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**ORIGINAL RESEARCH ARTICLE**

# Effect of seed heteromorphism and seed collection time on germination of the medicinal plant *Centella asiatica* (L.) Urban in sub-tropical plains of north India

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*Mandukparni*
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**ABSTRACT**

*Centella asiatica (L.) Urban (Family- Apiaceae) is a high-value medicinal plant that contains asiatic acid, asiaticoside, madecassic acid and madecassoside, etc. It is traditionally used as a wound healer and in various skin related diseases. It is propagated through stem cuttings or seeds. It has been observed that the seeds of this medicinal plant show considerable germination in laboratory conditions, whereas its germination is very low under natural environmental conditions. In the present study, efforts are made to distinguish between the viable and non-viable seeds of Centella asiatica (L.) Urban as the same plant bears seeds of different shapes and sizes. Colour thus may show seed heteromorphism. Seeds were collected at three time-frames; before the rain, at the initiation of rain, and during good rains in north India's sub-tropical climate, and were classified among seven different categories based on shape, size and color. Results indicated that maximum germination (24%) was observed in the seeds, that were dark brown, swollen and collected during the initiation of rains. The lowest germination was observed in light brown-colored seeds. In addition, it has also been observed that in some categories (yellow-coloured seeds with the flattened shape) there was no germination.*



## INTRODUCTION

*Centella asiatica* (L.) Urban, commonly known as *Mandukparni* or Indian Pennywort, enjoys a considerable reputation in the *Ayurvedic* tradition of India and Madagascar and is used as a diuretic and alternative tonic in treating leprosy and other skin related diseases (Gohil et al., 2010; Attre et al., 2014). It is a prostrate, faintly aromatic herb found as wild population throughout India and Ceylon up to 2,000 feet and attains a height of 15 cm; leaves are about 6.0 cm long and 1.5-5.0 cm wide with long petiole (Singh et al., 2010). The leaves are commonly employed in herbal medicines, but the use of the whole plant is

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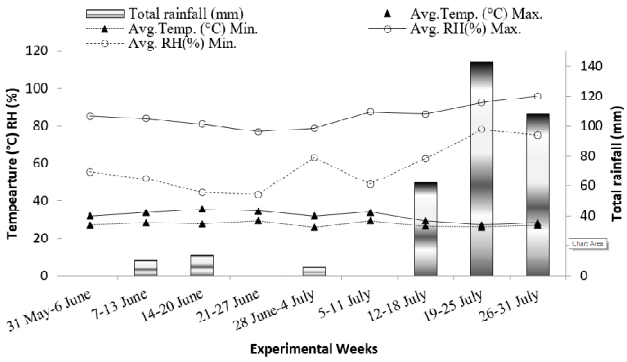
recommended. In India, this plant is found in abundance on marshy and moist clayey or sandy soils in big clumps, growing as a weed in crop fields. It accumulates a large number of pentacyclic triterpenoid saponins, which form the major storehouse of different metabolites providing active compounds that encourage cell rejuvenation, improving physical and mental health (Prasad et al., 2014; Attre et al., 2014). About 20 species related to *C. asiatica* (L.) Urban grow in most parts of the tropics or wet pantropical areas such as rice paddies and also in rocky slopes in higher elevations. This herb is propagated by seeds or stolon's and can be grown on various soils and shady places. In India, *C. asiatica* (L.) Urban frequently suffers due to overexploitation, increasing use of herbicides, drastically depleting water level in water bodies and high level of water pollution. Besides, low seed germination is also a big problem in the cultivation of this crop (Gandi and Giri, 2014). It has been reported that in laboratory experiments, about 82% of germination can be achieved by applying chemicals (Devkota and Jha, 2010), but seeds give poor germination when they are put in a nursery or natural soil environment. One of the reasons behind low germination may be seed heteromorphism because the plant found in moist and marshy places under shades, and it has been reported that seed heteromorphism is often associated with the halophytes and as an adaptation to the spatial and temporal variation of natural habitats (Ma et al., 2018). Many researchers have made efforts to express germination patterns and dormancy mechanisms in heteromorphic seeds of halophytes, particularly in the family *Amaranthaceae*. However, in some non-halophytic plants like *Aethione maarabicum*, seed heteromorphism is associated with some physiological and anatomical features between fruits and seed morphs (Liu et al., 2018). Seed germination and dormancy are also affected by seed collection time, i.e., the weather prevailing during the seed collection affects dormancy and germination, which differs from species to species. Thus, keeping in view the above points, experiments were planned to evaluate (i) the effect of seed heteromorphism and (ii) the time of seed collection on germination of *Centella asiatica* (L.) Urban in field condition of the sub-tropical climate of north India.

