ISSN (Online): 2349-1183; ISSN (Print): 2349-9265
TROPICAL PLANT RESEARCH
The Journal of the Society for Tropical Plant Research

6(1): 01–07, 2019
DOI: 10.22271/tpr.2019.v6.i1.001

Research article

Phenotypic plasticity of *Centella asiatica* (L.) Urb. growing in different habitats of Nepal

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Abstract: The plant growing in range of environmental conditions exhibits phenotypic plasticity that reflects the ability of the plant to allow its establishment in different areas. *Centella asiatica*, an important medicinal plant, is widely growing in tropical and subtropical belt of Nepal. We measured phenotypic characters (density, petiole length, stolon length, SLA, leaf number per ramet, plant biomass, flower number) and soil attributes (soil pH, soil nitrogen (N), soil organic carbon (OC), soil organic matter (OM) of 21 *C. asiatica* populations in three habitats (open grassland, open agricultural land, shady grassland) of Nepal. Ramet density (105 plants m^{-2}) and biomass yield (52.5 g m^{-2}) was found highest in partially shaded grassland with soil having 5.83 pH, 0.20% N, 4.26% OC and 7.38% OM. Leaves had 391 cm^2 g^{-1} SLA, 4.13 cm long petiole and 1.76% N. The three sites differed significantly ($p<0.001$) in petiole length, SLA, leaf N, soil N, soil OC and soil OM contents. Thus land uses had a significant effect on ramet density and leaf characters of *C. asiatica*. Phenotypic plasticity in leaf petiole length and number of flowers per inflorescence was observed, which appeared to be governed by light availability and height of associated species. In terms of yield partially shaded grassland was the most suitable natural habitat of *C. asiatica.* Evaluation of growth traits and yield in a different habitat help to find the suitable condition for growth of the plant in nature. This information is helpful in planning cultivation of *C. asiatica.*

Keywords: Phenotypic plasticity - Growth traits - Habitats - Yield.

[Cite as: Devkota A & Jha PK (2019) Phenotypic plasticity of *Centella asiatica* (L.) Urb. growing in different habitats of Nepal. *Tropical Plant Research* 6(1): 01–07]

INTRODUCTION

The plants occurring in varying environmental conditions (*i.e.* soil characteristics and/or light intensity) exhibit phenotypic plasticity in the form of various important ecological traits which reflects the adaptiveness of the plant in habitat (Tyler *et al*. 2007, Zhu *et al*. 2007, Zhao *et al*. 2010). *Centella asiatica* (L.) Urb., an important medicinal plant, belongs to family Apiaceae is widely distributed in tropical and subtropical region of Nepal. The plant is indigenous to the warmer regions of both hemispheres and found in different parts of the world including Africa, Australia, Cambodia, Central America, China, Indonesia, the Lao People's Democratic Republic, Madagascar, the Pacific Islands, South America, Thailand, Southern United States of America, and Viet Nam (Annonymous 1953, Iwu 1993). The plant flourishes in damp, moist and shady habitats and grows by producing stolons that are characterized by long internodes and nodes, on which are borne reniform-cordate leaves and sessile flowers in simple umbels. The plant has multiple uses in different parts of the world as vegetable in Asian and in some western countries (Peiris & Kays 1996), as medicine for treatment of leprosy and psoriasis, wounds and burns, and for insanity in traditional Indian and Chinese systems of medicine (Bose 1932, Kan 1986), as memory enhancing tonics, mental and stress-related disorders (Sharan & Khare 1991, Moharana & Moharana 1994), and as cosmetic industry for the preparation of hair oils, tonics and shampoos. Though *Centella asiatica* grows widely in a different habitat, evaluation of best habitat condition suitable for growth of the plant is not studied in detail. In view of this 21 different population of *C. asiatica* were identified representing different habitat (open grassland, open agricultural land, shady grassland) of Nepal. Evaluation of

Received: 19 June 2018 Published online: 28 February 2019
https://doi.org/10.22271/tpr.2019.v6.i1.001

growth traits and yield in a different habitat help to find the suitable condition for the growth of the plant in nature. This information is helpful in planning cultivation of *C. asiatica.*

MATERIALS AND METHODS

The Species

Centella asiatica (L.) Urb. a small creeping perennial herb belongs to family Apiaceae has following morphological characters: slender stem, creeping stolons, green to reddish green in color, interconnecting one plant to another with kidney-shaped leaves emerging alternately in clusters at the petiole. The runners lie along the ground and leaves with their scalloped edges rise above on long reddish petioles. The rootstock consists of rhizome growing vertically down (DPR 1986).

