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Chapter

Integrated Conservation Approaches for Rescuing, Regeneration and Adaptive Management of Critically Endangered Asteraceae Florae in Africa: A case of *Bothriocline auriculata* Species in Uganda

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Abstract

Among the 62 Bothriocline plant species, *Bothriocline auriculata* is the only endemic species in Uganda. Although this species is capable of thriving in diverse agroecosystems including mountainous areas, bamboo thickets, montane and tropical rainforests, it is only sited along Mt. Elgon slopes, the species' native ecosystem. Unfortunately, for the last two decades, the species' native ecosystem is undergoing very rapid deterioration as the increasing human populations have no option for survival but to clear protective forests and vegetation for arable farming, grazing and settlement. Despite the proven ecological importance of *Bothriocline auriculata* as a fodder plant, nutrient recycling and biodiversity in contributing to ecosystem balance, the species is declining at unprecedented rates. Consequently, the *Bothriocline auriculata* is now on the blink of extinction and is classified as critically endangered. This project aimed to rescue and conserve this species to ensure its full recovery, restoration and conservation under protected ecosystems. The specimens were rescued from the native ecosystem and multiplied into 150 juvenile seedlings. The seedlings exhibited a faster growth rate under well-nourished and moist soil conditions and vice versa. The data confirm the species' ability to thrive in protected ecosystems with favorable weather and soil conditions for ex-situ conservation.

Keywords: biodiversity, conservation, endangered species, endemic species, Africa

1. Introduction

1.1 Background and context

Bothriocline is a genus of angiosperms belonging to the Aster family called Asteraceae [1, 2]. The *Bothrioclines* are hairy herbs with purple flowers whose species range from annual, bi-annual and perennial growth cycles. There are 62 known *Bothrine species*, most of which are native to tropical Africa and a few are native to isolated islands of the Indian Ocean [2]. Among the 62 species [1], *Bothriocline auriculata* (M. Taylor) hereafter referred to as *BAT* is the only endemic species in Uganda [3]. The *BAT* (**Figure 1**) thrives well in the montane forest, mountainous areas and bamboo thickets. The only known locations of *BAT* in Uganda are within Mount Elgon areas between 2745 and 3150 meters above sea level [4]. **Figure 1** presents photos of the *Bothriocline auriculata* (BAT) species.

However, the BAT natural habitat (**Figure 1**) is undergoing an unprecedented rate of ecosystem deterioration and destruction over the last two decades. The rapid ecosystem loss is attributed to exponentially increasing anthropogenic activities from exponentially increasing local populations, who have no livelihood option but to clear the remaining forest and protective vegetation cover to open up more land for arable farming and human settlement [5]. There is also increased frequency and intensity of other anthropogenic activities mainly deforestation for fuelwood and timber as well as overgrazing of livestock along the Mount Elgon slopes, which further destroys the species and its native ecosystems. For instance, the increasing livestock numbers, – which surpass the carrying capacity of the traditional rangelands, force farmers to drive their livestock upwards on the Mount Elgon slopes in search of dense vegetation [5, 6]. Farmers have shifted cultivation upwards because of the virgin fertile lands along the mount slopes [6].

