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# Propagation potential for the conservation of *Brackenridgea zanguebarica* Oliv., a critically endangered plant species endemic to Vhembe District in Limpopo Province (South Africa)

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## Summary

*Brackenridgea zanguebarica* Oliv. is an important multipurpose tree valued for its medicinal uses in Vhembe District. The unsustainable harvesting coupled with poor seed germination in the wild is threatening its regeneration; which poses a challenge in efforts to its conservation. This study was conducted to identify suitable methods for propagating *B. zanguebarica* species using seeds and stem cuttings. Seeds propagation was carried out to evaluate the effect of various pre-treatments. Vegetative propagation was tested to assess if *B. zanguebarica* could be successfully propagated via stem cuttings with appropriate treatments. The results showed that *B. zanguebarica* seeds did not germinate at all under any of the conditions tested. Stem cuttings presented a possibility of propagating this species despite the poor results obtained, where 51% of cuttings across all treatment produced buds and 17% only developed leaves without any root development. The growth media had insignificant ($P > 0.05$) effect on some vegetative growth parameters, while growth hormones showed significant ($P < 0.05$) effect in all the vegetative growth parameters of stem cuttings where IAA performed better than IBA and NNA. However, their interaction were significant ($P < 0.05$) on all the growth parameters of *Brackenridgea zanguebarica* stem cuttings except on the percentage of cuttings that produced buds ($P = 0.107$). The findings showed that *B. zanguebarica* is difficult to propagate sexually and asexually, hence, further studies are needed to identify suitable methods for both seed and vegetative propagation of this plant.

**Key words:** Multipurpose tree; seed propagation; stem cuttings; auxins; growth media.

## Introduction

The on-going disappearance of biological diversity due to various anthropogenic activities has increased drastically during the last few decades, to the point where many species are now threatened with extinction (Hayes and Hayes, 2013). Many plant species are threatened due to indiscriminate overexploitation coupled with insufficient efforts made on the renewal of natural resources, (Chen et al., 2016). The most affected species are those with small populations and narrow geographical ranges (Wilson et al., 2004). This trend is alarming and immediate conservation measures are required to safeguard many of these species (Sharanappa and Rai, 2011). Many plant species have the potential to be propagated with both sexual and asexual means. These techniques play an important role in the conservation of numerous threatened plant species with poor natural regeneration and offer a promising way for saving plants from extinction.

In South Africa, *Brackenridgea zanguebarica* Oliv. is a species comprised of a small population of plant found only in Thengwe village within the Vhembe District of Limpopo Province. It has been identified as an endemic plant species of that region. It is locally known as “Mutavhatsindi” and commonly known as “Yellow peeling plane”. The plant belongs to the family Ochnaceae and can grow into a tree of 10 m high (Tiawoun et al., 2018). Its roots and barks are widely harvested and traditionally used in the treatment of amenorrhoea, swollen ankles, wounds, diarrhea and various others (Arnold and Gulumian, 1984; Möller et al., 2006; Bruschi et al., 2011). Due to its multiple uses, the indiscriminate extraction and overexploitation of this important plant is depleting its population at an alarming rate. It has now been categorized according to the International Union for Conservation of Nature (IUCN) Red List of South Africa as a Critically Endangered plant species (Williams et al., 2013). Detailed information concerning the propagation is required to increase the availability of this plant species and to expand its distribution to new areas in the region. Cultivation is crucial for *in situ* and *ex situ* conservation for this threatened plant species.

There is therefore a need to take an initiative for mass propagation and reestablishment of the species in areas it used to occur in nature. The natural regeneration of *B. zanguebarica* from seeds in the wild is unsatisfactory in generating adequate plant material (Mutshinyalo, 2011) and requires a long time to germinate. This is probably due to extremely complex seed dormancies that are not well understood and the vegetative regeneration process that has not yet been fully investigated. In order to minimize dependence of propagation by seeds, the use of growth media and hormonal supplements in vegetative propagation can be an effective alternative or supplementary method to seed propagation. Many studies showed that growth media and hormonal concentration for vegetative propagation has successfully improved root initiation on stem cuttings (Kassahun and Mekonnen, 2012; Kiuru et al., 2015).

