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Effect of substrate on germination, growth, and anthocyanin pigment accumulation in *Caralluma tuberculata (Gems of the Hills)*

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Abstract. Optimizing growing conditions is essential for the conservation and promotion of medicinal plant species such as *Caralluma tuberculata*, a succulent plant of great medicinal importance. This project, this particular research was done at the experimental station of the Faculty of Sciences in Oujda, aims to evaluate the impact of two types of substrates, peat and sand, on the morpho-physiological parameters and anthocyanin pigment accumulation in *Caralluma tuberculata*. The study compared the effect of these substrates on germination rate and kinetics, as well as aerial and root development and red pigment concentration in transplanted seedlings. The results show that the final germination rate is not significantly different between the two substrates (p=0.358), although peat promotes faster kinetics. On the other hand, peat proved to be significantly superior for growth, resulting in significantly greater stem height (8.49 cm) and root length (8.26 cm). Conversely, sand caused an extremely significant accumulation of anthocyanin pigments, reaching 100.00%, compared to 16.20% for peat. This accumulation is interpreted as a physiological defense response to the more pronounced environmental stress in the sandy substrate. These results suggest that the choice of substrate should be based on the cultivation objective. For biomass production, peat and sand should be used to induce protective secondary metabolites.

1 Introduction

Gems of the Hills (*Caralluma tuberculata*), this plant belongs to the Asclepiadaceae family, subfamily Asclepiadoideae, is a perennial, wild, branched succulent plant [1], [2]. The *Caralluma* genus comprises around a hundred species found mainly in the arid and semi-arid regions of Africa, Asia, and South America [3]. *Caralluma tuberculata* is a succulent,

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upright, fleshy herb with no leaves [2]. Its angular, leafless stem bears small flowers. Succulent, angular stem, can grow up to 15 cm tall [4].

Phytochemically, the plant is known to possess different biological constituents, which include volatile alkaloids, glycosides, flavonoids, saponins, sterols, and tannins [5]. Different chemical constituents have been extracted, which include terpenes (lupeol, α- and β-amyrin), Pregnanes (Caratubersides A to G), Sterols (β-sitosterol), and flavonoids (luteolin and kaempferol glycosides) [6].

For several centuries, *Caralluma tuberculata* has been utilized as a vegetable in some parts of Balochistan [3]. *Caralluma tuberculata* is also used in traditional medicine for the cure of several diseases like rheumatism, fever, paralysis, diabetes, leprosy, and arthritis [7], [8]. The plant is also known to possess carminative, stomachic, antipyretic, and hypotensive properties [9], [10]. Similarly, *Caralluma* extract has been proven to possess anticancerous and anti-inflammatory properties, which have been ascribed to pregnane glycosides [11].

Despite *Caralluma tuberculata's* widespread recognition for medicinal as well as nutritional benefits, few research studies have been undertaken to optimize conditions for growing this plant, primarily in terms of soil factors having an influence on plant development, as well as secondary metabolite generation of this plant species itself.

The purpose of this project is to assess the influence of two different substrates, namely sand and peat, upon the seed germination of *Caralluma tuberculata*, as well as upon the seedling development in terms of shoot and root size, as well as anthocyanin production in seedlings, in an attempt to optimize conditions for this species.

Despite its well-established medicinal and nutritional importance, few studies have focused on optimizing the growing conditions for Caralluma tuberculata, particularly the impact of edaphic factors on its growth and the production of its secondary metabolites.

2 Material and Methods

2.1 Plant material and substrates used

The seeds of *Caralluma tuberculata* were harvested in May 2024 from a mother plant located in the Berkane region, which showed optimal performance at maturity. The collection site is located in Laâtamna (Berkane Province) at the following geographical coordinates: 35° 00′ 34″ North and 2° 15′ 35″ West. The experimental work for this project was carried out at the experimental research station of the Faculty of Sciences of Oujda, at the geographical coordinates 34°39′151″ North and 01°53′595″ West.
The comparative substrates used were a substrate of organic origin (peat) and a substrate of mineral origin (sand).