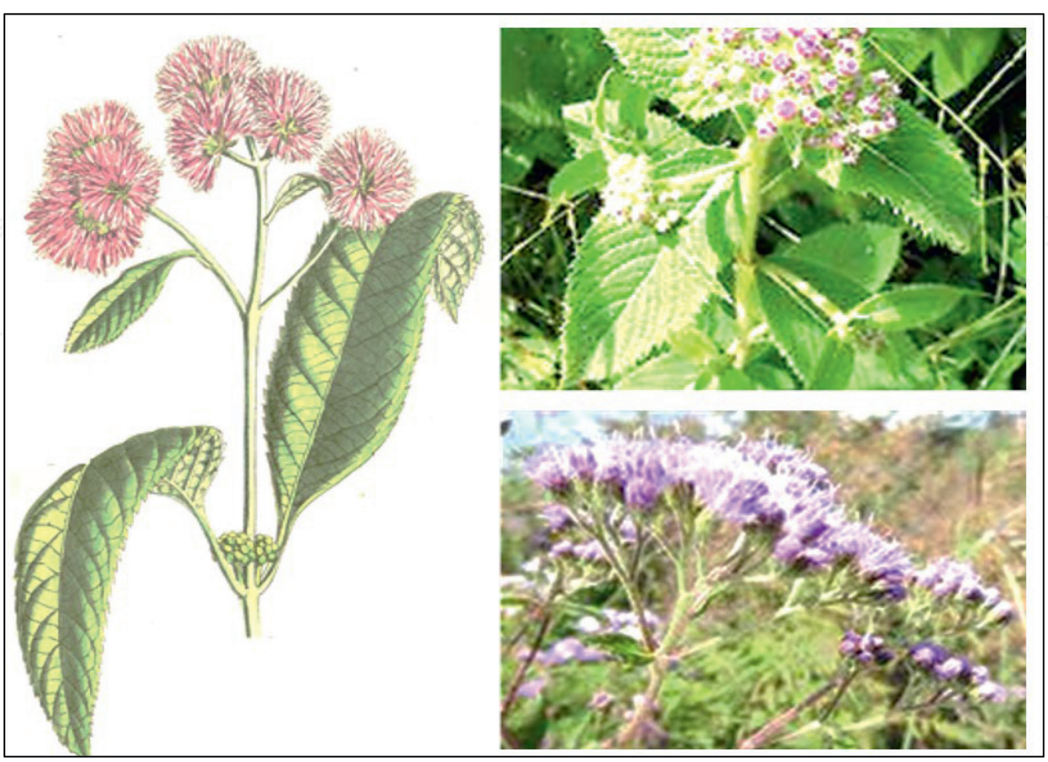

Figure 1.
Bothrine species in native Mount Elgon ecosystems, Eastern Uganda.

The aforementioned anthropogenic activities coupled with climate change-induced disasters like prolonged droughts, more frequent and intense flooding, landslides, and erratic precipitation are the leading threats to *BAT* species survival [5, 7]. The more severe and frequent flooding episodes within the species' natural habitat, – the Mount Elgon biosphere reserve, have not only damaged the micro-climate but also resulted in severe landslides that significantly destroy the species' protective vegetation [5, 6]. In summary, the aforementioned environmental challenges are responsible for the observed and continuing decline in the quality of native habitats for the *BAT* taxon, which is now categorized as critically endangered by the International Union for Conservation of Nature (IUCN): "Red List of Threatened Species" [4, 7].

1.2 Objectives of the action

The main project goal was to assess the potential of using integrated-conservation approaches in Rescuing, Regeneration, and Protection of critically-endangered plant species, using *Bothriocline auriculata* as a case study. In this project, the target Asteraceae florae species was the *Bothriocline auriculata* M. Taylor (BAT). The BAT species were regenerated into mass seedlings. The clean (disease-free) juvenile species seedlings were propagated back into native ecosystems. Sone of the species seedlings was simultaneously introduced into the new Mabira tropical rainforest, to ensure full recovery under a protected natural environment.

i. In light of the above, the specific objectives were to;

ii. Evaluate BAT species conservation status, spatial distribution, and richness;

iii. Identify and rank ecological threats to the survival of the BAT species;

iv. Rescue the BAT species from its native ecosystem and regenerate them into mass seedlings;

Propagate and assess the growth performance of the newly regenerated BAT species under the different ecosystems and conservation management approaches.

1.3 Justification of the interventions

The *BAT* plant species are ecologically important in providing soil cover against erosion and nutrient recycling and are also palatable fodder crops for livestock. The BAT plants are also among the herbal medicine used by the local population [8]. Under this project, the plant specimens from existing populations of the critically-endangered BAT species, which were on threatened with extension or were on the brink of extinction from their protective native ecosystem were surveyed and rescued from their harsh native ecosystems.

The rescued *BAT* species were regenerated and multiplied into mass seedlings, using germination and tissue culture techniques respectively. In order to support full species recovery and further in-situ conservation under the natural environment, the regenerated *BAT* seedlings were propagated and re-introduced back into their native ecosystems along Mount Elgon slopes. Similarly, replicate BAT species seedlings were introduced into a new forest ecosystem within Mabira tropical rainforest sites which have more favorable ecological conditions (e.g., soil fertility, moisture and weather).

The new sites are also protected from human activities and encroachment thereby providing suitable ground for mass regeneration and full recovery of the BAT species under natural environments.

Finally, the juvenile *BAT* species seedlings were shared with farmers as well as the national institutions mainly the National Environment Management Authority (NEMA), and the National Forestry Resources Research Institute (NaFORRI); as the key line stakeholders for inclusion in both the present and future conservation programs such as agroforestry, herbariums, gene banks, and re-afforestation programs.

2. Materials and methods

2.1 Study sites

In this project, field activities were conducted in 2 ecological sites; 1) Mount Elgon slopes, and 2) Mabira forest reserve in Eastern and central Uganda; respectively (**Figure 2**). The Mt. Elgon and Mabira forest areas are selected based on the availability of the surviving *BAT* species specimens, and relative climate suitability to regenerate the BAT species; respectively.

2.1.1 Description of site 1: Mount Elgon with montane

Site 1 (the species' native ecosystem): is located from mid to high slopes (2745–3150 meters) of Mount Elgon with montane forests and dense vegetation in Bududa districts, Eastern Uganda. The latitude and longitudinal spatial coverage of the study site stretch from 0°59'N, 34°17′ E to 1°04'N, 34°25′E (**Figure 2**). The area receives bimodal rainfall patterns with a mean annual rainfall of 1900 mm, where the long and short rainy seasons range from September to November, and March to April 2021–2022. respectively. The mean maximum and minimum daily temperatures are 23° and 15°C; respectively.

