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# THE EFFECT OF DIFFERENT SUBSTRATES ON MORPHOLOGICAL CHARACTERISTICS OF ACCLIMATIZED *BOWIEA VOLUBILIS*

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**ABSTRACT**
Before acclimatization of *Bowiea volubilis*, *in vitro* propagated bulbs were cleaned and separated into four sizes (from 6 to 15 mm). We formed a total of 10 groups with 30-30 individuals, distributing the sizes evenly. Four types of substrate (peat, perlite, coconut fiber, sand) and their mixtures of 50-50% were used. Previously we examined three parameters: length, diameter and weight of the bulbs; later the weight of the successfully acclimatized plants, the number and length of roots and leaves were measured. The acclimatization was done in one of the greenhouses of the Buda Arboretum, where the plants were grown with fleece covering till one month, irrigated every three days, without the use of nutrient replenishment and artificial lighting. After 3 month period, survived plants developed effectively on peat + perlite, sand + perlite and sand + peat mixtures. In these cases, we achieved the largest increases in roots, green parts, bulbs and total weight.

**Keywords**: *Bowiea*, acclimatization, substrates, morphological features

## INTRODUCTION

*Bowiea volubilis* is an endemic (South African), drought tolerant, traditional medicinal plant with large, poisonous, round shaped bulb, twisted, long (sometimes 3 m) green stems and inconspicuous, yellowish-green flowers (WATT AND BREYER-BRANDWIJK, 1962). The over-collecting of bulbs (which contains cardiac glycosides – CUNNINGHAM, 1988) drastically decreased wild populations, especially when tons of bulbs were sold every years (HANNWEG ET AL., 1996). Because of the small seed production, low germination ratio (DYER, 1964) and difficult division of succulent, fleshy bulbs (with slow regrowth), certain *in vitro* studies were carried out in order to multiply the plants effectively (HAVRANEK AND NOVAK, 1976). Sterilised segments of bulbs (JHA AND SHEN, 1985; COOK ET AL., 1988) or inflorescence stalks (HANNWEG ET AL., 1996) were placed onto MURASHIGE AND SKOOG (1962) media with different hormones (2,4-D, BA, NAA) and thereafter, during multiplication, rooting, acclimatization of shoots, hormone-free media, clean substrates (for example sand + peat) were resulted high volumes of plantlets, even 1000 specimens from 1 explant. In this work, the aim was to find morphological differences between the survived plants and ascertain the effects of different substrates on the success of acclimatization.

## MATERIALS AND METHODS

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### 3.1. Plant material, substrates, culture conditions

Before acclimatization, *in vitro* bulbs were cleaned (their roots, shoots removed, *Figure 1*), and classified into 4 sizes (6-8; 9-10; 11-12; 13-15 mm, *Figure 2*) in order to provide homogenous stocks in every groups. We formed 10 groups with 30-30 individuals, distributing the sizes evenly. Four types of substrate (peat, perlite, coir, sand) and six kinds of fifty-fifty percent combinations were used (*Figure 3*). We acclimatize the stocks in one of the greenhouses of the Buda Arboretum, where the plants grown with veil foil (fleece) covering until one month and during the whole period (3 month), irrigated every three days, without nutrient replenishment and artificial lighting.

