

Growth Performance and Nutrient Status of *Centella asiatica* (L.) Urban in Different Land Uses of Kathmandu Valley, Nepal

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ABSTRACT

Centella asiatica (L) Urban is an important medicinal plant of tropical and subtropical belts of Nepal. Ramet density, stolon length, petiole length, specific leaf area (SLA), leaf nitrogen content, number of flowers in inflorescence and soil nutrients (nitrogen (N), organic carbon (OC) and organic matter (OM) contents of populations of *Centella asiatica* growing under different land uses (grazing, non grazing and agricultural land) in Kathmandu valley were recorded. Ramet density was highest in non grazing land with soil with 0.134 % N, 1.52% OC and 2.58 % OM. Leaves had 401.45 cm² g⁻¹ SLA, 3.58 cm long petiole and 0.67 % N. The plants from 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 significant effect on ramet density and leaf characters of *Centella asiatica*. Phenotypic plasticity in leaf petiole length and number of flowers per inflorescence appeared to be governed by light availability and height of associated species.

Key Words: Ramet Density, Petiole Length, Specific Leaf Area, Soil Characters, Phenotypic Plasticity.

INTRODUCTION

Medicinal and aromatic plants (MAPs) in Nepal are the important non-timber forest products (NTFPs) contributing significantly to the national economy. In Nepal about 70-80 % of populations in the mountain region depend on traditional medicines for health care (Manandhar 1996). About 1700 species of MAPs have been recorded from Nepal (Baral and Kurmi 2006).

Protection of wild population and cultivation for commercial purpose are two important strategies, which can prevent the species from becoming extinct. Cultivation is the best option to decrease harvest pressure on the wild population. However, there are some major problems in cultivation such as the decline in medicinal value of plants from cultivation (Hamilton 2003, Schippmann et al. 2002). A successful cultivation without any decay in medicinal value of the plant may need replication of wild habitat condition in the farm land, which is virtually impossible but can be maximized if we have detailed information on habitat requirements and plant growth performance in different

habitats in wild condition. Biological study is a prerequisite to develop a management plan for medicinal plants (Schippmann et al. 2002). However, detailed ecological studies on medicinal plants of Nepal are lacking except a few studies (e.g., Ghimire et al. 1999, 2005, 2008, Shrestha et al. 2007). This study was undertaken to understand the growth performance (ramet density and morphology) and nutrient status (leaf and soil) of *Centella asiatica* in three land uses in Kathmandu valley, Nepal. The specific objectives were to understand variation in density, morphology (vegetative) and nutrient status of plants and soil in different land uses.

Centella asiatica (L.) Urban (syn. *Hydrocotyle asiatica*; family Umbelliferae; Ghod tapre in Nepali) is an important medicinal herb of tropical to subtropical regions, growing in moist places up to an altitude of 2200 m, and also on moist stone wall or other rocky sunny areas. It is a small creeping herb with heart or kidney-shaped leaves emerging alternately in clusters at the nodes. Taxonomic description of this plant can be found in Flora of Bhutan (Grierson and Long 1999).

Traditional uses of *Centella asiatica* in different parts of Nepal have been well documented (Mahato and Chaudhary 2005, Shrestha and Dhillion 2003, Manandhar 1996, 2002). Most commonly the aerial parts of the plant are used against high blood pressure, gastritis, uric acid, fever and headache. People collect plants for their use from the wild but the plant is not cultivated in Nepal.

STUDY SITES

The Kathmandu valley (85°30' E to 85° 40' E and 27°55' N to 27°35' N, 1350 m above sea level) is roughly elliptical in outline and covers about 339 km² area. The area is drained by Bagmati River and its tributaries. The climate is warm temperate, but influenced by the tropical monsoon with wet summer and dry winter. Maximum and minimum temperatures range from 30°C to 33°C in summer and -3 °C to 0 °C in winter (DHM 2006). The valley is surrounded on all sides by hills and mountains (maximum elevation 2720 m, Shivapuri hill). The soil texture in the valley varies from loamy to clayey.

After reconnaissance survey three sites: Godavari (1600 m altitude), Kirtipur (1350 m) and Matatirtha (1600 m) were selected for the study. At each site three different habitats (land uses) i.e. grazing, non grazing and agricultural land (in fallow period) were selected for sampling population, plant material and soil. The sites were:

1. Non-grazing land was comparatively undisturbed and densely vegetated area where grazing was prohibited, but other human activities like cutting and clearing of vegetation occurred. This site was shaded by trees and shrubs like *Pinus roxburghii*, *Prunus*, *Rubus*, *Populus*, etc. Associated species were, *Chromolaena adenophora*, *Cynodon dactylon*, *Setaria* sp., *Geranium* sp.