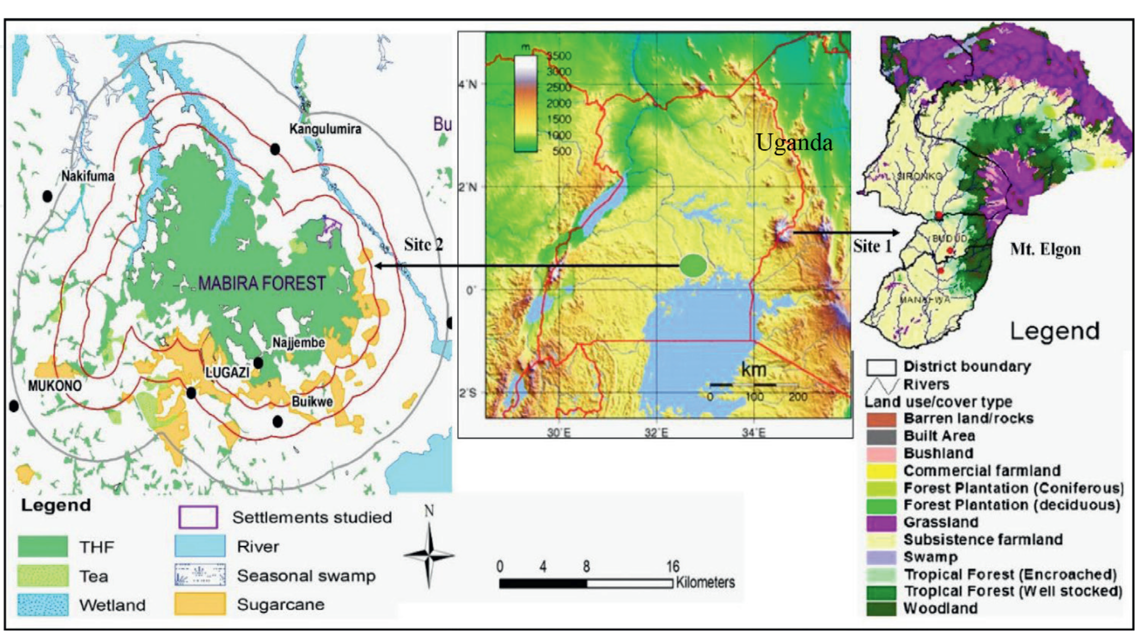

Figure 2.
Location of Mt. Elgon slopes (Site 1) and Mabira Forest (Site 2), Uganda.

Vegetation along the slopes of Mt. Elgon is associated with large mountain massifs and is altitudinally controlled zonal belts, which are classified into four zones namely; moorland - above 3500 m, bamboo and low canopy montane forest (from 2400 to 3000 m); and mixed montane forest -up to 2500 m elevation [9].

2.1.2 Description of site 2: tropical rainforest

Site Two (Tropical rainforest) is located in the Mabira forest reserve, the largest rainforest in Uganda covering over 30,000 hectares, in the Buikwe district of Central Uganda (**Figure 2**). The forest is located at latitude and longitudes of 0°23′54″N and 33°0′59″ E; respectively with several watersheds draining from Lake Victoria. The Mabila forest is elevated between 1000 and 1340 m A.S.L., and nearly 5% of the forest is made of four gently sloping hills: Dangala, Namusa, Ntunda, and Wakobe, which rise up to 1340 m [8]. The forest has an equatorial climate type with a bimodal rainfall pattern with two distinct short and long rainy seasons, from March to May, and September to November; respectively. The area receives a mean annual rainfall of 1300 mm, which is generally distributed throughout the year. The mean annual temperature within the Mabira forest ranges from 21 to 25°C, with minimum and maximum mean annual temperature ranges of 16–17°C, and 28–29°C; respectively [10].

Like the climate along Mt. Elgon slopes, the general climate of Mabira forest zones displays comparably small inter-seasonal variations in wind, rainfall, humidity, and temperatures throughout the year. This is because the forest is in close proximity to Lake Victoria and at an elevation of up to 1340 m, which moderates the area's microclimatic conditions (**Figure 2**), including the warming effect despite being within the equatorial region [10].

2.2 Methodology and activities undertaken

2.2.1 Objective 1: Evaluating the current conservation status, spatial distribution, and richness of the Bothriocline auriculata species

The conservation status, distribution and species richness of the *Bothriocline auriculata* (BAT) species were studied in its native ecosystem on the slopes of Mt. Elgon in the Bududa district (Site 1). The study site, site 2 (**Figure 2**) was subdivided into three big zones, separated by elevation and vegetation differences. In each zone, 10 quadrants of 250x300ft size (covering 1.7acres) were randomly distributed across each zone, from which the BAT specimens were collected and studied.

The spatial distribution and species richness of the *Bothriocline auriculata* species in each zone were recorded through a detailed ecogeographical field survey [11], in which the BAT species' geographical range and ecological status were defined and recorded. During the surveys, spatial locations of the BAT species were geographically marked with a new Global Positioning System, GPS datum WGS84 using Garmin 12XL receiver. Study sites were mapped based on the species' spatial occurrence, the frequency of habitation, mature and young individuals, and population size using ArcGIS 9.2 software. These parameters were used to estimate BAT conservation status, vegetation structure, spatial distribution and species richness using the IUCN criteria [7].

