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Vegetative propagation of *Echinops giganteus* using stem and root cuttings

Christiana Ngyete Nyikob Mbogue*, Anjah Mendi Grace and Nkemnkeng Francoline Jong

Department of Plant Biology, Faculty of Science, University of Dschang, Dschang, Cameroon.

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Echinops giganteus **(Asteraceae) is a shrub native to Cameroon. As part of a recovery effort focused on** ***Echinops giganteus*****, a vegetative propagation technique was developed. Plants were vegetatively propagated in and out of a non-midst propagator, juvenile stem and root cuttings from the nursery and plant growth regulator hormones of concentrations: 0, 0.5, 1, 2, 3, 4, 5, 8 and 10 g/L. The effect of the cutting positions from the mother plant (apical, medial and basal) and concentration of growth hormones (indole butyric acid and naphthaleneacetic acid) on rooting success were evaluated. Cuttings were dipped in into a commercial insecticide commonly called EAGROW before putting it into hormone concentrations; the dilution media for hormones was alcohol. Rooting occurred with and without auxin treatments but was greatest in the control concentrations (just alcohol) for both hormones; rooting was lowest when hormone concentrations were greater than 3 g/L. Rooting success was evaluated two months after the experiments were started. None of the stem cuttings survived. The control root head cuttings in the propagator had the lowest mortality rates than (NAA 11% and IBA 33%). The trails out of the propagator hard a 0% mortality rate. Vegetative propagation of** ***E. giganteus*** **will allow its large-scale regeneration for a sustainable management plan.**

Key words: Vegetative propagation, stem and root cuttings, indole-3-butyric acid (IBA), ∝-naphthaleneacetic acid (NAA).

INTRODUCTION

The environmental degradation in tropical world is an inevitable outcome of developmental activities. The ecosystem services are now shrinking due to erosion of genetic biodiversity in natural ecosystem. There is faster harvesting of natural resources due to increasing population and demand for material development. The economic growth depends much on the use of natural resources (Upadhyay et al., 2010).

The genus Echinops is of the Asteraceae family and consist of about 120 species distributed world-wide (Garnatje et al., 2004). *Echinops giganteus* has been designated a non-forest timber product (NTFP) in the Congo Basin and the part exploited is the root (Tchatat, 1999).The roots have diverse uses spanning from medicinal, culinary to industrial (Noumi, 1984; Menut et al., 1997). The root of this plant is used to treat heart and gastric troubles (Tene et al., 2004). The root has aromatic properties and has been collected and distilled to obtain essential oils which are used in synergy with those from other plants to eradicate weevils in stored grains (Ngamo

*Corresponding author. E-mail: christytafon@yahoo.com. Tel: (672087320).

et al., 2007; Pérez et al., 2010). This species is also of interest to the fragrance and flavor sectors Menut et al., 1997).

The main problem with *E. giganteus* is that, despite all its importance and its conservation status as a nearly threatened species, no implementation of conservation, management and sustainable use strategies have been put in place, due to lack or insufficient scientific data on their regeneration. This general lack of information is in particular related to lack in Sub- Saharian zone of expertise and infrastructures to carry out propagation experiments. Vegetative propagation is also a practical means for mass production of high quality regeneration stock. Unlike with sexual propagation, the new independent plant produced through vegetative means is a clone in which desirable traits of the donor are preserved (Santoso and Parwata, 2014). Moreover, vegetative propagation can bypass the germination phase to reduce the rotation period of the species. Stem cutting is the most common of vegetative propagation methods for herbaceous and woody plants. Reasons for the popularity generally revolve around the low cost (Waziri et al., 2015) and ease (Dawa et al., 2017) associated with the use of the technique. The success of cutting propagation may be confounded by the age of the donor plant (Ambebe et al., 2017), growth medium (Ashiono et al., 2017), type of cutting (Washa, 2014), phytohormones (Bhardwaj et al., 2017), size of the cutting and health of the donor plant (Kramer and Kozlowski 2014) among others. Furthermore, the responses of the cuttings to some of these factors are species sensitive (Hassanein, 2013). Rooting hormones are very important in the rooting process of cuttings (Wiessman-Ben and Tchoundjeu, 2000). Their beneficiary effect was also confirmed by Aminah et al. (1997), Arya et al. (1994), Al-Saqri and Alderson (1996) and Hartmann et al. (1997). According to Wiessman-Ben and Tchoundjeu (2000) hormones such as auxin (IBA, IAA, NAA) play an important role in root growth, whereas hormones like gibberellins are important in the physiological process of the plants such as in stem elongation and bud development. Among the exogenous rooting hormones, indole-3-butyric acid (IBA) and ∝-naphthaleneacetic acid (NAA) are two synthetic chemicals that have been found to be reliable in root promotion of cuttings. IBA is widely applied for general use because it can remain non-toxic within a wide range of concentrations and improves root initiation of cuttings for most plants species (Al-Barazi and Schwabe, 1982; Hartmann et al., 1997; Hartmann et al., 2002). This research work is therefore designed to come out with the best method for the propagation of *E. giganteus* in the Western Highlands of Cameroon.