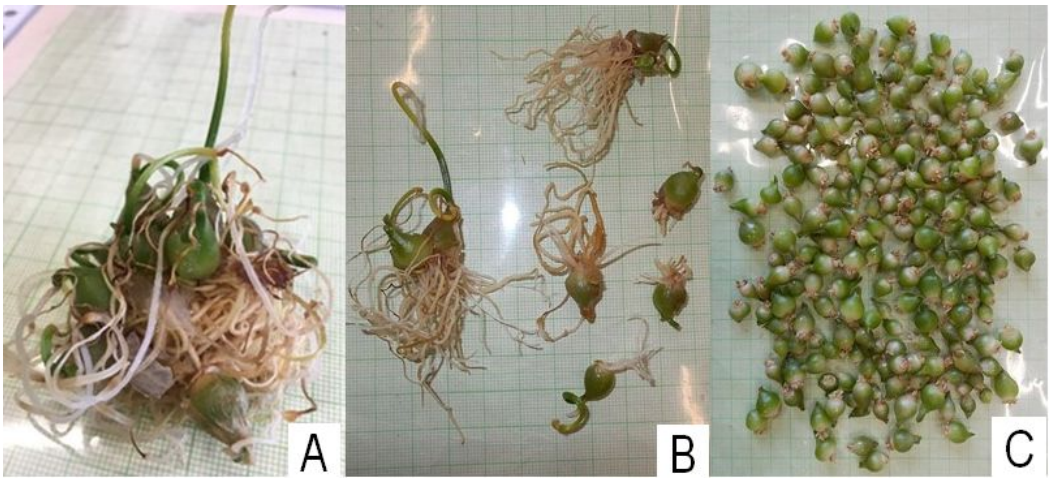


**Figure 1:** *Bowiea volubilis in vitro* bulbs in clusters before division (A), before (B) and after (C) cleaning

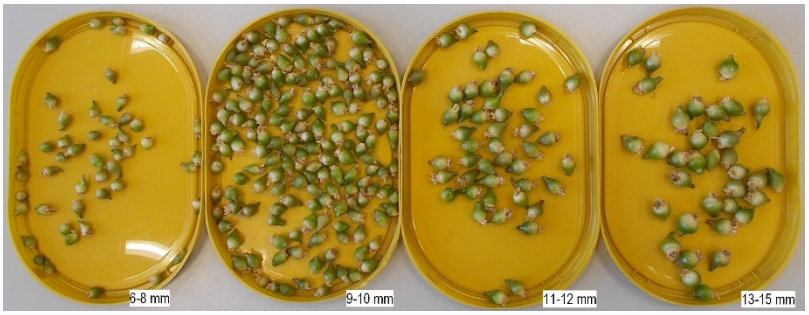


**Figure 2:** Classified *in vitro* bulbs (ready to acclimatization)

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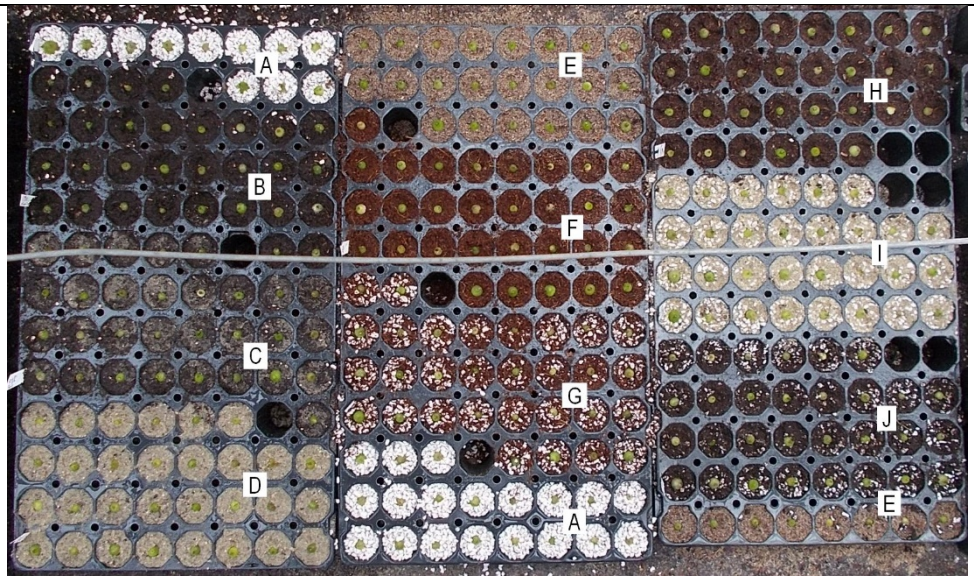


**Figure 3:** Start of acclimatization - newly planted bulbs in different substrates (A: perlite, B: peat, C: sand + peat, D: sand, E: sand + coir, F: coir, G: coir + perlite, H: peat + coir, I: sand + perlite, J: peat + perlite)

Previously we examined three parameters: length, diameter and weight of the bulbs; 3 months later the same bulb values and total weight of the successfully acclimatized plants, the number and length of roots, leaves were measured.

