Variation in Growth of *Centella asiatica* along Different Soil Composition

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**Abstract:** The effect of variation in soil composition on growth vigour of *Centella asiatica* (L) Urb. was investigated in greenhouse of Central Department of Botany, Tribhuvan University, Kathmandu. Variation in different growth traits of *Centella asiatica* was investigated using vegetative clone of genome from one population in Kirtipur, Kathmandu, Nepal. We chose soil composition type as the treatment factor to study variation in growth traits as well as to know the best type of composition of soil for cultivation purpose. We raised plants in each of six soil compositional type and examined an array of 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 *C. asiatica* plant can maximize growth and yield in habitat with sandy loam rather than clayey soil.

**Key words:** *Centella asiatica* • Growth traits • Soil Composition • Variation

**INTRODUCTION**

*Centella asiatica* (L.) Urban (Family: Apiaceae) is an important traditional medicinal plant [1]. The plant is native to India, China, Nepal, Indonesia, Sri Lanka, Australia, Madagascar and Southern and Central Africa [2]. It is found throughout India and Nepal in moist places up to an altitude of 2200m (tropical to subtropical region) and also on moist stone wall or other rocky sunny areas. It is a clonal plant colonising early in the abandoned jhum (slash and burn agriculture) [3]. The plant can grow in a variety of soils with moist, sandy or clayey loam, rich in humus. Observation of natural populations of *Centella asiatica* indicated extensive variation in its growth and reproductive traits.

Results of an extensive survey of populations of *C. asiatica* indicated significant effects of site on growth traits [4]. We investigated the amplitude of variation in growth traits with respect to soil type in controlled environmental conditions and choose soil type as the treatment factor. It is well established that soil texture governs most soil properties [5], including organic matter accumulation [6], retention and release of water [7] and the amounts of nutrients in plant-available forms [8]. We hypothesized that growth traits and yield of *Centella asiatica* varied significantly with soil type; and these variations underlies the adaptation of plant to less uniform size containing four leaved condition; were planted in earthen shallow pots filled with different types of soil composition and to identify soil type for cultivation to obtain high yield. In present paper, we report patterns of variation in growth traits and yield of *C. asiatica* in response to changes in soil composition.

**MATERIALS AND METHODS**

**Treatment Conditions:** A pot culture experiment in a completely randomized design, was established in the Botanical Garden, Central Department of Botany(CDB), Tribhuvan University, Kathmandu, (85º17.32'E Long; 27º40.20'NLat, 1350m asl), Nepal. The proportions of clay and sand varied by thoroughly mixing the fine soil (0% sand) (collected from agricultural land) with horticultural sand in different proportion. Thus six treatments (composition) reflecting a gradient of decreasing clay contents (%), 100 (S1), 80 (S2), 60(S3), 40 (S4), 20(S5) and 0(S6) were prepared (Table 1). The six soil types analyzed fall under the following textural classes clay, silt, loam soil, sandy loam, sandy soil and sand (Table 1).

**Plant Material:** Several plant cuttings of randomly sampled individual plants of *C. asiatica* were collected from same population from garden of CDB, TU, Kathmandu. The cuttings of plantlets were more or less uniform size containing four leaved condition; were planted in earthen shallow pots filled with different types of soil (S1 to S6) in green house. Altogether 300 plants;
Table 1: Soil textural class and characteristics

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Textural class</th>
<th>Bulk density (g cm⁻³)</th>
<th>Soil N (%)</th>
<th>Organic carbon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Clay (0 % sand)</td>
<td>1.55</td>
<td>0.25</td>
<td>4.5</td>
</tr>
<tr>
<td>S2</td>
<td>Silt (20% sand)</td>
<td>1.4</td>
<td>0.22</td>
<td>4.2</td>
</tr>
<tr>
<td>S3</td>
<td>Loam soil (40% sand)</td>
<td>1.35</td>
<td>0.19</td>
<td>3.8</td>
</tr>
<tr>
<td>S4</td>
<td>Sandy loam (60% sand)</td>
<td>1.05</td>
<td>0.12</td>
<td>3.58</td>
</tr>
<tr>
<td>S5</td>
<td>Sandy soil (80% sand)</td>
<td>0.99</td>
<td>0.09</td>
<td>1.31</td>
</tr>
<tr>
<td>S6</td>
<td>Sand (100% sand)</td>
<td>0.88</td>
<td>0.008</td>
<td>0.025</td>
</tr>
</tbody>
</table>