2. Grazing land - where grazing pressure by cows and buffaloes was high all round the year. This site was open and received full sunlight. The associated species of *C. asiatica* were *Cynodon dactylon*, *Parthenium hysterophorus*, *Breea arvensis*, *Paspalum* sp.

3. Agricultural land in fallow period was open area receiving full sunlight and the vegetation cover was sparse. Grazing by cows was frequent but the grazing pressure was lower than in grazing land. Associated species were *Bidens pilosa*, *Cynodon dactylon*, *Hydrocotyle* sp., *Setaria* sp., etc.

MATERIALS AND METHODS

Field Sampling

Because of the plant having rosette habit, each adult with a rosette of leaves and a root system was considered an individual. Population sampling and leaf and soil sample collections were done during May 2007. Quadrats (1m × 1m) were laid randomly in three different habitats (i.e. grazing, non- grazing and agricultural land). In each site thirty quadrats were randomly sampled in a large 40m × 40m plot for ramet density. From each site ninety matured leaves were collected for measuring petiole length, specific leaf area (SLA) and dry weight of leaf. Altogether 270 leaves were collected for this purpose. Soil samples (200 g) were collected from each plot at rooting depth (5-10 cm). Number of flower was counted in thirty inflorescences at each site; altogether ninety inflorescences were counted for this purpose. Leaf and soil samples both were air dried in shade for one week and stored in plastic bags until nutrient analysis. Stolon length of thirty plants at each site was measured; altogether, ninety plants were measured for this purpose.

Laboratory Analysis

Specific Leaf Area and Petiole Length

Petiole length, length and breadth of leaves were measured. Then the leaves were oven dried (60°C, 48 h) and mass of each leaf was weighed in electric balance (0.001g). Leaf area was determined by multiplying the product of length and breadth of leaves with a conversion factor (Zobel et al. 1987). The specific leaf area was calculated as the ratio of leaf area and leaf dry mass.

Leaf Nitrogen Content

Leaf nitrogen (N) content was determined by modified micro-Kjeldahl method following the procedure described by Horneck and Miller (1998). Thirty samples from each site were analysed.

Soil Analysis

Thirty soil samples from each site were analysed for soil organic carbon (OC), organic matter (OM) and nitrogen (N) contents. Air dried samples were passed through fine sieve (mesh size 0.5 mm) before analysis. Analyses were made following the methods described by Gupta (2000).

Numerical Analysis

The data were analysed for the significance of differences in measured attributes between the three sites by ANOVA and the Duncan's homogeneity test using SPSS (version 11.5, 2002).

RESULTS

Density

Three sites with different land uses differed significantly in density of ramets ($p < 0.001$) of *Centella asiatica* (Table 1). Non grazing land had highest (76 plants m^{-2}) ramet density. Despite lowest ramet density, agricultural land had the highest number of flowers per inflorescence. At agricultural land, more than 90% of the ramets bore flowers.

Morphological variation

The average number of flowers per inflorescence was 9.83 (Table 1). The plants at three sites were different ($p < 0.001$) in number of flowers per inflorescence. The number of flowers per inflorescence in agricultural land was four times and two times higher than in non grazing land and grazing land respectively. Specific leaf area ranged from 254.45 $cm^2 g^{-1}$ at agricultural land to 401.45 $cm^2 g^{-1}$ at non-grazing land (average 327.95 $cm^2 g^{-1}$). The difference in specific leaf area (SLA) among the sites was significant ($p = 0.026$). Petiole length ranged from 2.4 cm at agricultural land to 3.58 cm at non-

grazing land. The three sites differed significantly ($p < 0.001$) in petiole length. Average dry mass of a leaf was 19 mg and there was no significant difference ($p = 0.389$) among the sites (Table 1).

Nutrient content

Leaf N content ranged from 0.67 to 0.86% (average 0.75%). Three sites differ significantly ($p < 0.001$) in leaf N content. Soil N content ranged from 0.134 to 0.24 % (average 0.182 %) and there was significant difference ($p < 0.001$) among the three sites (Table 2). Soil organic carbon (OC) ranged from 1.16% at grazing land to 2.34% at agricultural land with average 1.68% for all sites. Mean soil organic matter (OM) was 2.96%. There was significant difference ($p < 0.001$) in soil OM and OC among the sites. C/N ratio ranged from 9.43 at agricultural land to 15.0 at non grazing land. There was no significant difference in C/N ratio among three sites.

