Growth and yield responses of three accessions of *Centella asiatica* grown in lowland under varied watering intensities

S Wulandari \(^1\), Y Widyastuti \(^2\), Pardono \(^3\) and A Yunus \(^4\)

\(^1\)Departement of Agronomy Graduate School, Universitas Sebelas Maret, Surakarta 57126, Central Java, Indonesia
\(^2\)Research Centre for Medicinal Plant and Traditional Drug, Tawangmangu, Karanganyar, Central Java, Indonesia
\(^3\)Departement of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta 57126, Central Java, Indonesia
\(^4\)Departement of Agrotechnology, Faculty of Agriculture; Research Center of Biotechnology and Biodiversity, Universitas Sebelas Maret, Surakarta 57126, Central Java, Indonesia

E-mail: yunus.uns7@gmail.com

**Abstract.** *C. asiatica* presented the best vigor when planted at an altitude of 2500 meters above sea level. Drought is the main environmental stress in *C. asiatica* cultivation. The objective of this study was to determine which accession of *C. asiatica* that grows and produces optimally under stressed conditions since no known accession can grow optimally with various drought stresses. The study conducted in a screen house, Jumantono, Universitas Sebelas Maret Surakarta, from 4 May to 5 August 2019. The research used a completely randomized design with a variety of *C. asiatica* accessions (accession 1, 2 and 3) as the first factor and variety of watering intensities (once a day, once every two days, once every three days, and once every four days) as the second factor. The results showed that there was an interaction between accessions and watering intensity only on the number of tillers. Accession 1 was a drought-tolerant accession because it grew optimally in almost all variables. Watering intensity once a day was the optimum treatment for *C. asiatica* growth.

1. Introduction

*C. asiatica* is a wild plant that contains a secondary metabolite, namely asiaticoside. The health sector used *C. asiatica* as regenerative medicine for injured skin, improve memory, and reduce symptoms of stress and depression [1]. *C. asiatica* also has the opportunity to cure HIV disease, especially to maintain endurance [2] and as a natural antibiotic [3]. In the last 2 years, the cosmetics business has used asiaticoside from *C. asiatica* as an ingredient in a mask, cream, etc [4]. Other important secondary metabolites in the centela asiatica are saponin, tannin and flavonoid [5] [6].
C. asiatica can easily grow and yield well in almost all types of dry land which has moderate clay content, thus allowing C. asiatica to grow well [7]. C. asiatica can grow optimally in highlands with an altitude of about 2500 meters above sea level with 30%-40% sunlight. C. asiatica can also grow as maximally by planting intercrops[8]. In Indonesia, C. asiatica has several accessions planted by farmers and each C. asiatica accession has almost different morphological characteristics. It grows extensively in marshy, damp and wet places and flowering occurs during April to June with white to purple or pink flowers. Fruits are approximately 2 inches long, globular in shape and strongly thickened pericarp[9].

Benefits offered from C. asiatica make its demand increase, yet its production insufficient. The demand equals 120 tons per year, while the production is only 60-80 tons with 7.5-10 tons (Simplicia) per year [10]. An effort to increase the production of C. asiatica is by expanding the planting area, but not all areas have a considerable quantity of water, thus, drought is the main difficulty in C. asiatica cultivation. Drought is an environmental stress that greatly affects the growth and production of C. asiatica that require a sufficient amount of water to grow optimally. In previous research show that if drought stress has an effect on growth and physiology of medical plant [11][12][13]

The research aimed to determine the standardization of growth and yields of C.asiatica accessions as a recommendation for farmers in under drought stress

2. Materials and method

The research at screen house belong to Faculty of Agriculture Universitas Maret in Jumantono, Karanganyar, and Central Java and the research start from May to August 2019. The research design used was a Completely Randomized Design (CRD) consisting of 2 factors. The first factor is accession of C. asiatica accession (accession 1, 2 and 3) The second factor is watering intensity (once a day, once every two days, once every three days, and once every four days) thus there were 12 combinations with 5 replications. The tool used is a set of UV-Vis Spectrophotometer, analytical scales, 7-liter plastic bucket volume, marker, rope, meter cloth, nail board, filter paper, funnel, and rotary evaporator.

The stages of work in this study began from the initial soil analysis to determine the field capacity of the soil. The preparation of seedlings from C. aciatica stem cuttings which were planted in small polybags for 2 weeks after leaving the roots was ready to be moved, then the preparation of planting media in the form a mixture of soil and manure 2: 1 which is then put into a bucket and carried out planting C. asiatica with the media, the next step is to conduct maintenance, observation and treatment of watering according to the treatment. If the plant has reached the age of 3 months then harvesting is carried out which is then followed by drying under the sun and oven. The material used was 3 accessions of C. asiatica, and chemical for Asiaticoside analysis. Observed variables were the number of leaves, leaf diameter, and petiole length, number of tillers, root length, fresh weight and dry weight of the plant. The results were analyzed using analysis of variance (ANOVA) followed with Duncan Multiple Range Test (DMRT) at 95% confidence level.