Conversely, soil samples from each zone were collected using a soil auger, packed in plastic bags and transported to Makerere University for analysis of biophysical and chemical characteristics, so as to determine the conditions needed for the growth and regeneration of BAT. Similarly, both weather and climate data from each study site/zone were collected from the nearest meteorological station. The vegetation structure, soil and climatic data were used to spot the best habitat preferences for BAT species to enhance in-situ conservation within the study sites.

2.2.2 Objective 2: identification and ranking of different threats to BAT conservation and their causes

Different threats to BAT conservation in its natural ecosystems and their causes, Mt. Elgon areas (Site 1) were identified in each zone and classified under three major threat categories, namely human-use threat, livestock threat and habitat destruction threat.

a. Human-use threat: any sign of BAT collection for purposes of human socio-economic use: medicinal trade, medicinal plant trade, fuel, agriculture use (as fodder, mulch, compositing etc.) were recorded. Additional assessments of the conservation threats and their causes were made through oral interviews and informal meetings with community stakeholders namely farmers and herbalists, within and around the habitat zones.

b. Livestock/wild threat: vulnerability to animal destruction was measured by the grazing level in each zone (overgrazing), which were determined spatially by dung presence, frequency and density methods of Omar *et al.* [12].

c. Habitat destruction: the level of anthropogenic habitat destruction for arable farming, infrastructural development (roads, urbanization), deforestation for fuelwood, human settlement, and other land use/change activities, were assessed using high-resolution satellite imagery of LANDSAT and google earth. Likewise, natural causes of habitat destruction such as floods, prolonged dry spells, and climate change (extreme shifts in temperature and precipitation) were identified using LANDSAT imagery from 1990 to 2017, and data processing in GIS.

In each study zone, the identified threats and their causes were arranged and ranked as; very high, high, medium, low and very low when the threat in question is capable of destroying at least 80%, 70–80%, 50–69%, 30–49%, and below 30% of in-situ BAT species, respectively [13]. The threats are ranked as Very High: when a very large area of conservation target species and site is likely to be destroyed and High: when many locations of the target conservation site and the number is likely to be destroyed. Medium thread occurs when it's localized in its scope with a moderate degradation level and affects limited locations of the target conservation zone. Low and very low threats occur when the threat only impairs small and insignificant portions of the target conservation area and species; respectively [13].

2.2.3 Objective 3: undertake the BAT species regeneration, and mass seedling propagation and test the in-situ rescue performance

Mass regeneration of BAT species was done by rapid in-vitro micropropagation, ex-vitro plantlet growth, and in-situ re-establishment of the species in the wild. The BAT plant materials: vegetative portions and mature fruits, were collected from the Mt. Elgon region (Site 1) and transported to Makerere University Microbiology

Laboratory where the species were regenerated into mass seedlings using micropropagation. The plant part(s) were cut open or seeds extracted from pods for fruits and rinsed in distilled water three times. The parts/seeds were washed in 2%(v/V) Tween-20 detergent solution for 8 minutes, and later sterilized with 1%NaClO solution containing 2-drops of Tween-20 for 15 minutes. The plant part tissues/seeds were re-washed with sterile distilled water under aseptic conditions.

The extracted plant parts and seeds were regenerated into mass seedlings using tissue culture and in-vitro seed germination techniques; respectively [14], – where they were cultured on growth media and incubated under aseptic conditions. The growth media were prepared by mixing equal volumes of 3%Sucrose and 0.8%Agar into and diluting to 50% concentration while keeping the pH at 5.8. About 20 ml of the growth media will each be dispersed into sterilized 100 ml culture jars, where the plant tissues/seeds were inoculated. Non-absorbent cotton wrapped in cheesecloth was plugged into the culture jars, and the jars were autoclaved at 1.06kgcm^{-2} and 121°C for 15 minutes [14]. The inoculated tissues/seed samples were kept under aseptic conditions, at a temperature of 25 ± 2°C and 16/8 light–dark photoperiod supplied by white fluorescent lights under tissue culture rooms, until full tissue regeneration/seed germination. At least 20 seeds and 50 plant tissues were regenerated during 1st micropropagation cycle and were exponentially increased for five successive seedling generations, so that mass BAT seedlings were rescued during the five micropropagation phases.

The newly germinated BAT seedlings, after 3–4 weeks from inoculation in the Tissue Culture Laboratory, were removed from the laboratory to artificially induced shoot and root formation. The regenerated shoot tips from the seedlings were cut using a sterile blade, and cultured in Murashige and Skoog (MS) medium supplemented with varying concentrations of BA/KA: 0.5, 1.0, 1.5 and 2 mg/l for 5–6 weeks [15]. The best resulting micro-shoots were incubated on a half-strength MS medium containing varying concentrations of indole-3-butyric acid (IBA)/naphthalene-1-acetic acid (NAA): 0.5, 1.0, 1.5 and 2 mgl^{-1} to induce root formation and growth [15]. After the mass micropropagation process, regeneration success was assessed as the percentage ratio of fully developed seedlings to the total number of seedlings cultured.