DISCUSSION

Density

Land use pattern had significant influence on ramet density of *Centella asiatica*. The ramet density depends on stolon length; shorter the stolon length, higher is the ramet density. Generally, stolon is long and ramet density is high in nutrient rich sites (Tworkosk et al. 2001). But in present study stolon length was highest (5.91 cm) and ramet density was lowest (32 plants m^{-2})

Table 1. Density and morphological characters of *Centella asiatica* in Kathmandu valley. 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 ^a	Nongrazing Land	Grazigland	Agricultural Land	Mean	F value	P value ^b
Ramet density (no m^{-2}) [*]	76 ± 35.28b	66 ± 28.7b	32 ± 8.84a	58 ± 0.03	23.21	<0.001
No of flowers/inflorescence [*]	4.65 ± 7a	8.2 ± 2.6b	17.07 ± 6.56c	9.83 ± 6.64	70.94	<0.001
Stolon length [*] (cm)	4.52 ± 0.93a	4.63 ± 1.27a	5.91 ± 1.22b	5 ± 1.6	12.77	<0.001
Petiole length [§] (cm)	3.58 ± 1.75c	3.06 ± 1.32b	2.40 ± 0.65a	3.01 ± 1.4	17.84	<0.001
SLA [§] ($cm^2 g^{-1}$)	401.5 ± 486.6b	380.1 ± 446.2b	254.5 ± 151.3a	345.3 ± 395.0	3.71	0.026
Dry mass per leaf (mg) [§]	20 ± 0.015a	20 ± 0.039a	16 ± 0.009a	19 ± 0.025	0.95	0.389

^a Samples size (n): * n = 30, [§] n = 90; b Bold number indicates significant difference among the mean, ± Standard deviation

Table 2. Leaf nitrogen (N) content and soil nutrients (nitrogen and organic carbon) *Centella asiatica* in Kathmandu valley. For each parameter significant difference between mean among the 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 ^a	Nongrazing Land	Grazingland	Agricultural Land	Mean	F value	P value ^b
Leaf N (%)	0.67±0.05a	0.72±0.15a	0.86±0.09b	0.75 ± 0.13	25.94	<0.001
Soil N content (%)	0.134±0.23b	0.13±0.05a	0.24±0.12b	0.18 ± 0.08	10.867	<0.001
Soil Organic carbon (%)	1.52±0.47a	1.16±0.51a	2.34±0.98a	1.68 ± 0.85	22.751	<0.001
Soil Organic matter (%)	2.58±0.82a	2.03±0.89a	4.23±1.73b	2.96 ± 1.54	26.675	<0.001
Carbon: nitrogen ratio	15.0±13.59b	11.66±10.99ab	9.43±3.59a	12.07±10.49	2.242	0.112

^a Samples size (n) for each site: 30; ^b Bold number indicates significant difference among the mean ±Standard deviation

in nutrient rich agricultural land. As agricultural land was an open area, received full sunlight and nutrient as well as less biotic interference causes guerilla ramets of *Centella* spreads horizontally rapidly with increased spacer i. e stolon length. *Trifolium repens* showed clonal foraging response to mild shading, with increased stolon lengths relative to no shading; however, with deep shading where growth was greatly reduced, this response was no longer shown and only short internodes were produced (Thompson 1993). Lowest ramet density of *C. asiatica* in agricultural land (Table1) could be due to periodic disturbance during agricultural practices. There appears trade off between densities of ramets and number of flower per inflorescence. The number of flower per inflorescence was highest at agricultural land where ramet density was the lowest. At this site more than 90% ramets bore flower but at non grazing land about 40% of the total ramets bore flowers. Failure of large proportion of ramets to bear flower at non grazing land may be due to density dependent factors such as competition for resources (eg. nitrogen), space as well as light factor. The lowest number of flowers per inflorescence in non-grazing lands may also be due to light factor. Dense growth of associated species could also be less favourable for the production of flowers. The plants of non grazing land tended to invest less in sexual fecundity and more in traits ensuring vegetative offshoots. Different patterns of ramet recruitment and growth in different habitats also underlie variation in population growth rates in other perennial herbs (Tictin and Nantel 2004).

