traits i.e. Leaf margin, leaf colour, leaf petiole and flower colour showed significant variations among the germplasm. The two germplasm IIHR CA-5 and IIHR CA-15 has distinct dentate leaf margin among the germplasm. The check variety VallabhMedhahas distinct red purple (59-C)flower colour and the germplasm IIHR CA-5 has white (NN155-D) flower colour among the germplasm.

6.1.1 PCV, GCV, Heritability, GA as percent of mean and correlation studies in *Centellaasiatica*L. germplasm

Phenotypic coefficient of variation (PCV) was found to be higher in magnitude than genotypic coefficient of variation (GCV) in respect of all the characters.

High estimates of GCV, PCV, heritability and genetic advance as per cent of mean was observed for shoot length, leaf length, leaf width, rosette diameter, petiole length, fresh leaf weight and dry leaf weight.

Studies on correlation indicated that shoot length, leaf length, leaf width, internode length, rosette diameter, petiole length, specific leaf weight and fresh leaf weighthave significant and positive association with dry leaf weight and can be considered in the selection criteria.

6.1.2 Genetic divergence using MahalanobisD^2 analysis

In MahalanobisD^2analysis, among the five cluster, cluster I with six germplasm formed the largest cluster. 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 include in 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 crop improvement programme.

6.1.3 Biochemical variability in *Centellaasiatica*L. germplasm

Biochemical variability of various terpenoid compounds in leaf of fifteen germplasm of *Centellaasiatica* with wide variation in contents of Madecassoside, Asiaticoside, Madecassic acid and Asiatic acid was noticed.

The Madecassoside content was highest in germplasmIIHR CA-14, IIHR CA-7,IIHR CA-1 and IIHR CA-13.Asiaticoside content was highest in IIHR CA-13, IIHR CA-14, IIHR CA-8 and IIHR CA-15.Madecassic acid content was found to be highest in IIHR CA-12, IIHR CA-10, IIHR CA-5 and Asiatic acid content recorded highest in IIHR CA-10, IIHR CA-9, IIHR CA-11 and IIHR CA-6,whereas the total terpenoidscontent was shown highest in IIHR CA-14, IIHR CA-13, IIHR CA-7 andIIHR CA-8.

6.1.4 Genetic studies by SSR marker

The SSR primers were found efficient for variability study among the germplasm of *Centellaasiatica.*Out of twenty primers, eighteen primers showed 100% polymorphism and found efficient for molecular variability studies in *Centellaasiatica*germplasm.

The Dendrogram showed two (I, II) major clusters at 10% and 66% similarity coefficient respectively, the major Cluster I with one germplasm. The major Cluster II was subdivided into three sub clusters b_1, b_2 and b_3. Sub cluster b_1 contains six germplasm. Sub cluster b_2 contains five germplasm. Thesub cluster b_3 includes three germplasm. The genetic similarity matrix generated by Jaccard's coefficient shown the extent of relatednessfrom 0.10 to 0.66.

The genetic similarity showed least 10% betweenIIHR CA-2 and IIHR CA-14. The IIHR CA-5 and IIHR CA-4 are closely related and found 66% similarity with each other. In this present study can conclude that there is a wider variability among the germplasm of *Centellaasiatica*.

FUTURE LINE OF WORK

Based on the information available and results obtained in the present study following future line of work are can be proposed.

1. There is needed to evaluate more germplasm and identify elite germplasm for confirming their superiority for yield and biochemical content.
2. Putative chemotypes for different terpenoids need to be confirmed further.
3. Qualitative traits which showed distinctness need to be tested for their stability and uniformity over different crop periods for utilizing them as DUS traits.
4. This diverse germplasmcan be utilized in breeding programmes to widen the genetic base in the crop.
5. Molecular diversity with more number of SSR primers with advance markers systems can be studied.

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*Originals not seen

ANNEXURE

Meteorological data recorded during the investigation period at ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru- 89, 2015- 2016

Month	Temperature (°C)		Relative Humidity (%)		Mean wind speed (km/h)	Total Rainfall (mm)
	Max.	Min.	7.30 hrs.	14.00 hrs.		
June	30.50	21.40	78.20	53.40	8.50	15.00
July	30.40	21.00	77.70	50.60	5.80	36.00
August	30.00	20.10	79.20	49.50	4.30	84.00
September	30.10	19.80	80.00	50.80	3.40	145.20
October	30.70	20.60	76.10	52.30	3.30	142.30
November	28.50	18.50	84.20	52.20	3.19	43.00
December	28.80	17.50	78.30	51.50	2.58	2.00
January	27.20	15.10	73.30	44.80	3.20	-