2.2 Test conditions

The study was conducted in two successive stages. A phytotronic chamber was used for analysis of germination, under controlled conditions of temperature at 25°C, daylength of 16 hours, and relative humidity of 90%. Seedlings emerged from seeds placed in equal quantities of either sand substrate, peat substrate, or both, numbering 54 in each treatment, arranged in three replicates.
The second phase started after the germination and seedling development stages, accompanied by potting into the same set of substrates, namely peat, as well as sand. Every growth treatment included three repeats, each relating to a tray full of 14 seedlings, amounting to a total of 42 seedlings for every substrate category. The measurements of

seedling development, as well as the production of pigments, were conducted using a glass greenhouse providing an optimal environment for seedling development.

2.3 Parameters studied

2.3.1 Morphological and Physiological Parameters

Final Germination Percentage (FGP): is the ratio of the number of germinated seeds on the total number of seeds [12]. The formula used to calculate the germination percentage was as follows:

$$FGP = \sum_{i}^{k} (n/N)x100$$

Where, *n* is the number of germinated seeds at each *i* (daily) till *k* (last day) counting and *N,* the total seeds in each treatment.

Germination Kinetics: written as the cumulative number of seeds germinated each day during the experiment.

Stem Height: measure from the collar to the tip of the stem using a ruler graduated in centimeters.

Root Length: After carefully excavating the underground portion, the length measurement of the major roots was conducted using a ruler calibrated in centimeters.

2.3.2 Quantification of anthocyanin red pigments

Anthocyanins are colored flavonoids whose accumulation is often induced by abiotic stress factors [13]. The concentration of red anthocyanin pigments was estimated using MATLAB R2025a software for image analysis. This program detects and quantifies pigments (green and red) based on color values (red, green, blue) to estimate their quantity (Fig. 1).

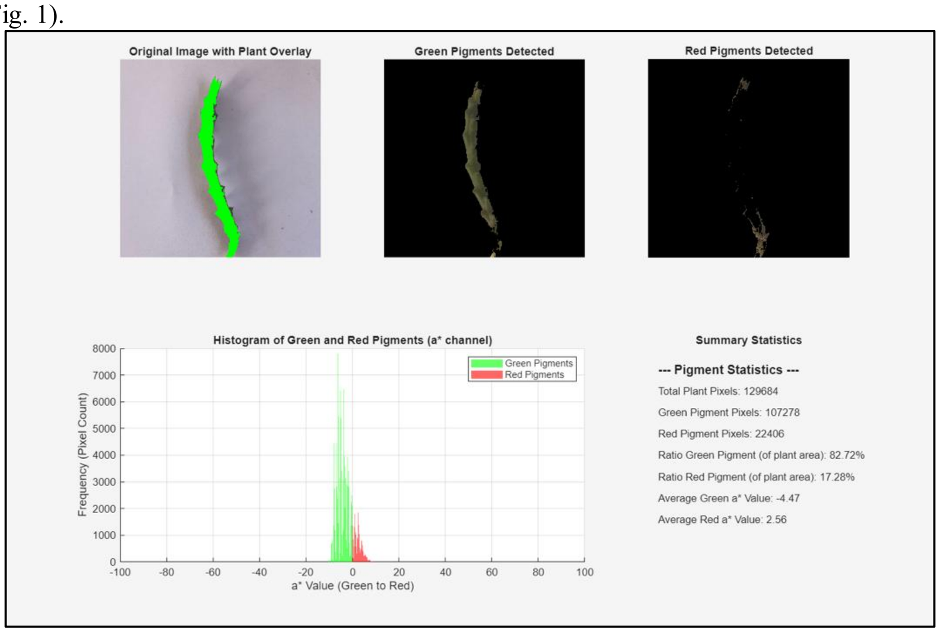

Fig. 1. Display of results.