To ensure the conservation and the perpetuation of *B. zanguebarica*, the present study was aimed at enhancing its propagation by developing an understanding of sexual and asexual propagation of this species. The specific objectives were to develop a protocol for seed germination by testing several pre-treatments requirements and assess *B. zanguebarica* stem cuttings’ response to various auxins in different growth media in order to develop an efficient method for vegetative propagation.

## Materials and methods

### Study area and plant collection

The collection of fruits and stem cuttings from *B. zanguebarica* trees was conducted in the Brackenridgea Nature Reserve (BNR) and transported to the University of Venda. This reserve is found in the Vhembe District of Limpopo Province, South Africa. It is located between the latitudes of 22° 24’ and 23° 36’S, and longitudes of 29° 12’ and 31° 12’E. The climate of the study area is semi-arid (Nel and Nel, 2009) with an average annual rainfall of about 350 mm (Mzezewa et al., 2010); and the mean annual maximum and mini-

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mum temperatures are 26.5 °C and 16 °C respectively. The soil type is mainly stony with shallow sandy loam (Mutshinyalo, 2011).
To assess the propagation capacity of *B. zanguebarica* seeds and stem cuttings, the experiments were carried out in the shade house at the School of Agriculture, University of Venda (South Africa).

### Seed propagation

The fresh and mature black fruits used in the experiments were picked from five different *B. zanguebarica* trees (Fig. 1a) on the 30th of January 2017. A total of 360 seeds used for all the pre-treatments including control, were manually de-pulped from the fruits (Fig. 1b), which were washed with distilled water and left to dry for 24 h at room conditions.


**Fig. 1:** *Brackenridgea zanguebarica* seed. (a) Fresh fruits (b) seed de-pulped from the fruit.

After this procedure, the seeds were subjected to nine pre-treatments:
**T1**: Seeds soaked in concentrated sulfuric acid (95-97%) for 30 min,
**T2**: Seeds soaked in concentrated sulfuric acid (95-97%) for 60 min,
**T3**: Seeds soaked in hot water (100 °C) for 5 mins,
**T4**: Seeds soaked in hot water (100 °C) for 10 min,
**T5**: Seeds soaked in cold water for 24 hs,
**T6**: Seeds soaked in cold water for 48 h,
**T7**: Seeds cold stratified at 4 °C for 60 d,
**T8**: Seeds cold stratified at 4 °C for 90 d,
**T9**: Control (untreated seeds).

Seeds treated with concentrated sulfuric acid (95-97%) were washed thoroughly under tap water to remove all the acid residuals.
Forty seeds were used for each pre-treatment and each pre-treatment had four replicates of 10 seeds. Each seed was sown to about the same depth as the size of the seed in a perforated black plastic bag containing the Hygromix growth medium, and placed on a metal bench in the shade house at the School of Agriculture on the 1st of February 2017. Watering was done when necessary every two to three days to maintain the moisture content for better seed germination conditions throughout the duration of the experiments of twelve months.

### Source of plant material and preparation of stem cuttings

Healthy semi hardwoods stem were collected in February 2018 from fresh branches of *B. zanguebarica* trees growing in the BNR. A total of 200 stem cuttings were uniformly trimmed into 10 cm long pieces, with a diameter of about 5 mm and each cutting having four to five nodes; these were used for all the treatments. After taking all the leaves off, the cuttings were treated with three types of auxins, namely, Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) at different concentrations and the controls were treated with distilled water. All the cuttings were then planted in two types of growing media – top site soils (collected in the reserve) and Hygromix.