**Data and statistical analysis**
Data were evaluated by SPSS. An analysis of variance (ANOVA) was conducted to calculate the statistical significance of all data presented. When significant differences between treatments were found, the means were separated by Tukey's test at $p < 0.05$.

## RESULTS

**Fresh total plant and bulb weight**
Averagely, the heaviest (2.05 g) plants (as measuring total plant weight) were found on peat + perlite and pure sand or perlite resulted significantly easier (0.75-0.78 g) specimens. If we combined sand with peat or perlite, plants with relatively good weight (1.67 and 1.74 g) were obtained. Investigation of bulb weight (certainly after defoliation and remove roots), we got similar tendency. Thus, plants developed the heaviest bulbs on peat + perlite (0.65 g), sand + perlite (0.64 g) and sand + peat (0.53 g), and the easiest bulbs on 100% sand or perlite (0.35 and 0.36 g, *Figure 4*).

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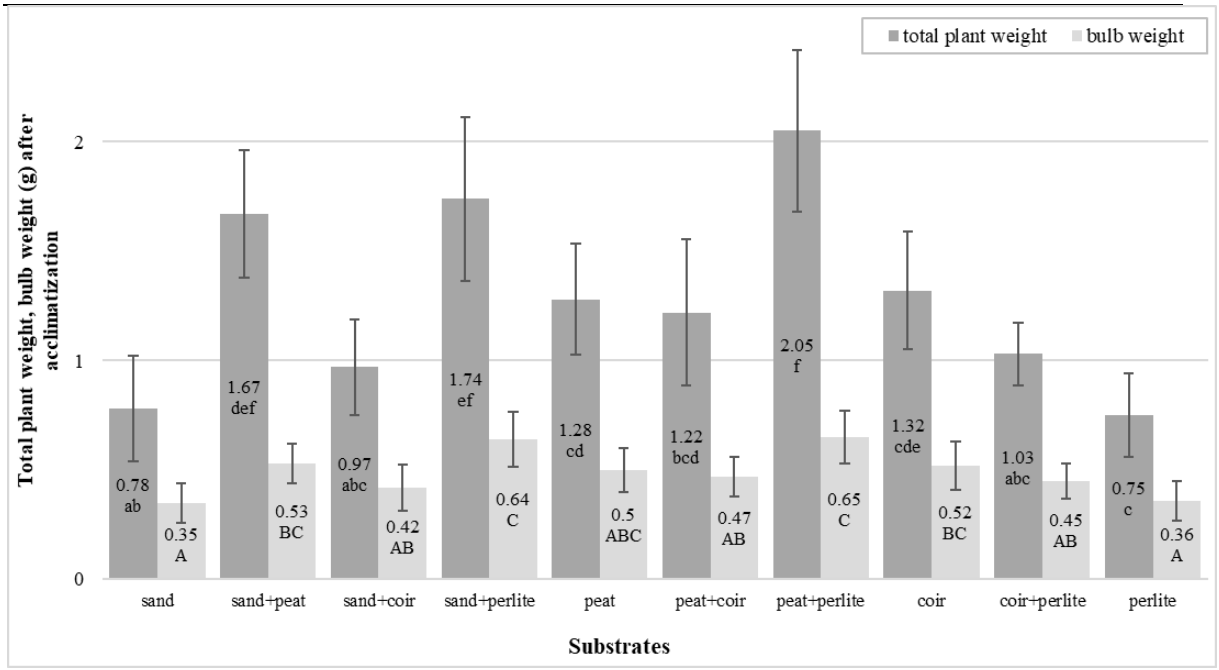


**Figure 4:** Total plant weight, bulb weight of *Bowiea volubilis* acclimatized plants. Means with different letter are significantly different by Tukey's test at $p < 0.05$

## Fresh bulb height and diameter

We noticed that during the acclimatization period, sizes (height and diameter) of bulbs decreased on average despite of their larger weight, probably due to water storing and tissue transformation. It was observed mainly in 100% peat with the lowest sizes (7.7 mm and 8.47 mm), although its bulbs mass values can be said to be average. On the other hand, sand + perlite and sand + peat resulted the largest height (10.9 mm) and diameter (10.27 mm) values after acclimatization (*Figure 5*).