2.4 Experimental setup and statistical analyses

The experimental design was based on a completely randomized block design, with three replicates of each treatment to ensure reliable representation and reduce experimental variability. The data collected was analyzed by SPSS statistical software version 26.0 (Statistical Package for Social Sciences) (IBM Corp., Armonk, NY, USA) using the t Student test for independent samples to compare the means between the two experimental groups (sand and peat). A difference was considered significant when the p-value was less than 0.05.

3 Results

3.1 Morphological and Physiological Parameters

Germination rate and kinetics

Despite a slightly higher average germination rate for peat (18.52%) compared to sand (16.33%), statistical analysis revealed no statistically significant difference between the two substrates (p=0.358) (Fig. 2a). The germination kinetics (Fig. 2b), however, show that peat promotes a faster start. These differences suggest that the properties of peat are potentially more conducive to early germination initiation.

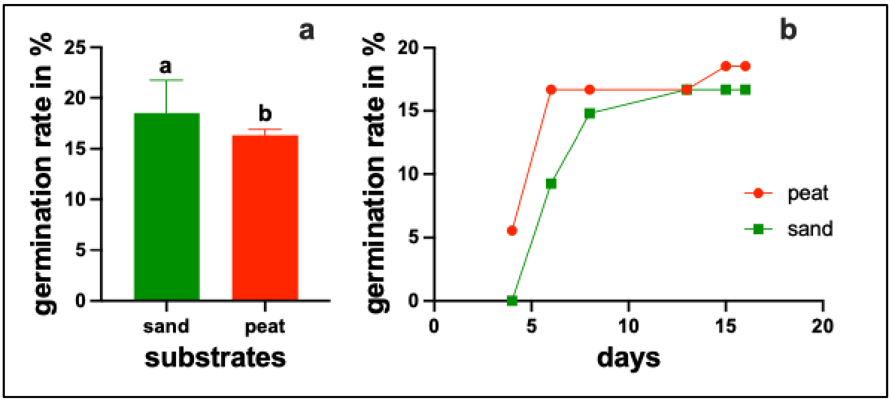

Fig. 2. Effect of substrate on the germination rate (a) and germination kinetics (b) of *Caralluma tuberculata*.

Stem height and root length

Figure 3a shows the impact of the growing substrate on plant height. Plants grown in peat reached an average height of 8.49±0.49 cm, which is significantly greater than the height of plants grown on sand (4.89±0.34 cm). An independent samples t-test also supported this highly significant result (p<0.001).

Likewise, data from Figure 3b showed that substrate significantly affects root development of the plants. The mean root development of plants grown in peat substrate measured 8.26±1.22 cm, which is significantly higher than plants grown in sand substrate, which measured 6.10±1.24 cm, as indicated by results from the t-Student test (p=0.024).

3.2 Concentration of anthocyanin red pigments

As is clear from Figure 3b, plants grown from seeds placed in sand showed an average value of red pigments of 100.00%, which is remarkably higher than in plants grown from seeds in peat (16.20%±8.11%). The result of t-Student test showed this difference to be highly significant (t =20.665; p<0.001).