fifty plants for each treatment were planted separately for experiment. Planting was done in October 2007 and equal amount of water was provided for irrigation purpose for each treatment. All pots and treatments were rotated each week to counter any positional effects of pots within treatments.

**Growth Measurement:** Data on yield and morphological traits were recorded in April 2008. Forty plants per replication, selected randomly, were used for the observations. Twenty quantitative traits pertaining to plant morphology and yield were measured.

Ninety mature leaves per treatment were measured for leaf length (LL), leaf width (LW), petiole length (PL), leaf area (LA), dry weight of leaf (LDW) and specific leaf area (SLA). Petiole length, length and width of leaves were measured in fresh leaves. Then these leaves were oven dried (60°C, 48 h) and mass of each leaf was weighed in electric balance (0.001g). Length and width of leaves were measured and multiplied by conversion factor following Zobel et al. (1987) [9] for determination of leaf area. SLA was calculated as the ratio of leaf area and dry mass.

Leaf nitrogen (N) content was determined by modified microKjeldahl method following the procedure described by Horneck and Miller [10]. Leaf N content was determined in twenty samples from each treatment. Chlorophyll a, Chlorophyll b and total chlorophyll content was determined following the method of Arnon (1949) [11] in five samples from each replication.

Number of nodes (NND) occurring along each primary branch were noted. Internodal lengths (IND) were also measured on primary branches arising from mature rosettes. The diameter of a mature leaf rosette (DR) indicated its spread and number of leaves (NLN) and primary branches (NBN) arising from it was also scored.

Inflorescences were measured for flower pedicel length (FPL) and total number of flowers per mature rosette. Fresh (FHY) and dry (DHY) herb yields per replication were obtained after harvest and moisture content (MC) calculated.

**Soil Analysis:** Air-dried soil samples (n =5), brought separately for each soil type for analysis. Organic C was determined by the Walkley Black rapid titration method and total N by the micro-Kjeldahl method [12].

**Statistical Analysis:** The significance of the difference between the mean of measured attributes among the soil type was analyzed by one way analysis of variance (ANOVA). The amount of variation in the parameters in response to the treatments was assessed by calculating the coefficient of variation (CV) computed as the standard deviation of the mean values in each of the six soil types divided by the overall mean of the six treatment means [13]. The treatment types were also compared by multiple range tests (Duncan Homogeneity test). Statistical Package for Social Science (SPSS, version, 11.5, 2002) was used for all statistical analysis.

**RESULTS AND DISCUSSION**

**Soil Texture and Fertility:** In the present study, clay content was significantly related to organic C ($r^2 = 0.88$, $P <0.001$) and total N ($r^2 = 0.85$, $P <0.001$). Studies have reported that, in soils with relatively high clay content, the stabilizing complexes are resistant to microbiological decomposition [14]. Furthermore, anaerobic conditions in fine-textured soils can increase denitrification losses [8, 15] and reduce mineralization of organic N [16]. Therefore, despite high organic C and total N, the amount of plant-available N would be lower in clay-rich soils. Sand would also be low in fertility because of small total N. Thus, the sandy loam (S4) would be expected to be the most fertile soil.

**Morphological Traits and Dry Mass:** All the measured traits of leaves varied significantly with soil type, however, the differences was only marginal for leaf dry weight and leaf area (Table 2). Among the leaf traits, the extent of variation was the highest in leaf dry weight (CV=1.48, Table 2) and lowest in total chlorophyll content (CV=0.043). Average number of leaves was 4.15 per ramet.
Table 2: Leaf characters of *Centella asiatica* in different soil textural type. For each parameter significant difference between mean among different 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).