3. Results and discussion

3.1 Number of leaves

Analysis of variance showed that the treatment of drought stress has a significant effect on the number of leaves. The accession treatment also shows a significant statistical value on accession 1. The results of the number of leaves presented in Table 1:
Table 1. Effect of C. asiatica accession and watering intensity on number of leaves

| Accession     | Number of Leaves |
|---------------|------------------|
| Accession 1   | 107.23 b         |
| Accession 2   | 85.48 a          |
| Accession 3   | 85.67 a          |
| Watering Intensity | Number of Leaves |
| Once a Day    | 102.88 b         |
| Once every 2 day | 93.85 ab      |
| Once every 3 day | 93.64 ab      |
| Once every 4 day | 80.80 a       |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%

Accession showed a significant effect, and the highest number of leaves found in accession 1. The diversity in the number of leaves in each accession was due to genetic variation. In line with the study in Plectranthus forskohlii (Wild) Briq (Lamiaceae), that there are morphological differences such as variations in the character of leaves, stems and roots in different accessions although grown under same field conditions [14].

Watering intensity showed a significant effect between the watering intensity once a day and once every 4 days. The treatment of watering once a day resulted in a higher number of leaves (102.88), while the treatment of watering once every 4 days resulted in a fewer number of leaves (80.80). This is in line with the research of [15] on cocoa which states that leaf loss due to water stress can be considered as an impact of water shortages and as a physiological response mechanism. [16] stated that the number of leaves will affect photosynthesis. The total number of leaves and no uniformity of canopy is the effect of water availability [17] [18] [19].

3.2 Leaf width

C. asiatica is a medicinal plant that used for its leaves and stems, so observation on the number and width of leaves is very important. The results of the analysis of variance showed that accession affects significantly on leaf width. The results can be seen in Table 2 as follows:

Table 2. Effect of C. asiatica accessions and watering intensity on leaf width

| Accessions   | Leaf width |
|--------------|------------|
| Accession 1  | 6.35 a     |
| Accession 2  | 6.54 a     |
| Accession 3  | 7.08 b     |
| Watering Intensity | Leaf width |
| Once a day   | 6.88 b     |
| Once every 2 day | 6.69 ab    |
| Once every 3 day | 6.78 b     |
| Once every 4 day | 6.28 a     |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%
Accession treatment had a significant effect on leaf width. Accession 3 presented a quite broad leaf width compared to others. This presumed due to the large leaf in accession 3 owned by B2P2T2OOT, so it had different leaf characteristics than other accessions. [20] assumed that the morphology and performance of each *C. asiatica* in Indonesia were varied. The watering intensity had a significant effect on leaf width. At the watering intensity of once, every 4 days resulted in the smallest leaf width due to shrinkage as the plant was lack of water. A plant in drought stress will cause the differentiation of new organs. The same research conducted by [21] stated that mild drought stress affected plant growth.

### 3.3 Petiole length

The results of the analysis of variance showed that accession and watering intensity treatment had a significant effect on petiole length. However, the interaction did not show a significant effect. The results presented in Table 3.

| Accessions  | Petiole length |
|-------------|---------------|
| Accession 1 | 14.61 a       |
| Accession 2 | 15.22 a       |
| Accession 3 | 17.12 b       |

| Watering intensity | Petiole length |
|--------------------|---------------|
| Once a day         | 17.48 b       |
| Once every 2 day   | 15.01 a       |
| Once every 3 day   | 15.12 a       |
| Once every 4 day   | 15.00 a       |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%

The effect of accession on the petiole length was very significant. The longest petiole performed by accession 3. Plant characteristics greatly affect the growth because the durability of each plant was not the same. By [22] that each plant has a different response in facing environmental stress. Watering intensity on petiole length showed a significant effect. The less water supplied to the *C. asiatica* plants, the shorter the petiole (Table 3). Water stress treatments affected leaf morphological characters, like petiole length and specific leaf area [23].