## MATERIALS AND METHODS

### *Experimental location*

Field experiments were conducted in the summer and initial rainy season of the year 2018 (June to July) at the experimental farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow. This site is located at 26°5´ N latitude and 80°5´ longitude and is situated about 120 m above the mean sea level, categorized under sub-tropical plains of North India with severe hot summers and fairly cool winters. The soil of the experimental plot has moderate pH (7.7) and sandy loam texture. In this region, monsoon sets typically from the last week of June and continues until the end of September with an average annual precipitation of 1000 mm. The prevailing weather conditions, i.e., temperature, humidity and total rainfall are presented in Fig.1.




RH: Relative Humidity

**Figure 1:** Weather data at the time of seed collection

### *Treatments and experimental design*

Seeds of *Centella asiatica* (L.) Urban were collected three times; before the rain (in the first week of June), at the initiation of rain (third week of June) and during rains (first week of July). The collected seeds were categorised based on their visual appearance of color, shape and size (Table 1). The seeds collected in the first week of June were categorized into seven types; those collected in the third week of June were categorized into only six types while only three types of seeds were

**Table 1: Morphological characteristics of *Centella asiatica* (L.) Urban seeds collected at different time-frames**

| Seed type / Time of collection | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|
| **Seed characteristics** | **First week of June** | | | | | | |
| Shape<br>Colour<br>Length (cm)<br>Width (cm) | Kidney, swollen<br>Dark Brown<br>0.36<br>0.22 | Kidney, swollen<br>Dark Brown<br>0.23<br>0.17 | Kidney, swollen<br>Light brown<br>0.33<br>0.20 | Kidney, swollen<br>Light brown<br>0.38<br>0.22 | Kidney, flattened<br>Very light brown<br>0.26<br>0.20 | Kidney, flattened<br>Dark yellow<br>0.25<br>0.21 | Kidney, flattened<br>Light yellow<br>0.24<br>0.17 |
| **Third week of June** | | | | | | | |
| Shape<br>Colour<br>Length (cm)<br>Width (cm) | Kidney, swollen<br>Dark brown<br>0.35<br>0.20 | Kidney, swollen<br>Dark Brown<br>0.23<br>0.17 | Kidney, swollen<br>Light brown<br>0.31<br>0.21 | Kidney, swollen<br>Light brown<br>0.37<br>0.21 | Kidney, flattened<br>Very light brown<br>0.26<br>0.21 | Kidney, flattened<br>Dark yellow<br>0.28<br>0.21 | NA |
| **First week of July** | | | | | | | |
| Shape<br>Colour<br>Length (cm)<br>Width (cm) | NA | NA | Kidney, swollen<br>Light brown<br>0.31<br>0.20 | NA | NA | Kidney, flattened<br>Dark yellow<br>0.27<br>0.21 | Kidney, flattened<br>Light yellow<br>0.24<br>0.18 |

**Table 2: Seed germination* (%) observed in different categories of seeds collected at three time-frames**

| Seed type | Time of collection | | |
|---|---|---|---|
| | **First week of June** | **Third week of June** | **First week of July** |
| **Type-1** | 13.10±0.65 | 24.00±1.20 | NA |
| **Type-2** | 9.00±0.45 | 17.56±0.87 | NA |
| **Type-3** | 7.50±0.37 | 17.50±0.87 | 15.60±0.78 |
| **Type-4** | 0.0±0.0 | 5.20±0.26 | NA |
| **Type-5** | 0.0±0.0 | 0.0±0.0 | NA |
| **Type-6** | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| **Type-7** | 0.0±0.0 | NA | 0.0±0.0 |

*Mean of triplicates ± standard deviation

obtained during rain (in the first week of July), thus making a total of 16 combinations of seed type and collection time (7 in the first collection time, 6 in the second collection time and 3 in the third collection time). Four replicates with 50 seeds each of heteromorphic seeds were tested in the germination experiment. Seeds were processed as per standard procedure and stored in paper bags at room temperature for three months. Nursery beds of 1 $m^2$ area were prepared and 50 seeds were sown in each bed. Each category of seeds was sown in three replicates in a randomized block design. All intercultural operations were carried out as per need. Germination percentage was calculated by counting the number of seedlings after $20^{th}$ day of sowing. The germination data were analysed statistically by using the analysis of variance technique for randomized block design as suggested Panse and Sukhatme (1985) at 5% (*P=0.05) level of significance.