Field sampling and analysis of plant and soil

Preliminary information on the range of ecological habitats for *C. asiatica* growth was obtained by reviewing literature (Malla *et al*. 1997, Press *et al.* 2000). Potential sites were then visited for field sampling between 2006 and 2008. Twenty-one different populations of *C. asiatica* were identified representing different habitats (open grassland, open agricultural land, partially shaded grassland) and soil characteristics from Nepal. Detail plant and soil chemical analysis were carried out from the selected habitats. In each population, 2–5 plots of 10 m × 10 m were subjectively chosen to represent the patches with relatively high density of *C. asiatica.* In each plot, 10 quadrats (1 m × 1 m) were sampled randomly. Altogether 54 plots (10 m × 10 m size) have been categorized into three different habitats considering 18 plots per habitat. Total number of individuals of *C. asiatica* was recorded from sampling plots. Because of the rosette habit, each adult with a rosette of leaves and a root system was considered an individual. Ramet density was calculated in number per square meter (plants m^{-2}). To determine aboveground biomass, all individuals of *C. asiatica* were collected from a single most densely populated quadrat of the large (10 m × 10 m) plot. Biomass was also estimated in 54 quadrats.

From each population ninety leaf samples of *C. asiatica* were collected (three from a single ramet) randomly from each subplot. Collected green leaf samples were shade dried and analysed for total nitrogen content.

Species specific traits: During field visit, individual plants of *C. asiatica* to study life history traits also sampled. Healthy and undamaged 40–50 individuals of *C. asiatica* were completely uprooted and out of which thirty healthy plants were selected to determine ramet diameter, number of leaves per ramet, number of primary branches, stolon length, petiole length, number of flowers borne by individual plant.

Ninety mature leaves, three from each plant per population were measured for petiole length (PL) and specific leaf area (SLA). Petiole length, length and width of leaves were measured in fresh leaves. Then these leaves were oven dried (60ºC, 48 h) and mass of each leaf was weighed in electric balance (0.001 g). Length and width of leaves were measured and multiplied by conversion factor following Zobel *et al.* (1987) for the determination of leaf area. SLA was calculated by dividing leaf area with per unit dry mass. The number of leaves (NLN) from each node was also scored. The total number of flowers per mature rosette was also scored. Dry mass of individual plant per replication was obtained after harvest. Leaf nitrogen (N) content was determined twenty samples per population as permodified micro Kjeldahl method following the procedure described by Horneck & Miller (1998).

Soil: About 200 g soil samples from the rooting depth (5–10 cm) of the plant was collected and analysed for soil pH, organic carbon (OC), organic matter (OM) and total nitrogen (N) content in air-dried soil samples after passed through fine sieve (mesh size 0.5 mm). A total of thirty soil samples were analysed following the method described by Gupta (2000).

Numerical Analysis

The significance of the difference in measured attributes among the three habitats was tested by one way analysis of variance (ANOVA). These three habitats were also compared by multiple range tests (Duncan Homogeneity test). The multiple range tests allow comparison of the pairs of sites for each attribute. Statistical Package for Social Science (SPSS version 11.5, 2002) was used for all statistical analysis.

RESULTS

Morphology, distribution and abundance of Centella asiatica

Centella asiatica was found throughout Nepal (eastern, central and western) from <85 to 2300 m asl in a wide range of habitats from open fallow land, to roadside to shady grassland (Table 1). Population distribution of *C. asiatica* in open agricultural fallow land was quite sparse (Table 2), whereas the population distribution was quite wide in open to shady grassland with sparse density of other associated species.