### 3.4 Number of Tillers

Analysis of variance showed that the combination of accessions and watering intensity had a significant effect on the number of tillers as seen in Table 4.
Table 4. Effect of combination treatment between C. asiatica accessions and watering intensity on number of tillers

| Accessions | Watering intensity |          |          |          |
|------------|--------------------|----------|----------|----------|
|            | Once a day         | Once every 2 days | Once every 3 days | Once every 4 days |
| Accession 1| 27.51f             | 26.7ef   | 22.86cd  | 20.39bc  |
| Accession 2| 26.71ef            | 25.43def | 17.98ab  | 16.34a   |
| Accession 3| 24.60de            | 22.89cd  | 21.27c   | 16.41a   |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%

Watering intensity of once every 4 days present a fewer number of tillers than different watering intensities in all accessions. This was because plants experience a lack of water, so it was difficult to grow or bear other activities. In contradiction, if the plants had adequate water, growth and other activities would have not disrupted. According to [24], plants in less optimal conditions will show a decrease in growth and yield. Water deficit not only affects growth[25] but also can damage plants [26]. Accessions 1 showed tolerance to drought since it has a higher number of tillers at each watering intensity than accessions 2 and 3.

3.5 Root length

The results of the analysis of variance showed that the root length was significantly affected by watering intensity. The variance analysis results shown in the following table.

Table 5. Effect of C. asiatica accessions and watering intensity on root length

| Accessions | Root length |
|------------|-------------|
| Accession 1| 33.17 a     |
| Accession 2| 32.40 a     |
| Accession 3| 30.27 a     |

| Watering intensity | Root length |
|--------------------|-------------|
| Once a day         | 28.77 a     |
| Once every 2 days  | 31.72 ab    |
| Once every 3 days  | 33.30 ab    |
| Once every 4 days  | 34.00 b     |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%

Watering intensity showed a significant effect on root length. At the watering intensity of once in 4 days resulted in the longest root than the other treatments (34.00 cm). Due to the lack of water, it presumed the roots would penetrate deeper to suffice their needs of water. The presence of water would encourage root cells to divide more quickly to uptake water and nutrients from the media. Low soil water content would reduce root extension, penetration depth, and root diameter [27]. Water deficit greatly limits the growth and survival of plants [28].
3.6  *Fresh weight and dry weight of the plant*

The negative impact of the lack of water is on the biomass and biochemistry activity [29], lack of water can reduce biomass until 17% [30]. The results of the analysis can be seen as follows;

| Table 6. Effect of accessions and watering intensity on fresh weight of *C. asiatica* |
|-----------------------------------|------------------|------------------|
| Accessions | Fresh weight of roots | Fresh weight of leaves+stems |
| Accession 1 | 35.35 b | 159.25 a |
| Accession 2 | 22.30 a | 145.20 a |
| Accession 3 | 21.75 a | 146.95 a |
| Watering intensity | Fresh weight of roots | Fresh weight of leaves+stems |
| Once a day | 29.80 a | 185.20 c |
| Once every 2 days | 22.27 a | 160.00 bc |
| Once every 3 days | 27.53 a | 135.00 ab |
| Once every 4 days | 26.27 a | 121.67 a |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%

| Table 7. Effect of accessions and watering intensity on dry weight of *C. asiatica* |
|-----------------------------------|------------------|------------------|
| Accessions | Dry weight of roots | Dry weight of leaves+stems |
| Accession 1 | 11.87 b | 34.94 a |
| Accession 2 | 7.00 a | 31.88 a |
| Accession 3 | 7.40 a | 33.55 a |
| Watering intensity | Dry weight of roots | Dry weight of leaves+stems |
| Once a Day | 8.81 a | 37.69 b |
| Once every 2 days | 7.80 a | 36.39 b |
| Once every 3 days | 9.31 a | 30.77 ab |
| Once every 4 days | 9.11 a | 28.97 a |

Note: the number followed by the same letter in the same column is not significantly different based from the DMRT test level 5%

Accession treatment had a significant effect on the root fresh and dry weight. The highest root dry weight yielded on accession 1. The wet and fresh weight of leaves and stems differed but were not significantly different. Differences in yield between plant accessions are due to genetic background whose general developmental growth, yield, and quality of herbs determined by genetic background [31].

Watering intensity to *C. asiatica* significantly affected the results of the fresh and dry weight of the roots. Dry and fresh weight of leaves and stems in watering intensity treatment were significantly different based on the results of the analysis of variance. Drought stress is one of the important growth limiting factors, which decreases plant growth during the vegetative stage and yield of biomass [23] [32]. The lowest fresh and dry weight resulted from the watering intensity of once every 4 days because plants
lacked water so that the photosynthetic was not optimal. According to [33] ginger in 75% drought stress had decreased photosynthesis resulting in assimilates were few. Deficit water can reduce yields because of plants difficult to uptake water and nutrients.

4. Conclusion

Conclusion from this research are accession and watering intensity significantly affected all observed variables. The interaction of C. asiatica accession and watering intensity significantly affected the number of tillers. Accession 1 with once a day resulted in the best growth on the number of leaves, number of tillers, root length, fresh and dry weight. Accession 3 resulted in the greatest leaf width, petiole length, and root length. The watering intensity of once a day delivered the best results on all variables.