### Preparation of plant hormones (auxins) and growth media

Each plant hormone (hormone powder) was prepared by dissolving a calculated amount of IAA, IBA and NAA in distilled water to obtain concentrations of 50 mg/ml, 100mg/ml, and 150 mg/ml respectively. The control solution was distilled water. The basal ends of about 2 cm of the cuttings were dipped for 30 sec in the prepared auxin treatment. Each treatment was replicated four times and each replication consisted of 5 cuttings. A total of 200 stem cuttings were used for this experiment and the treated stem cuttings were individually planted into a perforated black plastic bags filled with growing media (top site soil and Hygromix). Each growth medium had 100 cuttings that were maintained in the shade house and watered when necessary every two to three days, until the termination of the experiment (90 d).

**Tab. 1:** Total number of cuttings in different types and concentrations of auxins and different growth media.

| Auxins and Growth media | Concentrations of auxins (mg/ml) | | | | Total number of cuttings |
|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | |
| **IAA** | | | | | |
| Site soil | - | 10 | 10 | 10 | 30 |
| Hygromix | - | 10 | 10 | 10 | 30 |
| **IBA** | | | | | |
| Site soil | - | 10 | 10 | 10 | 30 |
| Hygromix | - | 10 | 10 | 10 | 30 |
| **NAA** | | | | | |
| Site soil | - | 10 | 10 | 10 | 30 |
| Hygromix | - | 10 | 10 | 10 | 30 |
| **Control** | | | | | |
| Site soil | 10 | - | - | - | 10 |
| Hygromix | 10 | - | - | - | 10 |

### Data recording

The following parameters were recorded:

(1) Days to first buds appearance: It was recorded from the day of planting to the minimum number of days taken to first buds appearance from one cutting of each treatment and then averaged to get the mean value.

(2) Percentage of cuttings that produced buds =
$$\frac{\text{Number of cuttings with buds}}{\text{Total number of cuttings planted}} \times 100$$

(3) Average number of buds per cutting: The total number of buds per cutting was counted in each treatment after 45 d and then averaged to get the mean value.

(4) Days to first leaves development: It was recorded from the day of planting to the minimum number of days taken to first leaves development from one cutting of each treatment and then averaged to get the mean value.

(5) Percentage of cuttings that produced leaves =
$$\frac{\text{Number of cuttings with leaves}}{\text{Total number of cuttings planted}} \times 100$$

(6) Average number of leaves per cutting: The total number of leaves per cutting was counted in each treatment after 90 d and then averaged to get the mean value.

(7) Percentage of cuttings that produced roots =
$$\frac{\text{Number of cuttings with roots}}{\text{Total number of cuttings planted}} \times 100$$

**Data analysis**

Two-way ANOVAs were performed to evaluate the effects of auxins concentration and growth media on the mean of each growth parameter mentioned above. The means of the significantly different growth parameters were separated using Tukey test Honestly Significant Differences (HSD), with $P < 0.05$ being considered as statistically significant. All statistical analyses were conducted using Microsoft Excel 2013.

## Results and discussion

**Seed germination**

In this study, *Brackenridgea zanguebarica* seeds were subjected to numerous pre-treatments known to enhance seed germination in other species. These were pre-treatments with sulfuric acid, hot water, cold water, and stratification at low temperature. However, none of the 360 seeds germinate within 12 months of the experiment. None of the treatments was able to overcome the seed dormancy of *B. zanguebarica*.

The inability of all pre-treated seeds to germinate might be due to the hard seed coat, which prevented water imbibition and gas exchange. *Brackenridgea zanguebarica* seeds seem to exhibit deep physical dormancy leading to poor seed germination. There are reports that revealed that seeds of many species showed delayed germination because of hard seed coats (El-Juhany et al., 2009; Babashpour-Asl et al., 2011; Zhang et al., 2015). This physical dormancy is clearly one of the problems that make this species difficult to propagate from seeds and this could explain partially the scarcity of this species in the wild.

Further experimentations should include the combination of treatments, as well as other pre-treatment methods should be tested. For example, physical dormancy can be broken by fluctuating temperatures, fire and passage through the digestive tracts of animals (Baskin and Baskin, 1998). Baskin (2003) revealed that the fluctuating temperatures promoted dormancy break in impermeable seeds of *Stylosanthes humilis* and *S. hamata* (Fabaceae). Additionally, the application of gibberellic acid (GA3) is also necessary in case this seed may present physiological dormancy.