Table 1. Collection sites and habitats of *Centella asiatica*.

S.N.	District	Locality	Elevation (m asl)	Eco-region	Habitat	Land use
1.	Ilam	Phidim	1250	E	Open Grassland	Grazing by cattle
2.	Jhapa	Bhadrapur	85	E	Agricultural fallow land	Fallow land, grazing of cattle
3	Sunsari	Inaruwa	96	E	Grassland	Cattle grazing was common
4.	Dhankuta	Hille	1800	E	Grassland	Cattle grazing was common
5.	Makwanpur	Daman	2300	C	Open Grassland	Grazing by cattle
6.	Makwanpur	Hetauda	650	C	Grassland	Grazing by cattle
7.	Chitwan	Gauriganj	250	C	Open Grassland, Agricultural Land	Fallow land, grazing by cattle
8.	Gorkha	Palungtar	600	C	Open Grassland, Agricultural Fallow land, Shady place	Grazing by cattle
9.	Lamjung	Way to Besisahar	740	C	Shady grassland	Cattle grazing was prohibited
10.	Lalitpur	Godavari	1550	C	Open Grassland	Grazing by cattle
11	Kathmandu	Kirtipur	1350	C	Open and shady Grassland, Agricultural fallow land	Grazing by cattle
12	Kathmandu	Matatirtha	1400	C	Shady Grassland, Open Agricultural fallow land	Fallow land, grazing by cattle
13	Kaski	Pokhara	850	C	Open grass land	grazing by cattle
14	Kaski	Dhampus	1800	C	Shady grassland, Agricultural fallow land	
15	Pyuthan	Ampchaur	1250	W	Open Grassland, Shady grassland	Fallow land, grazing by cattle
16	Dang	Lamahi	150	W	Open grassland	Fallow land, grazing by cattle
17	Surkhet	Birendranagar	650	W	Shady grassland	Cattle grazing was prohibited
18	Banke	Kohalpur	155	W	Open fallow land	Fallow land, grazing by cattle
19	Bardiya	Magaragadi	150	W	Shady grassland	Cattle grazing was prohibited
20	Kailali	Dhangadi	130	W	Open grassland	Grazing by cattle
21	Kanchanpur	Mahendranagar	150	W	Open grassland	Grazing by cattle

Table 2. Density and morphological characters of *Centella asiatica* in different habitats. For each parameter significant difference between mean among different sites are indicated by different letters (Duncan homogeneity test, $\alpha = 0.05$). F and P values were obtained by one way analysis of variance (ANOVA).

Attributes	Partially shaded grassland	Open grassland	Open agricultural land	N	Mean	F value	P value
Density (plants m^{-2})	105.24^c ±2.09	82.14^b ±1.43	32.12^a ±1.57	630	73.16±1.69	22.025	< 0.001
Petiole length (cm)	5.34^c ±0.25	3.38^b ±0.4	2.16^a ±0.89	1890	4.13 ±1.02	47.95	< 0.001
Leaf length (cm)	1.9^c ±0.53	1.63^b ±0.96	1.57^b ±0.34	1890	1.68±0.71	40.21	< 0.001
Leaf width (cm)	3.03^b ±0.90	2.59^a ±0.80	2.62^a ±0.60	1890	2.74±0.77	4.48	<0.001
Stolon length (cm)	6.08^b ±1.29	5.78^a ±0.98	5.25^a ±1.08	630	5.82±1.19	2.59	0.076
SLA (cm^2 g^{-1})	492^b ±41	322^a ±26	279^a ±29	1890	391±23	8.74	< 0.001
Leaf no/ramet	3.6^a ±0.89	3.67^a ±1.08	3.74^a ±0.82	630	3.69±0.96	0.154	0.857
Plant biomass (g m^2)	52.5^c ±0.89	38.23^b ±1.20	20.12^a ±0.28	630	36.95 ±0.79	18.50	< 0.001
Flower no/ramet	3.14^a ±1.14	6.78^b ±2.53	10.56^c ±2.36	630	6.61±2.01	33.35	< 0.001
Seeds/ ramet	0.24^a ±0.03	2.36^b±1.21	9.65^c ±0.62	630	4.8±0.3	28.14	< 0.001