Seedling hardening: the most vigorous BAT plantlets were transplanted into PE-plastic pots (dimensions: 10 × 15 × 7cm), filled with soil growth media artificially sterilized by autoclaving and mixed peat-moss, perlite and soil in ratios of 1:1:1(v/v). During hardening, the potted plantlets were irrigated with distilled water for about 10 days under culture room conditions before being moved to greenhouse and field weather conditions for further in-situ re-establishment.

2.2.4 Objective 4: propagating and assessing the growth performance of the regenerated BAT species under the different ecosystems and conservation management approaches

The growth performance of the regenerated and potted plantlets was assessed in terms of the quantity and quality of their morphological traits, re-established/grown under the completely randomized design (CRD) experiments. The three main in-situ conservation practices for BAT recovery management namely: active management, habitat restoration and habitat preservation. The conservation practices were classified based on their management intensity for the endangered species as high, medium, and low intensity for the active, habitat preservation and restoration management practices; respectively [4, 7].

2.2.4.1 Active management: Greenhouse production at Makerere University

About 100 BAT plantlets were transferred into a specially designed greenhouse, constructed at Makerere University, where they were monitored to full maturity. The plantlets, while still in pots, were regularly watered during the first 3–4 weeks (twice a day in the morning and evening), so as to get acclimatized to the harsh greenhouse weather conditions. Afterwards, the plantlets were transplanted from their conditioning pots, and re-planted about 5–10 cm in depth into previously fumigated soils, so as to grow under normal greenhouse soils and weather to maturity. Active management assumes that the species in question is critically endangered, and requires delicate management beyond its natural ecosystems to ensure rescue and full recovery [13].

2.2.4.2 Habitat preservation: BAT production in Mabira forest (Site 2)

At least a 1-acre area on one of the 4-gently sloping hills in Mabira forest, study site 2 (**Figure 1**) was randomly selected and isolated for BAT species growth trails. The plantlets were established in the preserved habitat of site 2, which are relatively identical soil and weather conditions of the BAT native ecosystem in site 1 using the same procedures in active management. The species under habitat preservation management were to grow under natural environment conditions: soil, temperature, humidity and rainfall to support species recovery. The habitat preservation management assumes that, if it is successful in supporting full species re-establishment, any other unoccupied ecological habitat with relatively similar environmental conditions will facilitate full species recovery by natural dispersal mechanism [11].

2.2.4.3 Habitat restoration: BAT production in Mt. Elgon slopes (Site 1)

About 1 acre of the experimental land in site 2 will also be isolated and BAT plantlets were planted in its native ecosystem using the same procedures described above in habitat preservation practices. The plantlets were monitored in their native environment until their full recovery and maturity. The habitat restoration management assumes that the endangered species is capable of full establishment once the native habitat is restored [11].

2.3 Data collection and analyses

Under each conservation management practice experiment, the 100 BAT plantlets were sowed at a standard spacing of 15 × 20cm, in a completely randomized design (CRD) experiment and were replicated three times [16]. Soil samples were collected and taken to Makerere University Soil Science Laboratory, for analysis of biophysical and chemical parameters such as pH, humus, electric conductivity, and nutrient (N, P, K and micronutrients), using the calorimetry method [17]. The daily ambient weather conditions, including the area temperature, rainfall and humidity were collected using digital thermometers, rain gauges and hydrometers; respectively.

Likewise, data on the morphological traits of the regenerated BAT plants such as leaf number and size, plant height, number of branches per plant and number of mature plants, as well as reproductive aspects of the mature plants (number of flowering stems, flowers, fruits and seeds per plant) were collected. The morphological and reproductive data were used for a detailed study of the species' recovery, regeneration and adaptation under the different in-situ conservation management practices and ecosystems.

The species' performance under the different in-situ conservation management practices was assessed by establishing the correlation relationships between the species' morphological and reproductive traits, and the management aspects or environment data: management practice, soil and weather parameters. Besides correlation relationships, Analysis of Variance (ANOVA) between the BAT species performance, morphological and reproductive traits under different conservation management against the soil and weather parameters were analyzed using Genstat statistical package [18]. The statistical significance for the data analyses was set at a 95% (p = 0.05) confidence interval. The most feasible management practice(s) for BAT species rescue, recovery and establishment was recommended not only for adaptive management and conservation of BAT species but also for conservation of other critically endangered plant species in Uganda.