MATERIALS AND METHODS

This study was carried out in Menoua, a sub-division fond in the Western Highlands of Cameroon.

Study site

Localization of the Menuoa division

The germination, transplant experiments and part of the floristic inventory was carried out in Dschang situated in the Menuoa Division in the Western Region of Cameroon (Figure 1). It has geographic coordinates, latitude 5° 26'N, longitude 10° 26'E and an altitude 1,400 m. According to the data of the meteorological station of the IRAD of Dschang, there is an equatorial climate characterized by an average annual temperature of 20.1°C and Annual rainfall is 2000 mm on average (Aghofack-Nguemezi and Tatchago, 2010).

The vegetation consists, to a large extent, of savannah grassland, with the Poaceae forming the main vegetation layer, interspersed with a few other annuals, biennials and perennials trees (Ngwa, 1979). According to Aswingnue (2003), the vegetation of this region is both natural and cultivated. The cultivated vegetation consists of planted trees like *Cola accuminata*, Eucalyptus globulus, *Raphia hookeri* and other fruit trees. Eucalyptus globulus lies mostly in the low lying plains, while woody valley and natural forest exist in the watershed area (Tematio et al., 2001). The soil texture is silt-clay-loam which makes it very fertile for agricultural activities in the area (Suh et al., 2015). The soil fertility is as a result of humus, which is a dark volcanic soil from the uplands/hilly areas that has been washed down from the hills and deposited on river banks or beds of streams (Tematio et al., 2001). Plant material: *Echinops giganteus* CD Adams

Seeds collection, selection and preparation

Seeds were collected from the Western Region of Cameroon, in fields where it grows naturally. Mature fruits were collected from the mother plant growing in the wild, dried for two weeks under natural sunlight then matured seeds with healthy grains were selected for germination. Some seeds were randomly selected for viability test by floatation method. The seeds were placed in a bucket of water at room temperature (Wamegni, 1991; Schaal, 2000). The seeds that sank down were classified as viable seeds, while those that floated were classified as non-viable.

Nursery construction

The field was cleared using a cutlass and ploughed with a hoe. Nursery beds measuring 1 by 4 m were established with distance of 50 cm apart. The entire nursery site was shaded with palm fronds. Seed sowing method was by line broadcast. The Blocks were 1 m apart for each nursery site. Nursery beds were monitored and watered after every one day.

Vegetative propagation of seedling cuttings

The non-mist propagator was partitioned into compartments each was constructed following the design modeled by Leakey et al. (1990). It was a wooden frame enclosed in a single sheet of polythene such that the base was completely water tight. The frame was covered tightly with a single piece polythene and closely fitting lid. The polythene base of the propagator was covered with a thin layer of sand to protect the polythene, and onto this was placed a layer of large stones to a depth of 10-15 cm. This was then covered by successive layers of small stones and gravels to a total depth of 20 cm. The space between stones and gravel was filled with water. The saturated layer of stones and gravel was covered by the rooting medium which consisted of a layer of fine river sand treated with fungicide. The rooting medium was made to remain moist by