<table>
<thead>
<tr>
<th>Attributes</th>
<th>0%sand (S1)</th>
<th>20%sand(S2)</th>
<th>40% sand(S3)</th>
<th>60% sand(S4)</th>
<th>80% sand(S5)</th>
<th>100% sand(S6)</th>
<th>Mean</th>
<th>CV</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petiole length (cm)*</td>
<td>11.5±1.47bc</td>
<td>12.54±6.83c</td>
<td>12.69±4.95c</td>
<td>12.38±3.57c</td>
<td>9.16±2.88ab</td>
<td>8.22±2.52a</td>
<td>10.9±4.62</td>
<td>0.42</td>
<td>3.604</td>
<td>0.005</td>
</tr>
<tr>
<td>Leaf length(cm)*</td>
<td>2.36±0.26</td>
<td>3.21±1.02</td>
<td>2.62±0.6</td>
<td>2.52±0.33</td>
<td>2.49±0.34</td>
<td>2.26±0.38</td>
<td>2.56±0.61</td>
<td>0.23</td>
<td>6.22</td>
<td>0.000</td>
</tr>
<tr>
<td>Leaf width(cm)</td>
<td>4.36±0.47a</td>
<td>4.41±3.23b</td>
<td>4.4±0.93a</td>
<td>6.13±0.6a</td>
<td>4.01±0.64a</td>
<td>4.03±0.75a</td>
<td>4.6±1.53</td>
<td>0.33</td>
<td>4.64</td>
<td>0.001</td>
</tr>
<tr>
<td>Dry wt of leaf(g)*</td>
<td>0.08±0.03a</td>
<td>0.083±0.041a</td>
<td>0.07±0.045a</td>
<td>0.05±0.02</td>
<td>0.11±0.24b</td>
<td>0.09±0.09a</td>
<td>0.09±0.12</td>
<td>1.33</td>
<td>2.52</td>
<td>0.004</td>
</tr>
<tr>
<td>Leaf Area(cm )*</td>
<td>0.17±1.33a</td>
<td>18.73±20.92b</td>
<td>19.84±5.43a</td>
<td>14.63±2.94</td>
<td>21.23±7.52a</td>
<td>13.62±20.17a</td>
<td>16.81±24.91</td>
<td>1.48</td>
<td>2.26</td>
<td>0.054</td>
</tr>
<tr>
<td>SLA(cm g)*</td>
<td>127.13±63.19</td>
<td>234.86±192.52c</td>
<td>283.58±70.72ab</td>
<td>292.2±43.69</td>
<td>193.55±34.87</td>
<td>151.33±56.34a</td>
<td>186.99±96.36</td>
<td>0.51</td>
<td>3.68</td>
<td>0.004</td>
</tr>
<tr>
<td>Leaf N (%) ^</td>
<td>1.69±0.31a</td>
<td>2.11±0.18</td>
<td>2.1±0.17</td>
<td>2.16±0.195</td>
<td>1.65±0.12</td>
<td>1.26±0.057</td>
<td>1.26±0.18</td>
<td>0.4</td>
<td>67.55</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* Sample size(n) for each treatment: *n=90 n=5 ^ n=20; Bold number indicates significant difference among the mean, ± Standard deviation, CV= Coefficient of Variance

Fig. 1: Effect of soil texture on root length of individual plant

There was no significant difference in leaf number among the treatments (Table 3). Diameter of rosette and root length of plants varied significantly (p<0.001) among treatments with longest (18.9cm) root found in S6 type of soil (i.e. pure sand; Fig1). In present study, plants had relatively low SLA if grown on sand as well as on soils with excessively high clay content. Plants grown at low nutrient availability show an increase in SLA [17, 18] partly as a result of the accumulation of non-structural carbohydrates or secondary compounds such as lignin or other phenolics [19]. Greater SLA values indicate more leaf surface per unit biomass and, thus, more area available for photosynthesis [20], which compensates for lower leaf area in plants, as found in this study for plants grown in 40% clay (S4).