Population density and above ground biomass yield varied with habitats (Table 2). Mean values of plant density and biomass across the habitats were 73 plants m^{-2} and 37 g m^{-2}, respectively. Highest density was shared by relatively undisturbed shaded grassland. Partially shaded grassland had the highest ramet density (105 plants m^{-2}) and biomass (52.5 g m^{-2}) yield. Open agricultural land had the least density (32 plants m^{-2}) and biomass (20 g m^{-2}) yield. Density and biomass production of *C. asiatica* increased relatively with increasing soil N and OC (Table 2 & 3).

There was a significant difference in growth (petiole length, specific leaf area, leaf mass and stolon length) and reproductive traits (flowers/plant, seeds/plant) of *Centella asiatica* in different habitats. Petiole length ranged from 2.16 cm at open agricultural land to 5.34 cm at partially shaded grassland (Table 2). There was a significant difference (*p* = <0.001) in leaf length and leaf width of individual leaf among the habitat. Specific leaf area ranged from 279 to 492 $cm^2 g^{-1}$ (mean 391 $cm^2 g^{-1}$, n = 1890). The average number of leaves per ramet was not affected by habitats. The number of flowers per ramet was highest (10.56) in open agricultural fallow land. The number of flowers per inflorescence in open agricultural land was three times and two times higher than in partially shaded grassland and open grassland, respectively.

Table 3. Leaf nitrogen (N) content and soil nutrients (nitrogen and organic carbon) in *Centella asiatica* habitats. For each parameter significant difference between mean among the sites are indicated by different letters (Duncan homogeneity test, α = 0.05). F and P values were obtained by one way analysis of variance (ANOVA).

Attributes	Partial shaded grassland	Open grassland	Open agricultural land	N	Mean	F value	P value
Leaf N (%)	1.95^{c} ±0.74	1.65^{b} ±0.63	1.52^{a} ±0.73	630	1.76±0.72	23.55	< 0.001
Soil pH	5.83^{b} ±0.94	5.57^{a} ±1.02	5.69^{a} ±0.7	630	5.63±0.7	8.62	< 0.001
Soil N (%)	0.20^{b} ±0.07	0.18^{b} ±0.10	0.16^{a} ±0.07	630	0.18±0.08	14.97	< 0.001
Soil OM (%)	7.38^{b} ±1.21	5.14^{b} ±1.86	4.41^{a} ±2.21	630	5.64 ±1.76	19.89	0.032
Soil OC (%)	4.26^{b} ±1.21	2.97^{b} ±1.26	2.55^{a} ±1.3	630	3.16±1.26	20.49	0.041
CN ratio	21.3^{a} ±2.65	16.5^{a} ±2.34	15.93^{a} ±4.32	630	17.91±3.10	0.95	0.386

Nutrient content of soil and plant parts

Leaf N content ranged from 1.52 to 1.95% (average 1.76%). Leaf N content differed significantly (*p* <0.001) along *C. asiatica* habitats. Soil pH ranged from 5.57(open agricultural land) to 5.83 (partially shaded grassland). There was a significant difference (*p*<0.001) in soil pH among the habitats. Soil N content ranged from 0.16 to 0.20 % (average 0.182 %) and there was significant difference (*p* <0.001) among three habitats (Table 3). Soil organic carbon (OC) ranged from 2.55 % at open agricultural land to 4.26 % at shady land with average 3.26 % for all sites. Mean soil organic matter (OM) was 5.64 %. There was a significant difference in soil OM and OC among the sites (Table 3). C/N ratio ranged from 15.93 at open agricultural land to 21.3 at shady grassland. There was no significant difference in C/N ratio among habitats.