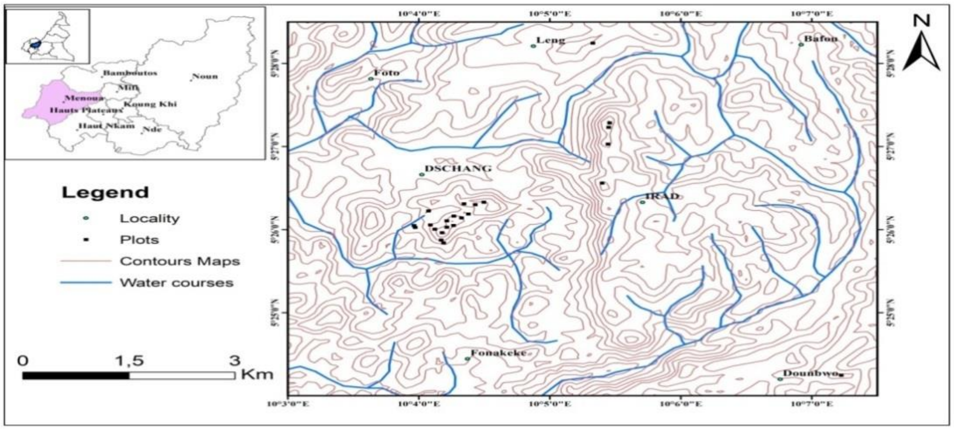

Figure 1. Map of study area in the Menoua Division.

capillarity and was dampened above as necessary. This resulted in a permanently humid environment throughout the propagation period. An open plastic cylinder (tube) of 25 cm long and 4 cm in diameter was vertically inserted in the propagator before filling. This cylinder was used as filling point for the water and allowed a regular check of the water table.

Preparation of cuttings

The plants were allowed to grow for two months at the transplanting sites from which five hundred mature uniformly healthy plants will be used to provide cuttings for this study. This time it was ensured that seedlings attain the necessary vigor. These mature plants were carefully uprooted and cuttings were obtained. Using a sharp knife, cuttings were collected at the stem base [SB], stem middle (SM), stem apex [SA] and root Apex [RA] of 4-6 cm in length each. The cuttings were deepened in to IBA and NAA hormones at different concentration and then put into the sand growth substrate.

For IBA (UN: 2811, LOBAL CHEMIE) 2g of powder hormone was put into 200 ml of alcohol to prepare the mother solution of 10 g/L which was further diluted with alcohol to prepare concentrations (0, 0.5, 1, 2 and 5) g/L; while for NAA (BDH Chemicals Ltd), 0.8 g of powder hormone was put into 80 ml of alcohol to prepare the mother solution of 8 g/L which was further diluted with alcohol to prepare concentrations (0, 0.5, 2 and 4 g/L). The growth hormone concentration range was large reason being that it is the first work done to evaluate the effect of hormone concentration on *E. giganteus* plant

Experimental design

A total of 558 cuttings set in a Complete Randomized Design were used with two Growth Regulator Hormones in the main blocks and four different cutting positions (SB, SM, RA and SA) were tested at the subplot level. At each level, treatments were assigned at random to experimental units so as to have a layout like this; 4 cutting positions *3 repetitions * 2 blocks. Growth hormones were used to enhance rooting; the experiment ran for 16 weeks.

Data collection

For the non-mist propagator experiment, cuttings were assessed for survival, mortality, number of roots, longest root length, shortest root length, and number of leaves.

Data analysis

Data was presented using tables and figures. Data on early growth parameters was subjected to Analysis of Variance (ANOVA) using the statistical programmer XLSTAT where the least significant differences (LSD) between the mean was detected and separated using the Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$.

RESULTS

Results on the vegetative propagation of *E. giganteus*

The results gotten for seedling Apex, Meddle and Base were negative since none of them reported (Figure 2), but never the less root apex cuttings had some results as presented.

Effect of NAA concentrations on growth parameters *E. giganteus* root apex

From the results, it was observed that there was no significant difference amongst the growth parameters that were measured, but there was a difference in mortality rate with respect to hormone concentrations. The control experiment that was without hormone gave the best results for NAA hormone which was significantly different from the rest of the other concentrations that had no significant difference amongst themselves (Table 1). For the mortality rate, the lowest mortality rate of 11% was in the control experiment, but when the NAA hormone was used, it was observed a mortality rate of 89% at concentration 1g/L and the rest of the concentrations gave a 100% mortality rate (Figure 3).

Effect of IBA concentrations on growth parameters *E. giganteus* root apex

From our results, we can saw that there was no significant

Figure 2. Stem cuttings sown after being deepened in hormones (a), stem cuttings not regenerated (b).