Growth of root was also affected significantly with soil type. Under extreme water stress; the roots more easily penetrated the loose-dry soil treatment, as supported by the observed root depth. More macropores allowed root penetration and soil particles were more easily pushed aside in the loose treatment due to higher porosity, possibly providing greater access to the little water available and promoting production as a result. Low nutrient supply results in an increased root length.
Fig. 3: Effect of soil texture on chlorophyll content of leaf of *C. asiatica*

ratio [21, 22]. Thus the longest root length in sand was attributed by low nutrient and water supply. Under arid and stressed condition, overall plant growth was reduced as a result of both biochemical disruptions and reduced cell enlargement, which in turn led to reduced leaf expansion and total leaf area and therefore reduced whole plant photosynthesis. That is the reason for low value of growth trait data and low yield in pure sand.

Further, inadequate contact of roots with the soil in sands could limit the uptake of water and nutrients, which in turn, appears to reduce the growth rates in *C. asiatica*. Sultan and Bazzaz [23, 24, 25] studied variation in growth traits in *Polygonum persicaria* in response to light, moisture and nutrient content of soil and they found marked morphological variation in leaf, stem, root and fruit and in structures related to reproductive support following changes in soil moisture [24].

Dry mass of plant was differed significantly among the soil type (p=0.035). It was higher in sandy loam (S4 type) of soil (Fig 2). Sandy loam soil with medium bulk density may have facilitated high biomass production. Compared to low and high bulk densities, the medium bulk density may provide longer retention of water in the soil and increase available water to plant due to the higher proportion of mesopores. Uptake of water and nutrients may also be improved by better root-soil contact [26]. In contrast to that soil having high (S1, 100% clay) and very low bulk density did not favor the growth of plants resulting low yield. Siemens *et al* [27] reported that limited resources (e.g. soil nutrients, water, air) can directly inhibit the rate of growth. According to Bazzaz [28], availability of soil resources, especially nutrients, critically influenced plant growth. Hence, comparatively low biomass exhibited on clay-rich soils (S1 treatment) suggests that *C. asiatica* had slower growth rates under soil having low available nutrient and air due to compactness of soil particles. i.e high bulk density.

Further, inadequate contact of roots with soil in coarse textured soil i.e. in sand; could limit the uptake of water and nutrients [29], which in turn, appears to reduce the growth rate and yield of *C. asiatica*. On the other hand, comparatively high yield and growth vigour observed in sandy loam (S2, S3 and S4) type treatments would enable the plant to pre-empt growth resources.

**Leaf Nutrient and Chlorophyll Content:** Leaf N content ranged from 1.46 to 2.11 %(average 1.26%) (Table 2). Soil textural type had significant influence (p<0.001) on the leaf N of *Centella asiatica*. Chlorophyll a content ranged from 3.25 at soil with no clay (S6) type to 13.17mg/g at 40% clay (S4 type) soil (average 8.37 mg/g) (Fig 3). Chlorophyll b content also ranged from 2.01 at S6 type to 7.88 mg/g at 60 % clay (S3 type) soil. There was significant difference (p<0.001) in chlorophyll a and b content among the treatments. Leaf N and chlorophyll content was the least in plants grown in pure sand (Table 2). Lowest value of leaf N (1.26%) and chlorophyll (6.23mg/g) content of *C. asiatica* grown in pure sand was due to poor nutrient (0.001% N) and less moisture content in that soil. The decrease of chlorophyll content in plants growing in sand may be associated with and most probably related to the decrease of plants water content (43.75%). The decrease of chlorophyll a content is highly related to the decrease of water content in plant leaves [30, 31]. Higher value of leaf N (2.16%) and total chlorophyll content (25.48mg/g; Fig 3) of *C. asiatica* plant grown in 40% clay was due to sufficient amount of air, nutrient and water.

In conclusion there was significant variation in many vegetative traits and yield of *C. asiatica* along different soil compositional type. The results also suggest that *C. asiatica* can maximize growth and yield in habitat with sandy loam type of soil rather than clay. This information can be used in planning cultivation of the plant.
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