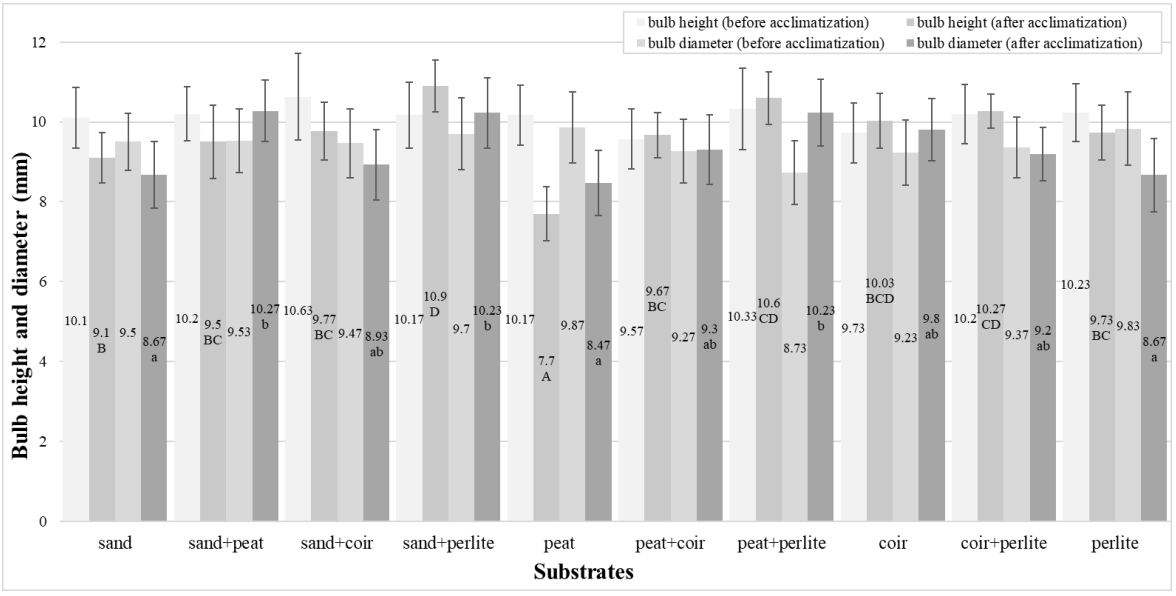


**Figure 5:** Bulb height and diameter of *Bowiea volubilis* acclimatized plants. Means with different letter are significantly different by Tukey's test at $p < 0.05$

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## Root number and length

According to the root number, all groups produced almost the same values (5-6) without considerable differences. By contrast (in case of root length), we found significantly longest (69 mm) roots on perlite combined with peat or sand and the shortest on pure sand (38 mm). We have good results (with roots longer than 60 mm) in the presence of 100% coir, 50-50% coir + perlite (*Figure 6*).