Table 1. Effect of NAA concentrations on growth parameters *E. giganteus* root head.

Concentrations	NL	NR	SRL	LRL
0 g/L	11.56 ± 5.42[a]	7.11 ±5.82[a]	0.38 ±0.41[a]	1.79 ± 1.62[a]
1 g/L	0.67 ± 1.89[b]	2.22 ± 6.29[b]	0.06 ± 0.16[b]	0.22 ± 0.63[b]
0.5 g/L	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
10 g/L	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
2 g/L	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
5 g/L	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
Pr > F	0.00	0.00	0.00	0.00
SD	yes	yes	yes	yes

***** Values indicated by the same letters within the columns are not statistically different at $P \leq 0.05$.
NL: Number of leaves, **NR:** Number of roots, **SRL:** Shortest root length, **LRL:** Longest root length

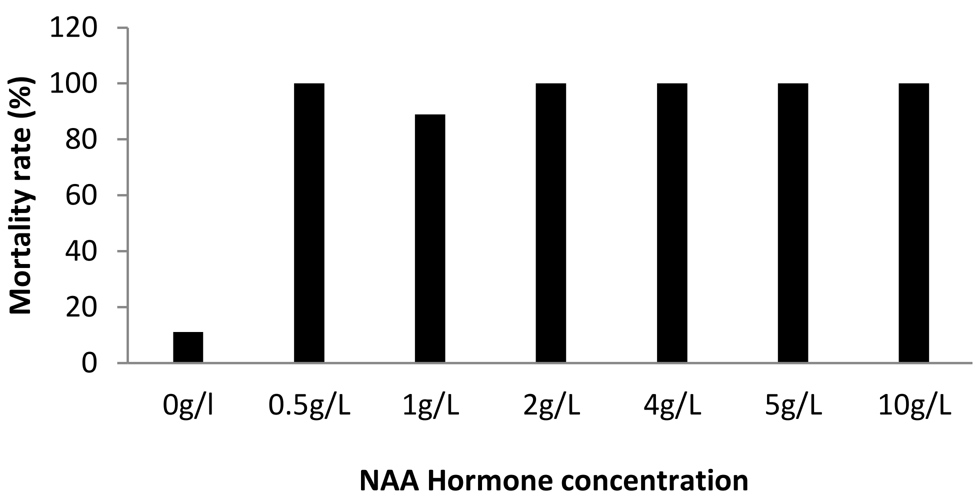

Figure 3. Effect of NAA concentrations on mortality rate of *E. giganteus* root head.

difference amongst the growth parameters that were measured, likewise in hormone concentrations. The control experiment that was without hormone gave us the highest results for IBA hormone which was not significantly different from the rest of the other concentrations that had no significant difference amongst themselves too (Table 2). For the mortality rate, the lowest mortality rate of 33% was in the control

Table 2. Effect of IBA concentrations on growth parameters *E. giganteus* root head.

Concentrations	NL	NR	SRL	LRL
0 g/L	5.89 ± 4.70[a]	2.89 ± 3.25 [a]	0.42 ± 0.86[a]	1.64 ± 2.13[a]
8 g/L	2.67 ± 4.99[ab]	2.00 ± 4.00 [a]	0.06 ± 0.16[a]	0.34 ± 0.97[b]
0.5 g/L	1.67 ± 2.49[b]	1.22 ± 2.39[a]	0.22 ± 2.39[a]	0.24 ± 0.69[b]
4 g/L	2.00 ±3.77[ab]	1.44 ± 3.17[a]	0.03 ±0.09 [a]	0.22 ±0.63 [b]
2 g/L	1.00 ± 2.00 [b]	0.89 ± 1.73[a]	0.16 ±0.44 [a]	0.23 ± 0.66[b]
Pr > F	0.11	0.71	0.58	0.08
SD	No	No	No	No

