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Influence of water stress on growth and yield of *Centella asiatica***

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A b s t r a c t. The influence of water stress on growth and yield of *Centella asiatica*, a traditional medicinal herb of Nepal, was carried out in a pot experiment. Variation in different growth traits of *Centella asiatica* was investigated using vegetative clones of one population. The 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). The experimental design was completely randomized and each treatment was composed of forty plants. An array of vegetative traits including: number of leaves, petiole length, specific leaf area, number of primary branches, and plant biomass was examined. Growth traits such as root length, leaf area and number of flowers per ramet demonstrated significant variation in response to water stress. The results suggested that plants irrigated to 100% pot water capacity showed highest growth and plant biomass production.

K e y w o r d s: *Centella asiatica*, growth, water stress, yield

INTRODUCTION

Centella asiatica L. Urban is one of the most important traditional medicinal herbs found in tropical to subtropical region of Nepal. This plant is widely used by locals as traditional medicine for the reduction of uric acid in blood, for the treatment of high blood pressure, and also as a memory enhancer as well as blood purifier (Devkota and Jha, 2008). Limited water supply is one of the most important environmental factors affecting productivity of crops and medicinal plants (Rahman *et al.*, 2004). Physiological changes in plants, which occur in response to moisture deficiency, decrease photosynthesis and respiration (Sarker *et al.,* 2005), and, as a result, overall production of the crop is decreased. Greater soil water stress decreased plant height and total fresh and dry mass of *Satureja hortensis* – a traditional medicinal herb of Iran (Baher *et al.,* 2002). It was shown that the number of stems per plant and plant dry mass was negatively related to water stress in a perennial weed *Eragrostis curvula* (Colom and Vazzana, 2002). Although the effects of water stress on growth and yield of different crops have been studied during the last years (Ahmad *et al.*, 2003; Kumaga *et al.*, 2003; Rahman *et al.*, 2004; Tahir and Mehdi, 2001), no such work has been done to study the effects of water stress on the medicinal herb *Centella asiatica* in Nepal.

The objective of the paper was to investigate the possible effect of water stress on growth traits and yield of *Centella asiatica*.

MATERIALS AND METHODS

A pot culture experiment in a completely randomized design was established in the Botanical Garden, Central Department of Botany (CDB), Tribhuvan University, Kirtipur, Kathmandu, Nepal (85º17'32"E, 27º40'20"N, 1 350 m a.s.l.). The growth medium utilized in the experiment was comprised of clay (garden soil), horticultural sand, and vermicompost in the ratio of 1:2:1.Variation in different growth traits of *Centella asiatica* was investigated using vegetative clones of plants from one population from the garden of CDB, TU, Kathmandu. Besides this, no fertilizer was added during the experimental period. Plantlets were collected from a mostly dense site within a 5×5 m area, to reduce the probability of genetic differences among the plantlets. The cuttings of plantlets were planted in earthen shallow pots (mean diameter: 20 cm), one cutting per pot, which were filled with the growth medium as described above. The pots were then transferred to a sort of green house at CDB, TU Kirtipur. The temperature of the green house was slightly

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higher than outer environment (+3 to 5°C more). Planting was carried out in October, 2007 and four different levels of water stress were applied (125% – surplus water, l00, 70, and 30% of pot capacity by mass; T1, T2, T3, T4, respectively). Forty plants for each treatment were planted. A total of 160 plants were planted separately for the experiment. The first treatment (125%) was applied at the beginning of the experiment. Drainage was prevented in those pots to represent the excessive water treatment by closing the hole lying at the bottom of each earthen pot. The pots were weighed every two days and water was added using a beaker to compensate for the water loss by evapotranspiration. The soil moisture in the other treatments was maintained every two days at 100, 70, and 30% of pot water capacity (PWC). Weeding was done as required. All pots and treatments were rotated each week to counter any positional effects of pots within treatments. Some of the experimental plants were damaged by insects. As a result, only thirty plants per treatment were randomly selected and were used for the observations. Data on yield and morphological traits were recorded in April, 2008.This experiment was repeated in the 2009 growing season.

Growth traits pertaining to plant morphology and herb yield were recorded. Ninety mature leaves per treatment *ie* three leaves per plant were measured for petiole length (PL) and specific leaf area (SLA). After the length and width of each leaf was measured, leaves were oven dried (60 ºC, 48 h) and the mass of each leaf was weighed with an electric balance (to 0.001 g). The length and width of each leaf was multiplied by a conversion factor to calculate total leaf surface area (Zobel *et al.,* 1987). Specific leaf area was obtained as the ratio of total leaf surface area and dry mass of each leaf.