Lack of information about seed propagation of *Brackenridgea zanguebarica* remains an immense challenge to developing a successful technique for promoting germination and conserving this plant species. Thus, at the current state, preference should be given to vegetative propagation rather than propagation from seeds for large-scale plant production.

**Effect of various auxins concentration and growth media on the vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings.**

Stem cuttings of *B. zanguebarica* with four to five nodes each were treated with three kinds of auxins at three concentrations each, to evaluate the hormonal effect on vegetative growth. The cuttings was planted in soil from the collection site or Hygromix, monitored for 90 d and several growth parameters were recorded.

For all the treated cuttings including the controls, the first bud emergence (Fig. 2a) was in the range of 9.5 to 13 d (Tab. 3). The effects of auxins concentrations were highly significant ($P < 0.001$) for days to first buds emergence while the effects of growth media on days to first buds development were not significant ($P > 0.05$). However, significant interaction effects ($P < 0.05$) were observed between auxins concentrations and growth media for days to first buds appearance (Tab. 2).

Percentage of cuttings that produced buds was significantly influenced by growth media and auxins concentration but not significantly affected by their interaction (Tab. 2). The highest percentage of cuttings that produced buds was recorded under site soil than those observed under Hygromix (Tab. 3).

Cuttings from all treatment groups developed buds and the average number of buds per cutting varied from 0.4 to 3.7. Average number of buds per cuttings after 45 d was significantly affected by both hormone concentration and growth media. Their interaction also had significant effect on the average number of buds per cuttings (Tab. 2). Most of the buds wilted and started dying during their development and by day 45 no further bud growth was observed in any of the treatments and control cuttings. Only a few buds were able to survive and produce leaves (Fig. 2b).

There was no significant ($P > 0.05$) effect of growth media on the average day to first leaves development. But, day to first leaves development reveals highly significant ($P < 0.001$) effect of auxins concentrations. The interaction between auxins concentrations and growth media showed significant difference ($P < 0.05$) on time taken for first leaves development (Tab. 2).

Percentage of cuttings that produced leaves was significantly ($P < 0.05$) affected by growth hormone and the interaction between growth hormone and growth media. But insignificant ($P > 0.05$) effect of growth media (Tab. 2). The highest percentage of cuttings that produce leaves was observed in IAA100 (40%) when propagated in both site soil and Hygromix; whereas, no cuttings produced leaves in IBA150 and NAA100 (Tab. 3).

At the end of the experiments (90 d) some leaves were well expanded (Fig. 2c). The number of leaves per stem cutting treated with different auxin, including control, and planted in different growth media varied from 0.1 - 2. There was significant ($P < 0.05$) effect of hormones application on the average number of leaves per stem cutting with no significant ($P > 0.05$) effect of growth media. The average number of leaves per stem cutting was affected by the interaction of various auxins and growth media at $P < 0.05$ (Tab. 2).

Overall, two-way ANOVAs (Tab. 2) showed that various auxins concentrations were highly significant ($P < 0.001$) in the mean number of all the vegetative growth parameters tested while only the percentage of cuttings that produced buds and the average number of buds per cuttings after 45 d were significantly affected by the growth media. However, the interaction of auxins concentrations and growth media were significant ($P < 0.05$) on the growth parameters of *Brackenridgea zanguebarica* stem cuttings except on the percentage of cuttings that produced buds ($P = 0.107$).

Remarkably, none of the cuttings had produced roots by the end of the experiment after 90 d (Tab. 3).

With auxin type and concentration, and their interaction with growth media, it was possible to generate from *B. zanguebarica* stem cuttings, some of the vegetative growth parameters. However, based on the performance of the untreated cuttings in all the parameters studied, it was clear that the application of all these treatments did not considerably confer any advantage for the vegetative growth parameters. All treated and untreated cuttings were ineffective to induce root growth on stem cuttings. Even though, the cuttings were more responsive to auxins treatment than control, the difference was not statistically significant indicating that *B. zanguebarica* stem cuttings can develop buds and leaves without the requirement of hormones. Similar results were also found in *Lippia javanica* and *Vitex leucoxylon* stem cuttings which showed no significant differences between cuttings with growth hormone application and untreated cuttings on some vegetative growth parameters studied (Tiwari et al., 2015).