References

[1] Y L, Kuo P L, Lin L T and Lin C C 2005 J. Pharm and Expe. Therapeutics 313 333-344.
[2] Manullang, W W S, Napitupulu J A, Mariati dan Noverita S V 2013 J Ekoteknologi 2 474-486.
[3] Ramadhan, S.N., R. Rayit, and Ematrisy. 2015. Antibacterial test of gotu kola leaf extract against Staphylococcus aureus and Salmonella typhi bacteria by Bioautographic method. Andalas Health Journal 4 (1): 203-206
[4] Koyel Kundu, Arpita Roy, Gaurav Saxena, Lakhan Kumar and Navneeta Bharadvaja 2016 Medicinal and Aromat Plants Vol5 (4) 1-4
[5] Robert Azerad 2016 Fitoterapi vol 114 168-187
[6] Santhi C and Thomas MT South Indian Journal Of Biological Sciences Vol 2(1)169- 173
[7] Darwati, I Pribadi, E R and Makmun 2012 (Bogor: Balai Penelitian Tanaman Obat dan Aromatika) p 1.
[8] Sarjan Alatas, Isryadi Siradjuddin, Mokhamad Irfan and Aulia Rani Annisava 2019 Journal of Agroteknologi Unisuska Riau Vol 10 (1) 23-32
[9] Tripathi G, Mishra S, Upadhyay P, Purohit S, Dubey GP 2015 Asian Journal of Pharmacology and Toxicology 3: 49-53.
[10] Januwati, M and Yusron M 2005 (Bogor: Balai Penelitian Tanaman Obat dan Aromatika) p 1–5
[11] Hetty M, Wawan K, Irawan W K and Marjenah J. Hort. Indonesia 10(1) 55-62
[12] Yuyun Y, Sheli M D, Warid A Q, and Anne N 2019 Jurnal Agro universitas islam negeri sunan gunung jati bandung vol 6 (1) 35-48
[13] Natasya G S , Parluhutan S and Nio S A 2019 JURNAL MIPA UNSRAT 8 (2) 55--58
[14] Ganapathy M A L, Selvarasuviasuki M 2014 Letters of Natural Sciences 28 47-54
[15] David M. 2008. Resilience Study on Initial Growth of Cocoa (Theobroma cacao L.) Clones to Drought Stress. Thesis (Surakarta: Universitas Sebelas Maret)
[16] Sezen S M, Seral Y, Servet T, Mehmet Y 2009 J Agri.Water Management 221 211–219
[17] Wang J, Xi H, Lin L J, He H, Liang D and Lv X L 2016. J Anim Plant Sci 26 1326–1333.
[18] Wang Y J, Shi X P, Wu X J, Meng X F, Wang P C, Zhou Z X, Luo F L, Yu F H, 2016. Sci. Rep 6 35459.
[19] Zhang Q Y, Peng, L and Ning W 2009 Ecol Res 24 617–625
[20] Munif G, Sandra A A and Nurfliani B 2007 (Bogor: IPB Laboratories)
[21] Derantika C. and Nihayati E 2018 J of Agriculture Science 3 78-84.
[22] Humphreys M O and Humphreys M W 2015 Food Production Press (England: Oxford)
[23] Musyarofah N, Susanto S, Aziz S A and Kartososewarno S 2007 Bulatn Agronomi.35 217-224
[24] Hossein H, Bijan S and Akbar A 2018 Industrial Crops & Products 124 600–606
[25] Hibat A B, Lorena B, John M and Annie D 2019 Annals of Forest Science 76 21
[26] Nahum C, Mugnisjah W Q, Yahya S, Sopandie D, Idris K, and Sahar A 2006 Agricultural Development: Potential Technologies and Production Organizations
[27] Tabar H, Abghari H and Hosseinzadeh T P 2012 Hydrol Process 26 3351–3361.
[28] Qing W, Qian L, Yu J, Kenian L, Ningfei L and Jin-song C 2019 Flora 256 36–42
[29] Marjan S H, Davood S, Morteza E, Javier A, and Morteza Phytochemistry 156
[30] Karik U, Tinmaz A B, Kürkçüoğlu M, Baser K, and Tümen G 2007 Horticulture 36 37–48.
[31] Pablo C. Salazar, Rafael M, Navarro C, Gastón C, Nora G and Rafael 2019 Journal of trees 3 1409–1422.
[32] Chaves 2002 Annals of Botani 89 907-916.
[33] Ramos M L, Parsons R, Sprent J I and James E K 2003 Pesq agropec. Bras 38 3