*****Values indicated by the same letters within the columns are not statistically different at P ≤ 0.05.

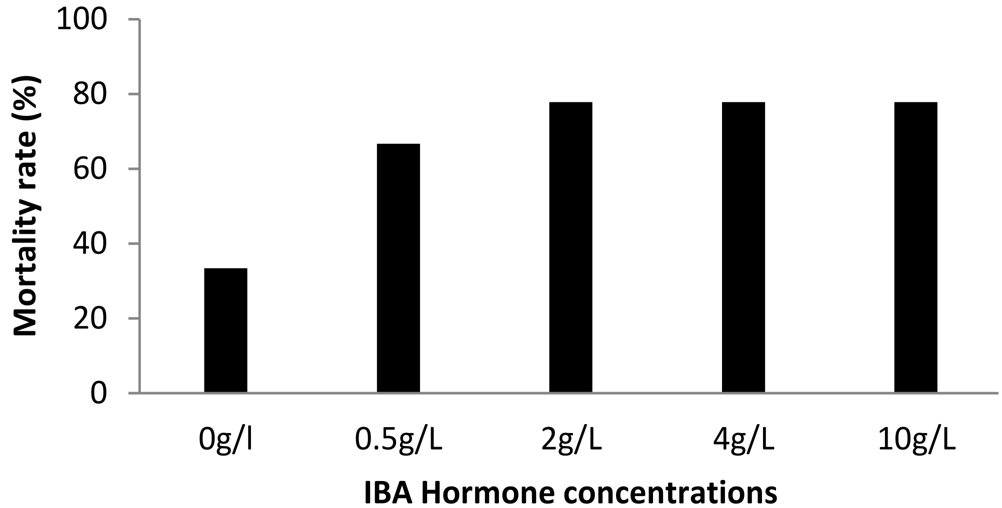

Figure 4. Effect of IBA concentrations on the mortality rate *E. giganteus* root head.

experiment, but when the IBA hormone was used, we observed a mortality rate of 67% at concentration 0.5g/L and the rest of the concentrations gave a 78% Mortality rate each (Figure 4).

Comparism between experiments in and out of the propagator

Table 3 shows that we had significant differences for growth parameters; the number of leaves and longest root length for both experiments and we also saw that when the experiment was carried out of the propagator and without (WOUT) the use of any plant growth hormone, the results were best (Figure 5). This is clearly explained by their mortality rates. For the mortality rate, the lowest mortality rate of 0% was in the experiment out of the propagator, followed by NAA control experiment in the propagator with a mortality rate of 25%. Also, root development was better for root apex cuttings out of the propagator than in the propagator (Figure 6).

DISCUSSION

Target 8 of the Global Strategy for Plant Conservation has a stated goal of ‘‘At least 75% of threatened plant species in ex situ collections, preferably in the country of origin, and at least 20% available for recovery and restoration programs of them included in recovery and restoration programs’’ (Sharrock, 2012). As Pence (2011) noted, Target 8 encompasses the following two goals of ex situ conservation: propagation of plants for recovery of wild populations and to provide backup collections of plants for restorations should wild plants be lost. Seed-based methods are the most efficient for ex situ preservation provided there is enough seed available and germination procedures are adequate (Pence, 2011). Additionally, preservation can be accomplished using propagation by cuttings or other vegetative methods.

In the experiments, hormone concentrations inhibited rooting and regeneration of buds but increased mortality in all cutting positions (Apex, Middle, Base and Root Apex) of *E. giganteus*. The differences in root growth may

Table 3. Comparism between experiments in and out of the propagator.

Parameter	NL	NR	SRL	LRL
WOUT	13.28±7.75 [a]	5.06 ±5.40[a]	1.59 ±2.05[a]	5.73 ±4.44[a]
NAA	11.56± 5.42[b]	3.33 ±3.19[a]	0.39 ±0.41[a]	1.79 ±1.62[b]
IBA	5.89±4.70 [b]	1.67±1.83[a]	0.42 ±0.86[a]	1.64 ±2.13 [b]
Pr > F	0.04	0.18	0.09	0.01
SD	yes	No	No	yes