Leaf nitrogen (N) content was determined by a modified micro-Kjeldahl method (Horneck and Miller, 1998). For leaf nitrogen analysis, leaves collected from 20 individuals per treatment were taken. Five samples from each treatment were taken for measurement of chlorophyll-*a*, chlorophyll-*b*, and total chlorophyll content (Arnon, 1949).

Number of nodes (NNB) occurring along each primary branch was noted. Internodal lengths (IND) were also measured on primary branches arising from mature rosettes. The number of leaves (NLN) and primary branches (NBN) arising from each rosette were also scored. The total number of flowers per mature rosette was also noted. The total fresh and dry masses of each were measured after harvest, and the total moisture content (MC) of plant was measured. Root length of each individual was measured. Whole soil of pot together with plant was dropped out from it to prevent destruction of root parts, while uprooting roots only.

The significance of the difference in measured attributes among the treatments was tested 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 percentage ratio of standard deviation of the mean. The treatment types were also compared by Duncan multiple range tests. The multiple range tests allow comparison of the pairs of treatment for each attribute. SPSS version 11.5 (2002) was used for all statistical analysis.

RESULTS

All morphological traits were affected significantly by the treatments (Tables 1 and 2). Leaf number per ramet ranged from 5.40 to 11.11 (average 7.50). All treatments differed (p<0.001) significantly in leaf number per ramet (Table 1). An effect of different water stress treatments on leaf morphological characters, like petiole length and specific leaf area, was noted during the experimental period. Among the leaf traits the extent of variation was the highest in leaf area (CV = 45.78) and lowest in number of leaves per ramet (CV = 12.29). The longest petiole length (5.67 cm) was observed at 100% PWC and the shortest (2.05 cm) at 30%. Similarly the highest value of SLA (417.3 cm^2 g^{-1}) was obtained at 30% PWC and the lowest (136.5 cm^2 g^{-1}) at 70% PWC. Leaf N content ranged from 1.4 to 2.54%

T a b l e 1. Growth, yield and some chemical characters of *Centella asiatica* leaves in different water stress condition (average from 2008-2009)

Attributes[a]	(T1)	(T2)	(T3)	(T4)	Mean	CV	F value	P value
Number of leaves per ramet[$]	6.90a	11.11b	6.60a	5.40a	7.50	12.29	10.27	0.001
Petiole length (cm)[#]	4.12c	5.67c	3.21b	2.05a	3.54	29.09	31.4	0.001
Dry mass of leaf (g)[#]	0.023b	0.021b	0.027c	0.012a	0.021	18.33	21.38	0.000
Area of leaf (cm^2)[#]	5.27c	5.1c	3.73b	2.46a	4.15	45.78	50.257	0.000
Specific leaf area(cm^2 g^{-1})[#]	217a	261a	136.5a	417.3b	257.95	19.23	4.3	0.003
Leaf N (% d.m.)*	1.68a	2.25b	2.54b	1.4a	1.77	27.11	21.19	0.000
Total chlorophyll* (mg g^{-1})[+]	10.15a	25.75b	25.43b	9.76a	17.27	13.27	9740.612	0.000

For each parameter significant difference between mean among different treatments are indicated by different letters (Duncan test, α = 0.05). Means marked by the same letter are not significantly differed, F and P values were obtained by one way analysis of variance.
[a]Sample quantity (n) for each treatment: [#] n = 90;* n = 20; [+]n = 5; [$] n = 30.

T a b l e 2. Growth traits and yield of *Centella asiatica* plant in different water stress condition (average from 2008-2009)

Attributes[a]	(T1)	(T2)	(T3)	(T4)	Mean	CV	F value	P value
Number of primary branch per ramet[$]	5c	5.5c	2.8b	1.3a	3.65	21.34	37.92	0.000
Number of nodes[$]	2.62b	4.44c	3.03b	1.66c	2.99	51.5	24.13	0.001
Length of internode (cm) [$]	5.077b	5.21b	3.84a	3.8a	4.52	19.24	46.91	0.001
Number of flowers per node[$]	5.11b	17.66d	6.37c	0.0a	7.28	80.9	58.5	0.001
Root length[$] (cm)	8.2a	8.82ab	7.1a	10.56c	8.58	15.64	7.5	0.001
Dry mass of individual plant[$] (g)	0.31a	1.44c	0.83b	0.23a	0.67	24.56	46.56	0.000

For each parameter significant difference between mean among different treatments are indicated by different letters (Duncan test, α = 0.05). Means marked by the same letter are not significantly differed. F and P values were obtained by one way analysis of variance (ANOVA). Explanations as in Table 1.

(average 1.77%). All treatments differed (p <0.001) significantly in leaf N content. Total chlorophyll content ranged from 9.76 to 25.75 mg g^{-1} with an average value of 17.27 mg g^{-1}. There was significant difference in total chlorophyll content among treatments (p<0.001) (Table 1).