## RESULTS

Seeds were first categorized based on their color and visual size, and then other biometric observations were recorded. Table 1 shows the observed parameters of the seeds. It was observed that seeds of all categories (total seven) were of the same outer shape, i.e., kidney shape; however, their color varied according to a category. It has also been observed that dark brown to light brown seeds were swollen, whereas the seeds which were dark yellow to light yellow in color were flattened in shape. The length and width of seeds presented in Table 1, are the average of 50 seeds chosen on similarity basis in each category.

Results on germination percentage indicated that maximum germination was observed when seeds were collected at the initiation of rain i.e., in the third week of June (Table 2). However, the germination pattern was such that it was maximum (24.0 %) in the darkest seeds i.e., the first category (Type 1), which was kidney-shaped and swollen. For the second and third category seeds, germination was 17.56% and 17.50%, respectively, whereas for the type 4 seeds, only 5.0% germination was observed. From category 5 to 7, no germination was observed irrespective of the time of seed collection. In all three seed-collection times, germination was maximum in the second time of collection i.e., in the third week of June. During the third seed-collection time, i.e., first week of July, only three categories of seeds were obtained- type 3, type 6, type 7. Among them, only the type 3 seeds showed germination (15.6 %) whereas no germination was observed in type 6 and type 7 seeds. The seeds which were collected in first week of June were categorized in all seven categories, but germination was obtained only in the first three categories.

Thus, the results indicated that the seed categories which have dark brown to brown color seeds with swollen shape showed maximum germination irrespective of the time of seed collection; however, among different seed collection time, the seeds collected during the initiation of rains i.e., the third week of June, showed maximum germination percentage.

## DISCUSSION

Higher germination percentage in seeds which were collected during the third week of June, i.e., initiation of rain, maybe due to the appropriate seed development phase and time of seed collection, which allow the seeds to store more food for maintaining their viability. The time of collection did not allow deterioration of physical and physiological conditions and hence, maximum germination was observed. It has also been noticed that the seeds which were dark brown to light brown in color and with swollen shape showed higher germination as compared to light-coloured seeds with flattened shape. It may be due to the heteromorphic nature of the seeds. Several other researches have previously reported seed heteromorphism, in which the same plant can bear different types of seeds that may or may not germinate. Seeds heteromorphism is not reported in *Centella asiatica* (L.) Urban till date. The external morphological features of heteromorphic seeds may show differentiation in the size and shape of the seeds, and sometimes the nature of seed dispersal may also vary. The weather conditions of natural habitat may also have a significant effect on seed heteromorphism. The temperature, rainfall, soil nutrients also possess a considerable effect on seed morph, ratio, or size and the germination and dormancy pattern of a number of seeds. Seed morphology of heteromorphic seed is often associated with the variation in seed dormancy, which is another adaptation to the heterogeneous environment of many wild seed plants population (Ma et al., 2018). Seed germination may also be affected by the maternal environment of a particular plant; in continuation with this, it has also been reported that the mother plants receive signals which has generated and transmitted due to stress condition and as a result plant produces subsequent generation with variable phenotypes which is an adaptive strategy towards stress condition for survival (Yang et al., 2015).

## CONCLUSION

*Centella asiatica* (L.) Urban, is propagated by vegetative means at present, which is a costly and challenging method. If germination can be enhanced under field conditions, cultivation of the crop may become more feasible and economically viable. Results of the present investigation suggest that brown coloured swollen seeds which are collected during the initiation of rains show more germination tendency over other categories. It is possible to enhance seed germination under field conditions by conducting more experiments employing techniques that are applied to other similar types of crops. Due to over-exploitation of *Centella asiatica* (L.) Urban, the plant has also been categorised under threatened species. Thus, it is essential and need of time that research focusing

on alternate methods of propagation of this high valued medicinal plant should be carried out.

## DECLARATION OF THE INTEREST

The authors declare that they have no recognized competing financial interest that could have seemed to impact the work reported in this paper. There are no conflicts of interest that appear for the manuscript submission.

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