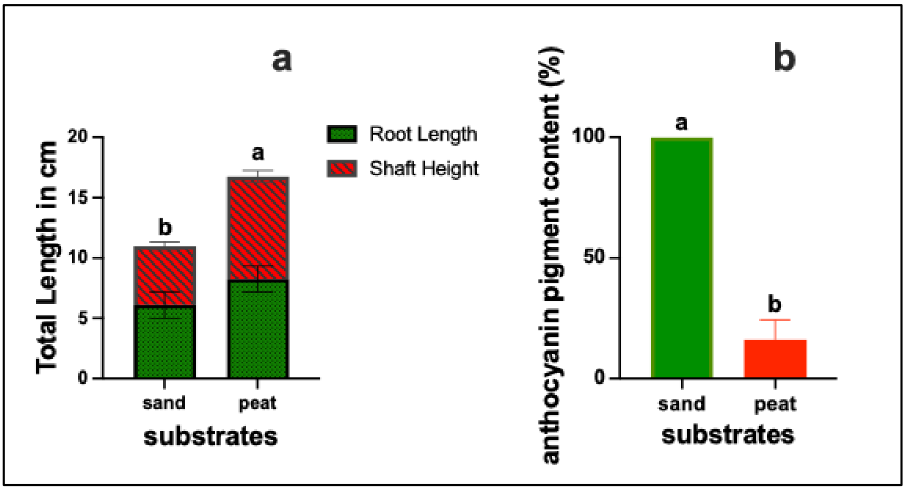

Fig. 3. Effect of substrate on the height, the root length, and total length (a) and concentration of anthocyanin red pigments (b) in *Caralluma tuberculata.*

4 Discussion

This study investigates the differences in influence between sand and peat in plant development of young *Caralluma tuberculata* plants, which showed significant variations between them.

Concerning germination, analysis of data showed that there is no significant difference between sand and peat in relation to final germination percentage (p = 0.358). However, analysis of germination dynamics indicated that peat has better start-up dynamic, which facilitates an earlier beginning of germination processes. This is an indication that seeds in peat experience more favorable conditions for an early initiation of germination processes, which may be related to water retention capacity in peat.

Peat was much more conducive to plant growth, resulting in taller plants (8.49 cm) and longer roots (8.26 cm) in comparison to those grown in sand. This is due to the better attributes of peat, which include higher water retention, good aeration, and presence of organic matter, which provides an ideal condition for above- as well as below-ground growth [14]. Sand, having low water retention capacity, hence, actually retarded plant growth to an extent. This finding is in line with previous research work, which indicated better growth in an organic medium [15]. However, better growth in an inorganic substrate has also been indicated by previous work [16], [17].

Contrary to plant growth, the amount of anthocyanin pigments accumulated in plants grown in sand (100.00%) was significantly higher than those grown in peat (16.20%). Anthocyanin pigments function as protective pigments in plants. High anthocyanin pigments in plants grown in sand imply that plants experienced more environmental stress, which might be caused by water or nutrient stress, as a result of the limiting nature of sand substrate. The stress, in this case, is a result of adverse environmental conditions to which plants respond biologically by increasing anthocyanin pigments as a defensive mechanism to protect themselves from those conditions. This also supports an earlier study, that showed increased stress leading to a higher concentration of anthocyanin pigments in plants [18]. In plants, anthocyanin pigments play a vital role in protecting plants from excessive light, which is harmful to plants as they contain excess energy that, when accumulated, affects plant cells; therefore, anthocyanin pigments work as protective agents in plants in two ways. First, they screen plant cells from excessive light to minimize photoinhibition, which leads to a decrease in the yield of photosynthesis as indicated by a decrease in photosynthetic quantum yield. Secondly, they protect plant cells by functioning as antioxidants in plants, which defend plant cells from damage by eliminating reactive oxygen species in plant cells, an aspect that increases plant cells [20], [21], [22].

5 Conclusion

This comparative study has laid down a clear distinction between the agronomic performance and physiological attributes of *Caralluma tuberculata* based upon the substrate for which it is grown in. From our study, it is clear that peat is better for vegetative growth, as it supports higher values of both shoot and root elongation, which is attributed to better physico-chemical attributes of this substrate. Though it failed to give higher values for final germination, it showed better values for emergence rates in comparison to sand, which increased speed of emergence.

On the other hand, environmental stress caused by cultivating peat moss resulted in an extremely high level of anthocyanin pigments. While this is harmful for biomass production, it is essential for analysis related to biotic stress response.

Finally, in regard to culture substrate for *Caralluma tuberculata*, it is difficult to generalize which substrate to use. For instance, if one seeks to maximize biomass or survival rates in the nursery, they should use peat as a substrate. Conversely, if they wish to stimulate the secretion of a secondary metabolite like anthocyanin, which has potential as an antioxidant, they would find sand to be a better substrate to work with. Such recommendations form a good platform for optimization of protocols for utilization of this plant resource.

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