Different auxins have been successfully used for the rooting of many plant species. According to Stuepp et al. (2017), plant growth hormones control all phases of growth and development from embryogenesis to senescence. The same authors reported that, auxins are critical in the regulation of many aspects of plant growth and development and their endogenous levels are considered important in the process of root induction. Root growth, however, was not recorded in

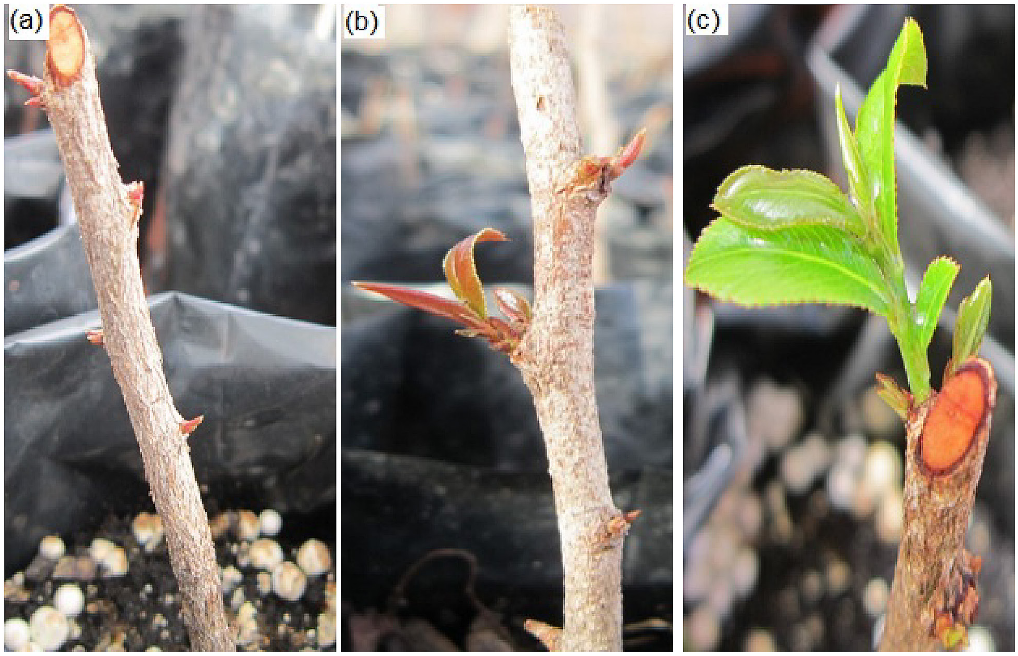


**Fig. 2:** Example of different stages of vegetative growth parameters used to score the response of *Brackenridgea zanguebarica* stem cuttings to different treatments in both growth media. (a) Buds emergence; (b) leaves appearance; (c) leaves expansion.

**Tab. 2:** Two-way ANOVA of the parameters of *Brackenridgea zanguebarica* stem cuttings showing the effects of various auxins concentrations and growth media and their interaction.