Morphological Variation

Land use history had significant influence on the morphological characters such as petiole length and SLA but no effect ($p=0.389$) on leaf dry mass (Table 1). The shape of leaf did not vary with land uses. There was significant difference ($p>0.001$) in SLA among three populations. The variation in SLA may be due to different light intensity (Hughes and Cockshull 1972, Nederhoff et al. 1972 Heuvelink and Marcelis 1996) or may due to variation of leaf nutrient. The highest SLA value ($401.45 \text{ cm}^2 \text{ g}^{-1}$) of *C. asiatica* in non grazing land may be due to reduction in available light to the leaves when the plant density was high. A positive effect of plant density on SLA has been found in other crops, e.g. potato (Vos 1995), tomato (Heuvelink and Marcelis 1996) and *Impatiens capensis* (Maliakal et al. 1999); Plants 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 no consistent pattern of variation in morphological characters among the three sites. Significant difference in petiole length ($P<0.001$) among sites could be due to different light conditions. Petiole length of *Centella asiatica* in non grazing land was the longest (Table 1). Long petiole raises the leaf lamina and enables the plant to receive adequate light when density and height of associated species is 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 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 plants in response to contrasting exposure to light (Niklas 1999, Niinemets and Fleck 2002).

All the morphological characters measured in this study depend on the size of leaves and number of flowers per inflorescence. Since size of organs is much plastic than the number of organs (Harper 1977) most morphological characters showed variation among the sites. Plastic responses of morphological characters to environmental factors such as light and nutrient availabilities are the major cause of intraspecific variation in clonal traits (Birch and Hutchings 1994).

Nutrient Status

Land use history and ramet density of *Centella asiatica* had significant influence ($p < 0.001$) on the leaf N content of this species. Leaf N content increased with decreasing ramet density at these three sites. The agricultural land with highest leaf N content had lowest ramet density, highest number of flower per inflorescence and relatively high soil N content, where as opposite were the cases at non grazing land. Lowest value of leaf N content (0.67%) of *C. asiatica* at non grazing land may be due to shaded site. Shaded leaves have a lower N concentration than exposed leaves (Lusk 2002). Plant density did not vary significantly with soil nitrogen ($p > 0.05$). It appears that high ramet density and low soil N content may be responsible for low leaf N content and high proportion of flowerless ramets at non-grazing land. Similarly, sexual reproductive effort was highest at N rich site, i.e. the agricultural land.

Leaf N of agricultural land was higher than other sites as soil N content was also higher at this site (Table 2). That might also be due to less biotic competition for nutrient resorption and periodic input of fertilizer at agricultural land. Though leaf N appeared to be independent of soil N (correlations, $p > 0.05$), the leaf N content of *C. asiatica* was 4-5 times higher than soil N content. This could be possible because the performance of a ramet of a physiologically well integrated plant is not governed by local conditions and ramet can exceed patch nutrient availability (Niva et al. 2003).

Average leaf N of *Centella asiatica* was less than the range of values for tropical grasses (0.9–2.3%, Jha 2003) as well as the median range (0.2–6.4%) of the global data set for 2548 species (Wright et al. 2004).

Land use history had significant impact on soil N and soil organic carbon (OC) (Table 2). High soil OC in agricultural land could be due to addition of organic fertilizer during cropping period. Low OC in soils of non grazing land could be the result of continuous utilization of resources of soil by plant and not further addition of fertilizer as well. Besides these, removal of plant biomass by human activities like cutting of grasses also the cause of low soil nutrient at non grazing land. Likewise low OC in soils of grazing land may be due to removal of above ground biomass by livestock, which removed plant biomass and dilutes the soil OC. Soil OM of present sites was slightly higher than the values (2.32%) reported for waste lands of riverside in the same valley (Koju 2005). Soil OC of the present study sites lies within average values (1.34–3.35%) reported for soil of tropical zone of eastern Nepal (Jha 2003).

Average soil N content of the study sites was lower than the global average of soil N content which is 2g/kg (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 (e.g. Banarjee et al. 1989, Paudel and Sah 2003, Jha 2003).

CONCLUSIONS

In conclusion, land uses had significant effect on ramet density, petiole lengths, number of flower per inflorescence, leaf N, soil N, OC, OM and stolon length but had no effect ($p = 0.389$) on dry mass of leaf. Long petiole and less number of flower at non grazing land and short petiole with more number of flower per inflorescence at agricultural land of *C. asiatica* indicate light demanding nature of the plant. Leaf petiole length and number of flowers per inflorescence showed plastic responses, which appeared to be governed by light availability and is the major cause of population variation in *C. asiatica* plant.

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