***Values indicated by the same letters within the columns are not statistically different at P ≤ 0.05.

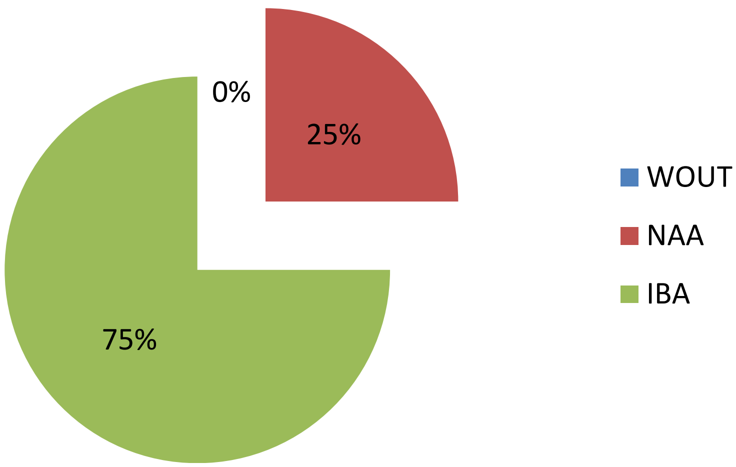

Figure 5. Comparism of mortality rate between experiments in and out of the propagator.

Figure 6. Root head results out of the propagator (a) and in the propagator (b).

be due to the differential effects of the growth regulators on metabolites translocation and carbohydrates metabolism (Abubakar et al., 2019). This was contrary to the results of Monteiro et al. (2010) in evaluation of sweet potato cuttings in sub soil with five concentrations of IBA, Kanmegne et al. (2015) on *Cola anomala* cuttings who reported that apical cuttings result in lower mortality than basal cuttings and those reported on the vegetative propagation by cuttings of *Lovoa trichilioides* (Tchoundjeu and Leakey, 2001) which show that the mortality rate increases from the apical nodes to the basal nodes.

However, our results for vegetative propagation of *E.*

giganteus are in line with those reported on *Khaya ivorensis* cuttings (Tchoundjeu and Leakey, 1996) which suggest that the mortality rate of apical cuttings is higher than that of basal cuttings. These different results indicate that there are interspecific variations in the response of cuttings to different positions on the mother stem, but the determinism of these variations is not well known (Kanmegne et al., 2015). High concentrations of IBA would therefore have a toxic effect on the survival of *E. giganteus* cuttings. This hypothesis is supported by the work of Ezzili and Bajouai (2000) and Houar et al. (2014) on the effect of different types of auxins (IAA, IBA, and NAA) on the rooting of *Simmondsia chinensis* cuttings. This work reveals that the survival of cuttings is inversely proportional to the rate of applied auxins.

In their work, Tchoundjeu and Leakey (2001) have shown that in *Lovoa trichilioides* cuttings from apical knots take root much better than cuttings from basal knots of the same stem. In *E. giganteus*, the rooting rate did not increases with the concentration of IBA, not sharing the same idea with researchers who said IBA has a positive effect on the induction of adventitious root formation (Leakey, 2004; Kanmegne et al., 2017). The results are in line with those obtained by Mapongmetsem et al. (2012) where IBA did not improve the percentage of rooting of cuttings in *Vitex doniana*. These contradictory results indicate that the need for exogenous auxin supply for rooting induction varies from one species to another. This variation may be partly due to differences in the concentrations of endogenous auxin in different plants at the time of excision of cuttings (Leakey, 2004; 2014).

Conclusion

Vegetative propagation is possible for *E. giganteus* without the use of any hormone. This method appears to be promising for establishing cultivated stands, because it permits the increase of improved lines. Traits such as resin content and composition, and regrowth after harvest are some of the most important characteristics to select for. The vegetative propagation process through cuttings of *E. giganteus* is most recommended out of the propagator in an open field during the raining season, without any growth hormone or fertilizers. This results to a great percentage of survived cuttings, rooted cuttings, cuttings with buds, producing a good number of leaves and a healthy plant. Inadequacies of quality planting materials remain the major constraints for establishing large scale plantation programs of *E. giganteus* in Cameroon and other tropical countries. Due to scarcity of seeds, vegetative propagation through root head cutting can be a better and helpful option of multiplication. Considering farmers perception regarding the regeneration and domestication of *E. giganteus,* quality planting materials of *E. giganteus* will inspire farmers to plant more *E. giganteus* at their homesteads as well as to establish nurseries for commercial production.

Recommendation

The Ministry of Environmental Protection should provide uniform training to those involved in the regeneration of *E. giganteus* with a clearly defined organizational framework within which there should be more collaboration among the bodies involved (those from the ministry and the worker) to ensure easy accessibility to resources for effective work done, information flow, supervision and monitoring.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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