2.4 Justification of the methodology and approach taken

Bothriocline auriculata (BAT) species is endemic in Uganda and is exclusively sited along Elgon slopes (Site 1), where field surveys were conducted. But because the species is near extinction, we envisage collecting a few species samples which were regenerated into mass seedlings to support full species recovery. The critically-endangered species have not been studied until now, and hence no scientific data: mainly on the species reproductive modes, germination and seed regeneration capacity, species plant physiology, growth performance and adaptive capacity to abiotic stress etc., exist to guide the species conservation actions. Faced with this uncertainty, natural germination and tissue culture methods were employed to regenerate the species samples into mass seedlings [15]. The tissue culture method was used as a backup against natural germination which takes an extended time and its success is not 100% guaranteed.

While on the contrarily, the tissue culture method is capable of exponentially multiplying a few plant tissues into mass seedlings under aseptic conditions in a short time and produces disease-free seedlings [15]. However, artificial crossings between the plantlets produced by tissue culture and a few produced by the germination process were made to address the challenge of genetically identical seedlings produced via tissue culture and simultaneously increase of genetic diversity of the species' offspring over successive generations during conservation.

The BAT taxon is expected to have varying adaptive capacities to abiotic stress and growth rates when subjected to varying weather and soil conditions during its in-situ conservation, as well as under different ecosystems and conservation management practices. Therefore, the BAT species' growth performance was monitored from sowing to maturity, under CRD field experiments [16]. The CRD trials were set up in the species' native habitat and Mabira tropical rainforest, as the 2 sites coincidently have nearly identical weather patterns, vegetation types, soil conditions, and hydrological regimes, – which are likely to favor BAT species' successful growth. For instance, both sites receive bimodal rainfall patterns with a mean annual rainfall of 1300 mm, which is evenly distributed within the two distinct long and short wet seasons from September to December, and March to May, respectively. The mean annual temperatures of both sites range from 21 to 25°C, with both the minimum and maximum mean annual temperature ranges of 16–17°C, and 28–29°C, respectively [19].

Data on BAT species morphological traits: leaf number/size, vigor, seedling rate, plant number/height, branches per plant as well as reproductive traits; flowering stems, flowers, fruits, and seeds per plant were recorded on a bi-weekly basis for growth performance analysis. Soil samples were collected and analyzed for pH, EC, and nutrient composition using the calorimetry method [16], and the weather variables: temperature, rainfall and humidity etc., were collected from site weather stations. The soil and weather parameters will depict local soil health and weather conditions that are suitable for the successful in-situ conservation of the BAT species in the study sites.

Using GenStat software [18], Spearman's correlation was employed to assess the relationship between species growth performance, soil parameters, and weather variables under different conservation management practices and the environment. Analysis of Variance (ANOVA) was employed to determine statistical significances ($p = 0.05$), and model relationships between different conservation practices (as the independent variables), and soil and weather parameters, — as the dependent variables. The ANOVA test will identify the most responsive conservation management practice(s) that support the BAT species rescue, recovery and regeneration in the natural environment.

3. Results and discussion

3.1 Description of the suitability of the ecosystems

Study site: although study site 1 is located along the slopes of Mount Elgon at higher altitudes from 2745 to 3150 m while study site 2 is within Mabira Forest is located at a significantly lower altitude from 1000 to 1340 meters Above Sea Level, both sites coincidently have nearly identical weather patterns, vegetation types, soil and hydrological regimes. Thus the environmental conditions of the 2 sites are most likely to favor the successful regeneration and conservation of the target species: *Bothriocline auriculata* (BAT) species, and hence their choice.

For instance, both sites receive bimodal rainfall patterns with a mean annual rainfall of 1900 mm and 1300 mm for sites 1 and 2 respectively, where the long and short rainy seasons range from September to December, and March to May, respectively [19]. The mean annual temperatures for the two sites also range from 21 to 25°C, with minimum and maximum mean annual temperature ranges of 16–17°C, and 28–29°C, respectively [19]. Like climate along Mt. Elgon slopes (Site 1), MWE [10] independently observed that the general climate of the Mabira forest area (site 2) displays comparably small inter-seasonal variations in wind, rainfall, humidity, and temperatures throughout the year. This is because the forest is in close proximity to Lake Victoria and at an elevation of up to 1340 m, which moderates the area's microclimatic conditions including the warming effect despite being within the equatorial region [10]. It is against this background, that the two sites were selected for the study and conservation of the BAT species.

3.2 Conservation status of the *Bothriocline auriculata* species in terms of the spatial distribution, species richness and abundance of the individual plants

Like many endangered species, BAT species is susceptible to many environmental threats within its natural ecosystems. Thus, the ecological threats to the species' survival within its native ecosystem (site 1), were identified and classified under three major categories namely human-use threat, livestock threat and habitat destruction threat, using the frequency and density method [12].

Conservation status, distribution and species richness of *Bothriocline auriculata* species were studied in its native ecosystem on the slopes of Mt. Elgon (Site 1) following an eco-geographical field survey [11, 20]. The survey method was successfully used by Choudhury and Khan [20] used the survey method to assess the population structure, species richness and conservation status of endemic and critically endangered plant species of *Aquilaria malaccensis, Gleditsia assamica*, and *Gymnocladus assamicus,* in India.