Root length of plants in each treatment was highly significant (p = 0.003) (Table 2). Longest root length (10.56 cm) was observed with the plant at 30% PWC. There was significant difference (p < 0.001 in number of primary branches and number and length of stolons among the treatments. A significant effect of different water stress treatments on number of flowers per plant was noted during study period. The highest number of flowers (17.66 flowers per ramet) was obtained at 100 % and the least number of flowers was at 30% PWC. A significant effect of different water stress treatments on yield of *Centella asiatica* plant was also found during the study period. The yield of plants in the different water stress treatments revealed that the highest drymass (1.44 g $ramet^{-1}$) was obtained at 100% and lowest (0.23 g $ramet^{-1}$) at 30% PWC.

DISCUSSION

Drought stress is one of the important growth limiting factors of *Centella asiatica*, which decreases plant growth during the vegetative stage. We observed effects of water stress upon most vegetative traits. An effect of different water stress treatments on leaf morphological characters, such as petiole length and specific leaf area, was noted during the experimental period. The largest leaf area was recorded at 125% PWC treatment. Leaf area decreased with increased water stress, and the minimum leaf area was observed in the 30% PWC treatment. The smaller leaf area transpires less water and this reduction in leaf area can therefore be considered a first line of defence against drought. This reduction in leaf area under water stress is similar to studies on *Vigna subterranea* (Mwale *et al*., 2007; Vurayai *et al*., 2011). Number of leaves per ramet was also the lowest at 30% PWC. These results are consistent with the work of Khalil *et al.* (2010) and Moeini *et al.* (2006) in *Ocimum basilicum* plant. Reduction of leaf area by severe water stress can be considered as an adaptive mechanism, which helps to reduce water loss from the plant (Turk and Hall, 1980).

Leaf N and chlorophyll content were lowest in plants grown in soil having 30% PWC. The lowest value of leaf N and chlorophyll of *Centella asiatica* at T4 (PWC 30%) was due to low moisture content in the soil. Low moisture content in soil causes an inability of plants to get all available nutrients in the soil, and consequently, it causes low levels of water and nutrients in a plant. The decrease of chlorophyll content in plants growing at T4 (PWC 30%) might be most probably related to the decrease of the water content of plants (43.75%). Wang and Nii (2000) stated that the decrease of chlorophyll *a* content is highly related to the decrease of water content in plant leaves. Root growth was also affected by water stress. Under extreme water stress, the roots more easily penetrated the soil, possibly providing greater access to the little water available and promoting production as a result. This might be the reason for having the longest roots at 30% PWC.

Dry matter content of plants differed significantly (p = 0.001) among water stress treatment. The highest value of dry mass was observed in the 100% PWC followed by 70% pot treatment. The total dry masss of plant decreased with exposure to high water stress (30%) or excessive water (125%). This could be the result of a reduction in chlorophyll content and, consequently, photosynthesis efficiency, as reported by Khalid (2006) in *Ocimum* sp., Said-Al Ahl *et al.* (2009) in *Origanum vulgare* and Khalil *et al.* (2010) in *Ocimum basilicum* respectively. This result could be due to that one of the first signs of water shortage was the decrease of turgor which resulted in decrease in growth and development of cell especially in leaves (Alishah *et al.*, 2006). When the leaf level decreases, the light draw decreases and the total capacity of photosynthesis decreases, so plant growth became less and plant performance decreases which leads also to the decrease in dry matter production. This result agrees with findings of Alishah *et al.* (2006) in *Ocimum basilicum* and Khalid (2006) in *Ocimum* sp., respectively.

Reduction of fresh and dry matter content and yield of a medicinal plant, *Mentha arvensis*, due to water stress has also been reported by Misra and Shrivastava (2000). The higher value of crop yield obtained at 100% PWC might be due to more frequent application of water resulting in more adequate moisture in the active plant root zone and better utilization of nutrients. Under arid and stressed conditions, 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 (Layer and Boyer, 1992; Mal and Lovett-Doust, 2005). That is the reason for low values of traits associated with growth and low yield in 30% PWC. At 30% PWC low crop yield obtained might be due to infrequent application of water resulting in a lack of moisture in the active crop root zone. Inadequate moisture may cause an insufficient amount of available nutrients for plants leading to low yield.

CONCLUSIONS

1. Water stress affected yield and growth of *Centella asiatica* L. Urban.

2. The highest growth traits like leaves number per ramet, petiole length, number of flowers per ramet were obtained at 100% PWC.

3. The highest dry mass of plant was obtained for the treatment irrigated with 100% PWC, while the least was obtained at 30% PWC.

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