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| **Days to first buds appearance** | | | | | | |
| Growth media | 1.8 | 1 | 1.8 | 3.375 | 0.071 | 4.001 |
| Auxins concentration | 51.2 | 9 | 5.689 | 10.667 | 0.000 | 2.040 |
| Growth media × Auxins concentration | 10.2 | 9 | 1.133 | 2.125 | 0.041 | 2.040 |
| Within | 32 | 60 | 0.533 | | | |
| **Percentage of cuttings that produced buds** | | | | | | |
| Growth media | 1445 | 1 | 1445 | 10.081 | 0.002 | 4.001 |
| Auxins concentration | 16725 | 9 | 1858.333 | 12.965 | 0.000 | 2.040 |
| Growth media × Auxins concentration | 2205 | 9 | 245 | 1.709 | 0.107 | 2.040 |
| Within | 8600 | 60 | 143.333 | | | |
| **Average number of buds per cuttings after 45 d** | | | | | | |
| Growth media | 2.926 | 1 | 2.926 | 9.377 | 0.003 | 4.001 |
| Auxins concentration | 61.145 | 9 | 6.794 | 21.772 | 0.000 | 2.040 |
| Growth media × Auxins concentration | 6.190 | 9 | 0.688 | 2.204 | 0.034 | 2.040 |
| Within | 18.723 | 60 | 0.312 | | | |
| **Day to first leaves development** | | | | | | |
| Growth media | 0.0125 | 1 | 0.013 | 0.020 | 0.889 | 4.001 |
| Auxins concentration | 1584.563 | 9 | 176.063 | 276.176 | 0.000 | 2.040 |
| Growth media × Auxins concentration | 1669.363 | 9 | 185.485 | 290.956 | 0.000 | 2.040 |
| Within | 38.25 | 60 | 0.6375 | | | |
| **Percentage of cuttings that produced leaves** | | | | | | |
| Growth media | 180 | 1 | 180 | 3.6 | 0.063 | 4.001 |
| Auxins concentration | 10580 | 9 | 1175.556 | 23.511 | 0.000 | 2.040 |
| Growth media × Auxins concentration | 1520 | 9 | 168.889 | 3.378 | 0.002 | 2.040 |
| Within | 3000 | 60 | 50 | | | |
| **Average number of leaves per cutting after 90 days** | | | | | | |
| Growth media | 0.144 | 1 | 0.145 | 1.040 | 0.312 | 4.001 |
| Auxins concentration | 29.470 | 9 | 3.275 | 23.558 | 0.000 | 2.040 |
| Growth media × Auxins concentration | 2.823 | 9 | 0.314 | 2.257 | 0.030 | 2.040 |
| Within | 8.34 | 60 | 0.139 | | | |

**Tab. 3:** Effect of auxins concentration and growth media on vegetative growth parameters of *Brackenridgea zanguebarica* stem cuttings. Data are shown as mean values of four replicates ± SE.