In this study, the type of host vegetation and flora at the landscape level are very important ecosystem components in the study of the conservation status of the inhabiting species, including species diversity, spatial distribution and ecological patterns in spatial variability. *Bothriocline auriculata* species host native ecosystems along the slopes of Mount Elgon differ considerably from the rest of the mountainous zones across Uganda due to the unique climatic conditions and physiography. The type of vegetation and flora diversity also vary with the elevation along the slopes of Mount Elgon. There are seven main classes of vegetation namely; mixed montane forest, bamboo, shrubs and thickets, low canopy montane forest, high canopy montane forest, and low, mid and high moorland ecosystems [3].

Table 1 presents the current *Bothriocline auriculata* (BAT) species conservation status in terms of relative abundance and spatial distribution of the individual plants within the host native ecosystems and vegetation types along the slopes of Mount Elgon, Uganda.

The number of *Bothriocline auriculata* plants was more abundant in the midland altitude and ecosystems having bamboo, shrubs and tickets followed by the high mixed montane forest vegetation (**Table 1**). The *Bothriocline auriculata* exhibited a very low species richness and limited ecological range along the slopes of Mount Elgon because the remaining plant populations were on the verge of extinction from their native ecosystems. The results are consistent with the findings by the International Union for Conservation of Nature [3, 7] who reported that the *Bothriocline auriculata* species are critically endangered and are on the verge of existing. The IUCN [7] also reported that *Bothriocline auriculata* species was also endemic

Zones ID	Vegetation type and ecosystems	Elevation (m)	Number of plants (n)
1	Mixed montane Forest	500–2000	43 ± 1^a
2	Bamboo, shrubs and thickets	2001–2500	52 ± 1^b
3	Low canopy montane forest	2501–3000	19 ± 1^c
4	High canopy montane forest	3001–3500	6 ± 1^d
5	Moorland – Low	3501–3800	1 ± 1^e
6	Moorland – Middle	3801–4000	0 ± 1^e
7	Moorland – High	Above 4000	0 ± 1^e

Values are arithmetic means with standard deviation (±SD) computed for values taken from every sampling area or zone along the line transect. Comparisons were made between the sampling zones, and n = number of plants collected per zone. Means in the same row bearing different superscript alphabetic letters are significantly different at a 5% (p = 0.05) confidence level.

Table 1.
Spatial distribution of the Bothriocline auriculata plants in the species' native ecosystems.

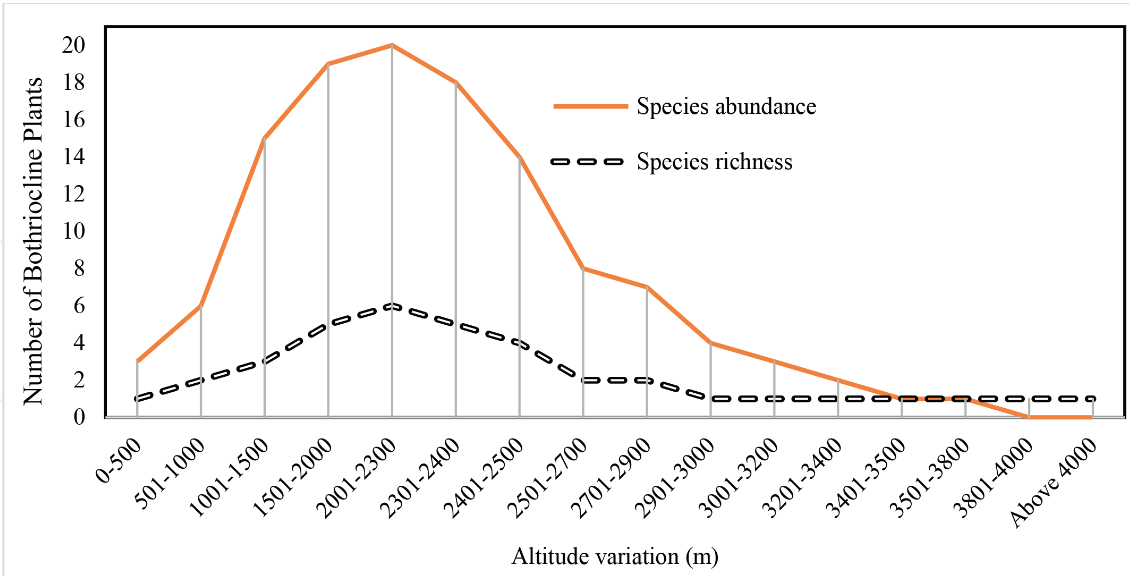

Figure 3.
Distribution of plant species in different altitudes of Mt. Elgon.

in Uganda whose ecological range was restricted to a few sites along the slopes of Mount Elgon between 2745 and 3150 meters Above Sea Level (A.S.L).