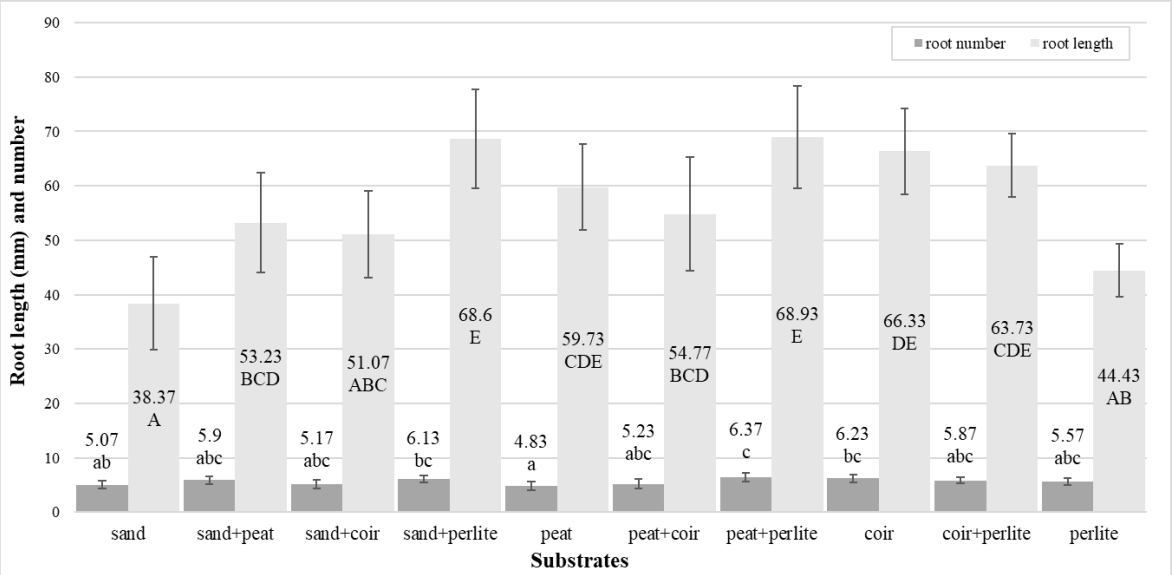


**Figure 6:** Root number and length of *Bowiea volubilis* acclimatized plants. Means with different letter are significantly different by Tukey's test at $p < 0.05$

## Leaf number and length

Normally, young *Bowiea volubilis* specimens can produce only one or two (rarely three) simple, straight leaves without generative organs. For this reason, we found almost the same leaf number in all group without great differences. In case of leaf length, peat + perlite resulted the longest (272 mm) leaves, and the shortest ones (128 mm) on 100% sand. Compare with nearly all the other group, the latter substrate has negative effect with significant differences (*Figure 7*).

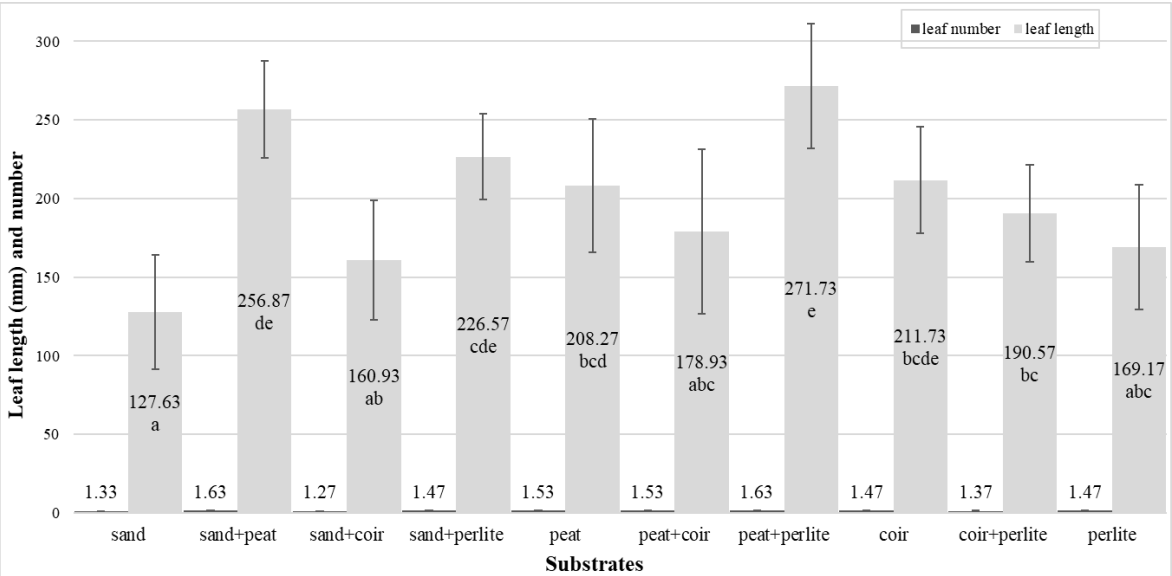

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**Figure 7:** Leaf number and length of *Bowiea volubilis* acclimatized plants. Means with different letter are significantly different by Tukey's test at $p < 0.05$