| | | | Growth Parameters | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Buds | | | Leaves | | | Roots |
| Growth media | Auxins concentration | | Days to firs buds appearance | % of cuttings that produced buds | Average number of buds per cutting after 45 d | Day to firs leaves development | % of cuttings that produced leaves | Average number of leaves per cutting after 90 d | % of cuttings that produced roots |
| | IAA | 50 | 10.5 ± 0.6[abc] | 70 ± 16.3[efg] | 3.1 ± 0.8[hij] | 21 ± 1[cd] | 25 ± 4[de] | 0.5 ± 0.3[ab] | 0 |
| | | 100 | 09.5 ± 0.6[a] | 60 ± 0.0[cdef] | 3.7 ± 0.6[j] | 18.5 ± 1.3[b] | 40 ± 8.1[f] | 2 ± 0.7[d] | 0 |
| | | 150 | 11.5 ± 0.6[cde] | 30 ± 11.5[a] | 0.4 ± 0.3[a] | 21 ± 0.8[cd] | 15 ± 4[bcd] | 0.4 ± 0.2[ab] | 0 |
| site soil | IBA | 50 | 11.5 ± 1.3[cde] | 60 ± 0.0[cdef] | 2.9 ± 0.6[ghij] | 20.5 ± 1[c] | 15 ± 4[bcd] | 0.25 ± 0.1[a] | 0 |
| | | 100 | 11 ± 0.8[bcd] | 65 ± 25.8[defg] | 3.5 ± 0.7[ij] | 22.5 ± 0.6[de] | 20 ± 11.5[cd] | 1.5 ± 0.6[cd] | 0 |
| | | 150 | 13 ± 0.0[f] | 40 ± 16.3[abc] | 1 ± 0.5[abc] | 0 ± 0.0[a] | 0 ± 0.0[a] | 0 ± 0.0[a] | 0 |
| | NAA | 50 | 11.5 ± 6[cde] | 60 ± 11.5[cdef] | 1.6 ± 0.5[abcde] | 21.5 ± 1[cde] | 10 ± 8.1[abc] | 0.7 ± 0.6[ab] | 0 |
| | | 100 | 10 ± 0.0[ab] | 30 ± 11.5[a] | 0.9 ± 0.4[ab] | 20 ± 0.8[bc] | 10 ± 4[abc] | 0.6 ± 0.4[ab] | 0 |
| | | 150 | 12 ± 0.8[cdef] | 55 ± 11.5[cdef] | 2.6 ± 0.4[efghi] | 23 ± 0.8[e] | 35 ± 8.1[ef] | 1 ± 0.5[bc] | 0 |
| | control | 00 | 12 ± 0.8[cdef] | 85 ± 12.2[g] | 2.8 ± 0.7[ghij] | 20 ± 1[bc] | 15 ± 10.8[bcd] | 0.5 ± 0.2[ab] | 0 |
| | IAA | 50 | 10.5 ± 0.6[abc] | 50 ± 4[abcde] | 2.7 ± 0.8[fghij] | 20.5 ± 0.6[c] | 35 ± 0.0[ef] | 0.7 ± 0.2[ab] | 0 |
| | | 100 | 10 ± 0.8[ab] | 60 ± 0.0[cdef] | 2.7 ± 0.5[fghij] | 18.5 ± 1.3[b] | 40 ± 8.1[f] | 1.6 ± 0.6[cd] | 0 |
| | | 150 | 10.5 ± 1[abc] | 30 ± 11.5[a] | 1 ± 0.4[abc] | 21.5 ± 0.5[cde] | 5 ± 0.0[ab] | 0.1 ± 0.1[a] | 0 |
| Hygromix | IBA | 50 | 11.5 ± 1.3[cde] | 60 ± 0.0[cdef] | 2.1 ± 0.5[defgh] | 21 ± 0.8[cd] | 20 ± 11.5[cd] | 0.15 ± 0.1[a] | 0 |
| | | 100 | 11 ± 0.8[bcd] | 35 ± 7[ab] | 2.7 ± 0.7[fghij] | 22.5 ± 0.6[de] | 5 ± 5.8[ab] | 1.5 ± 0.6[cd] | 0 |
| | | 150 | 12.5 ± 0.6[def] | 40 ± 16.3[abc] | 1 ± 0.5[abc] | 20.5 ± 0.6[c] | 10 ± 8.1[abc] | 0.2 ± 0.1[a] | 0 |
| | NAA | 50 | 11.5 ± 0.6[cde] | 40 ± 11.5[abc] | 1.2 ± 0.5[abcd] | 21.5 ± 1[cde] | 10 ± 8.1[abc] | 0.1 ± 0.2[a] | 0 |
| | | 100 | 10.5 ± 0.6[abc] | 30 ± 11.5[a] | 1.5 ± 0.4[abcd] | 0 ± 0.0[a] | 0 ± 0.0[a] | 0 ± 0.0[a] | 0 |
| | | 150 | 11.5 ± 0.6[cde] | 45 ± 11.5[abcd] | 2 ± 0.4[cdefg] | 20 ± 0.0[bc] | 25 ± 8.1[de] | 1.8 ± 0.5[d] | 0 |
| | control | 00 | 10 ± 0.0[ab] | 75 ± 10.8[fg] | 1.8 ± 0.7[bcdef] | 22.5 ± 0.6[de] | 5 ± 7[ab] | 0.1 ± 0.1[a] | 0 |
| | ***Mean*** | | ***11.1*** | ***51*** | ***2.06*** | ***18.8*** | ***17*** | ***0.69*** | ***0*** |

Means followed by the same letter within each column are not significantly di ferent at $P < 0.05$ according to Tukey HSD Test.

this study after the application of auxins to the stem cuttings. Puri and Swamy (1999) and Guo et al. (2009) state that, the effects of auxins application on stem cuttings vary between species because of their genetic differences.