DISCUSSION

Distribution of Centella asiatica

Centella asiatica has wide distribution in terms of elevation (< 95m to about 1900 m asl), habitat types (open agricultural land, grassland to shady fallow land) and physiography (eastern to western Nepal, Terai plain to midhills of Mahabharat range in Nepal (Table 1). It was also reported from wetland system of the Terai region of Nepal (Burlakoti & Karmacharya 2004).

This species has been reported from the shady marshy ground and roadside ditches of Sikkim and Bhutan from altitudinal range 400 m to 1500 m asl (Grierson & Long 1999). In open agricultural land *C. asiatica* distributed sparsely, while in grassland it grows vigorously. Abundant distribution of the species in the swampy areas of India, the Islamic Republic of Iran, Pakistan and Sri Lanka up to an altitude of approximately 700m have been reported (Tyler *et al*. 1988, Farnsworth & Bunyapraphatsara 1992, Iwu 1993).

It appears that deep shade within tall tress was not a suitable microhabitat for *C. asiatica*. In partially shaded grassland; density of *C. asiatica* was higher than in nearby open grassland and open agricultural land (Table 3). Thus the distribution of this plant in nature is appeared to be determined by light availability.

Abundance

Population density and above ground biomass varied with habitats (Table 2). Mean values of density and biomass across the habitats were 73 plants m^{-2} and 37 g m^{-2}, respectively. Highest density was found in relatively undisturbed moist partially shaded grassland. Partially shaded grassland had the highestramet density (105 plants m^{-2}) and biomass (53 g m^{-2}) yield. The highest ramet density and biomass production in partially shaded grassland could possibly due to high nutrient content (Table 2). Lowest ramet density of *C. asiatica* in agricultural land could be due to periodic disturbance during agricultural practices. Given the highest plant density and biomass production in partially shaded grassland, this habitat can be considered as the most suitable habitats for the growth of this plant under natural conditions. Comparable data on population and abundance of this species is not available from Nepal and elsewhere (Devkota & Jha 2008); but experimental shading has shown that plant biomass yield of *C. asiatica* was the highest under partial shading condition (Mathur *et al*. 2000).

Variation in growth characters

Variation in growth characters and reproductive outputs among the individuals of *Centella asiatica* growing in different habitats (open agricultural land, open grass land and partially shaded grassland) (Table 2) represents growth responses to resource availability. The variation in petiole length was adaptive response to light. Plasticity of petiole helps to place the lamina in areas of high light (de Kroon & Hutchings 1995). Herbaceous dicot plants growing in open habitat often respond to shading by elongating internodes and petiole length (Huber 1996), and improves access to light (Falster & Westoby 2003). Long petiole raises the leaf lamina and enables the plant to receive adequate light when density and height of associated species are high. This is a common strategy of light-demanding herbaceous species. Shortest petiole length of *C. asiatica* at agricultural land was due to less density of associated species and more open area as it received a sufficient amount of light. There is no need to develop long petiole for the plant. Plasticity in lamina and petiole form occurs both between and within plantsin response to contrasting exposure to light (Niklas 1999, Niinemets & Fleck 2002). Due to open land both in agricultural as well as grassland, plant easily get sufficient light so no need of the extension of cell of petiole as well as stolon. This explains the short petiole length in the leaf of *C. asiatica* in open habitat.

There was a significant difference ($p>0.001$) in SLA among three habitats. The variation in SLA might be due to different light intensity (Hughes & Cockshull 1972, Heuvelink & Marcelis 1996) or may due to the variation of leaf nutrient. The highest SLA value (492 $cm^2\ g^{-1}$) of *C. asiatica* in shady grassland may be due to the reduction in available light to the leaves when the plant density was high. A positive effect of plant densityon SLA has been found in other crops, *e.g.* potato (Vos 1995), tomato (Heuvelink & Marcelis 1996) and *Impatiens capensis* (Maliakal *et al*. 1999). Plants were grown in high light generally have thick leaves with low SLA (Bjorkman 1981). Average SLA of *Centella* lies near the median range (14 to 150 $g\ m^{-2}$) of global data set for 2548 species compiled by Wright *et al*. (2004).