Larger, older bulbs develop longer, twisted adult shoots with small, inconspicuous, greenish-yellow flowers. In our trial, only few individuals (*Figure 8: A*) produced this kind of shoots (because of small, young bulbs with mainly juvenile leaves, *Figure 8: B*), but as it turned out, bulbs with originally larger weight have better chance to develop curly, flowering stalks and achieve larger plant weight. However, production of adult typed shoots required higher energy from the bulbs; so, lower bulb weight gain was typical in these cases (*Figure 9*).


**Figure 8:** Acclimatized *Bowiea volubilis* plants with adult shoot (A), juvenile leaves (B)

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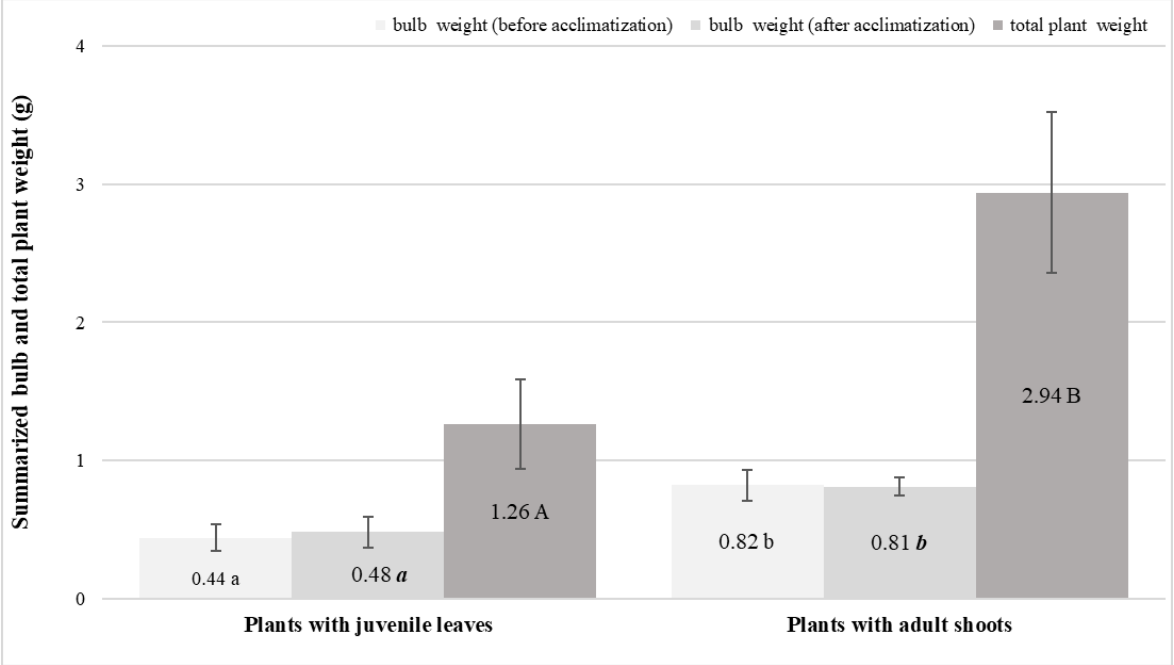


**Figure 9:** Summarized *Bowiea volubilis* bulb weight (before and after acclimatization), total plant weight of plants with juvenile leaves and adult typed shoots

## DISCUSSION

All in all, our results showed that the plants developed efficiently on peat + perlite, sand + perlite and sand + peat mixtures. In these cases, we achieved the greatest increase in bulbs and total weight, as well as the most intense shoot and root formation. Plants grew the least in the case of 100% sand or perlite, but interestingly, the mixture of these substrates yielded significantly better weight values than using only pure sand or perlite. Furthermore, a larger bulbs weight have a better chance to develop curly, flowering adult stalks.

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