A growth medium with an adequate supply of nutrients is important as it promotes the growth and development processes of plants. The mean values for the different parameters showed that the cuttings of *B. zanguebarica* responded positively to both the growth media used in this study (Table 3). From these results, it is clear that the growth and development of *B. zanguebarica* stem cuttings may not only depend of soil nutrients. According to Takoutsing et al. (2014), a good growth medium must have low water holding capacity and have a considerable level of porosity.

Stem cutting is the most frequent technique used for vegetative propagation of many plant species (Hassanein, 2013) and growth regulators and growth media were found to be effective factors for rooting many plant species (Akinyele, 2010). The performance of untreated cuttings in all the parameters demonstrated that auxin application and growth media may not necessarily be the major factor influencing buds and leaves growth on stem cuttings of this species. Wahab et al. (2001); Hartmann and Kester (1990) stated that the development of buds and leaves from stem cuttings can be attributed to high levels of plant carbohydrate reserve, however, Leakey and Coutts (1989), reported that carbohydrate content can quickly become depleted in cuttings without leaves or with very small leaf area, but increases if leaf area is appropriate; at least until roots start to develop. Stem cuttings without leaves were used in this study and the rapid buds and leaves development was probably due to the use of high carbohydrate reserve and slow reconstitution by stem cuttings, leading to the absence of root development. Similar findings have been obtained by Takoutsing et al. (2014) in leafless cuttings of *Garcinia lucida*, therefore, the type and concentration of auxins applied on *B. zanguebarica* stem cuttings may not have any direct effect on bud and leaf development. These results are similar to the findings of Ullah et al. (2005) who reported that different growth regulators did not have significant effect on the shoot development of *Psidium guajava*. Even though growth regulators and growing media are found to be effective factors for root development of many plant species, several researchers reported that root development from treated cuttings may also depend on a number of factors, such as type of cuttings, growing media, growth regulators, size of cuttings, season of planting (environmental conditions), cutting source (young seedlings or juvenile) and leaf area, (Tchinda et al., 2013; Naidu and Jones, 2009; Takoutsing et al., 2014). The inability of root development in *B. zanguebarica* from stem cuttings may be due to some of these factors and also to the application method of the growing media and growth regulators that did not produce the expected effects. For instance, Ahmad (2006) reported that dipterocarp species failed to root when the stem cuttings dropped their leaves one or two days after planting. Similarly, several studies have reported that stem cuttings without leaves presented a high mortality rate in vegetative propagation (Ofori et al., 1996; Akinyele, 2010). However, many other studies revealed that the presence of leaves on cuttings is necessary to stimulate root initiation (Hartmann et al., 1990; Zem et al., 2016; Belniaki et al., 2018). Therefore, buds and leaves development on stem cutting of this species was an important stage to stimulate root initiation; this has shown the possibility to propagate this species by stem cuttings if appropriate treatments are provided. For this reason, more studies with different interactions of growth media and hormones need to be conducted as these factors may promote root growth and development of *B. zanguebarica* stem cuttings.

## Conclusion

This is the first study to have reported on the propagation by seeds and stem cuttings of *B. zanguebarica*, with the aim of improving and promoting its conservation. The results of this study demonstrated that seeds subjected to different pre-treatments exhibited difficulties in germination under all the tested conditions and this may partially explain the scarcity of this plants species in the wild. Based on the trends observed, the protocol for vegetative propagation has shown, for the first time, promising results despite the very low growth parameters recorded. Despite the unsatisfactory results from both techniques, this study, nevertheless has improved the scientific understanding of seeds and vegetative propagation of *B. zanguebarica*. The nature of seed dormancy of this species is not well understood and further research need to define the type of dormancy before *B. zanguebarica* can be propagated. For vegetative propagation, mixture of auxins, reliable growth media, size of cuttings, environmental conditions and cutting source are required. Tissue culture propagation should be tried as another means for mass production.

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**Conflict of interest**

No potential conflict of interest was reported by the authors

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