There was a significant difference in the number of flower per ramet among the habitats (Table 2). There appears trade-off between densities of ramets and number of flower per inflorescence. The number of flower per inflorescence was highest at open agricultural land where ramet density was the lowest. Failure of the large proportion of ramets to bear flower at shady grassland may be due to density dependent factors such as competition for resources (*e.g.* nitrogen), space as well as the light factor. The lowest number of flower per inflorescence in shady grassland might also be due to the light factor. Dense growth of associated species could also be less favourable for the production of flowers. The plants of shady grassland tended to invest less in sexual fecundity and more in traits ensuring vegetative offshoots. Differential patterns of ramet recruitment and growth in different habitats also underlie variation in population growth rates in other perennial herbs (Tictin & Nantel 2004).

Nutrient status

i. Leaf nitrogen content: Land use history and ramet density of *Centella asiatica* had significant influence ($p<0.001$) on the leaf N content. Leaf N content increased with increasing ramet density at these different habitats. The open agricultural land with lowest leaf N content had lowest ramet density, and relatively low soil N content, whereas opposite were the cases at shady grassland. Shaded leaves have a higher N concentration than exposed leaves (Lusk 2002). Plant density varied significantly with soil nitrogen ($p>0.05$).It appears that high ramet density and high soil N content may be responsible for high leaf N content at shady grassland. Though leaf N appeared to be dependent of soil N (correlations, $p > 0.03$), the leaf N content of *C. asiatica* was 8–9 times higher than soil N content. Average leaf N content of *Centella asiatica* (1.76%) lies within the range (0.2–6.4 %) of global data set for 2548 species compiled by Wright *et al*. (2004). It was also comparable to other tropical herbs in Nepalese grasslands (0.9–2.3 %, Jha 2003). Leaf N content from 1.5–2.0 % (15–20 $mg\ g^{-1}$) has been considered as adequate for growth of most plants (Chapin & Cleve 1989). Leaf N content of *C. asiatica* lies within this range.

ii. Soil nutrient: Different habitats had a significant impact on soil N and soil organic carbon (OC) (Table 3). High soil OC in shady grassland could be due to the addition of organic fertilizer by litter decomposition. Increase in total nitrogen is due to the increase in organic matter content of the soil (Hatton &Smart 1984). Low OC in soils of open agricultural land could be the result of continuous utilization of resources of soil by crop. Likewise, low OC in soils of grazing land may be due to the removal of above ground biomass by livestock, which removed plant biomass and dilutes the soil OC. Soil OC of *C. asiatica* habitat lies within average values (1.34–3.35%) reported for the soil of tropical zone of eastern Nepal (Jha 2003).

Average soil N content of the study sites was comparable to the global average of soil N content which is 2 $gk\ g^{-1}$ (0.2%) (Larcher 1995), while the value was close to soil N content of warmer climates (tropical and subtropical) where soil N is generally >0.1 (Banarjee *et al.* 1989, Paudel & Sah 2003, Jha 2003). Average C/N ratio of the soil of *C. asiatica* habitat is higher than the upper limit of C/N ratio for fertile soil with stable organic matter (10–12, Biswas & Mukherjee 1999). That was due to the low amount of total nitrogen in the soil.

CONCLUSION

Centella asiatica exhibits significant differences in certain morphological characters like (ramet density, petiole lengths, number of flower per inflorescence and stolon length) and species-specific traits (leaf N content) in relation to habitat conditions (light intensity and soil N, OC, OM) but had no effect on leaf number per ramet. Long petiole with less number of flower at partially shaded grassland and short petiole with more number of flower per inflorescence at open agricultural land of *C. asiatica* indicate light demanding nature of the plant. In terms of yield partially shaded grassland was the most suitable natural habitat of *C. asiatica*.

ACKNOWLEDGEMENT

We are thankful to Dr Bharat Babu Shrestha for helping in statistical analysis. Partial financial support for this research from University Grants Commission, Nepal, is thankfully acknowledged.

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