Figure 3 shows variations in the spatial distribution of *Bothriocline* plants with the altitude along the Mount Elgon slopes. The *Bothriocline* species richness and abundance increase with altitude along the gradient transect, and are the highest mid-altitude zones between 1500 and 2500 m above sea level. The mid-altitudes are composed of the mixed montane forest, bamboo, shrubs and thickets, as well as low canopy montane forest with a more favorable protective vegetative cover and microclimate as opposed to the highland altitudes (**Figure 3**).

On the other hand, the *Bothriocline* species richness and abundance begin to decline at higher altitudes from 2501 m and above. The vegetation type at higher altitudes is dominated by low and high canopy montane forest and moorland coupled with mild to cool temperatures; that is not favorable for the protection and proliferation of the *Bothriocline* species (**Figure 3**).

Overall, the richness of *Bothriocline auriculata* species is low with the narrow ecological range within the species' native ecosystems along the slopes of Mount Elgon (**Figure 3**). The species' spatial distribution and plant populations are restricted to mid-altitudes having mixed montane forest, bamboo, shrubs and thickets. Previous studies also confirm that the *Bothriocline auriculata* species thrive well under tropical ecosystems with dense vegetative cover, and the species' plant populations are abundant in the montane forest, mountainous areas and bamboo thickets [3, 7].

The low species richness and narrow ecological range suggest the high vulnerability of the *Bothriocline auriculata* species which could be primarily driven by environmental and human-induced threats to the species' survival, existing population and protective ecosystem. The possible environmental or natural factors could be soil biotic and abiotic factors (fertility, pH, microbiota, moisture etc.), extreme weather episodes like heat waves or high temperatures, erratic rainfall and humidity.

Similarly, the likely human-induced threats to the *Bothriocline* species' survival and population could include among others, anthropogenic climate change episodes such as dry spells, flooding coupled with mismanagement of the fragile ecosystems namely, over-harvest/grazing, arable farming and deforestation for timber and fuel wood; that often destroy the species protective ecosystems.

In this context, future studies should focus on profiling the environmental and human-induced threats that compromise the survival of the *Bothriocline auriculata* species, as well as species richness, spatial distribution and ecological range across its native protective ecosystems. If so, the data will inform future species conservation options and adaptive management practices within the same ecosystems or find better alternative host environments.

3.3 Rescue the critically-endangered *Bothriocline auriculata* species from its native ecosystem

Intensive eco-geographical surveys and scouting were performed along the slopes of Mount Elgon, the species' native ecosystem as well as on nearby farmlands, bushes and thickets to find, locate and rescue any remaining species specimens. Despite several repetitive surveys, 114 species specimens were found and positively identified within its native ecosystem along the slopes of Mount Elgon.

Regrettably, the species plants did not have seeds and seedlings in the wild so seed germination or artificial propagation of seedlings could be carried out for conservation purposes. Failure to produce seeds suggests that the *Bothriocline auriculata* species does not attain a complete growth cycle where the species enter flowering phases to produce seeds after attaining full maturity but rather remains in the vegetative phase. Besides being a survival mechanism in a fragile ecosystem, unfavorable weather mainly dry spells and humidity put additional stress on the plants, suppressing hormones for flowering and seed production thereby remaining in vegetative phases ([7] and IUCN, 2020).

Failure to enter into the flowering phase and complete the growth cycle could partially explain the low species richness and limited ecological, as well as the fast declining *Bothriocline auriculata* plant population within its endemic and native ecosystems; where the species is classified by the IUCN as critically endangered ([7] and 2020). In response, future studies should identify both human-induced and ecological factors that affect the growth performance of *Bothriocline auriculata,* and propel the species not to enter into flowering phases but rather remain in vegetative phases. Ecological biotic and abiotic factors limit the species' dispersal and geographical range.

Due to the small populations of the *Bothriocline auriculata* species and the absence of the seeds, only a few vegetative portions of the species plants were collected from the species. For this study, 15 vegetative portions (leaves and branches) of the specimens were collected, and the donor parent plants were left living in the wild. As such, the 15 specimen portions were rescued and regenerated into mass seedlings using the lab tissue culture and micropropagation protocols at Makerere University.

3.4 Perform regeneration of the rescued *Bothriocline auriculata* species specimens into many seedlings and propagation of the species seedlings for in-situ conservation

The extracted BAT plant parts and seeds were regenerated into mass seedlings using tissue culture and in-vitro seed germination techniques respectively [14]. But because the species is endemic and critically endangered, a few plants were got whose seedling and multiplication rates by the natural process of germination, – to raise the required number of seedlings for in-situ conservation, is not 100% guaranteed.

Thus, artificial micropropagation of the collected seedlings or plant tissues by tissue culture [15], were included as a backup process just in case the seeds generated by germination are not enough to raise the required number of seedlings over successive generations. But because the tissue culture method despite multiplying seedlings in mass numbers at a faster rate and producing disease-free plantlets, the method is challenged by the production of genetically identical seedlings [15]. To increase genetic diversity, artificial crossings between the tissue cultured plantlets with those produced from the natural germination process were made. Afterwards, the progeny/F1 generation of the crossed seedlings was back-crossed with germinated parents [21], to increase genetic diversity and encourage additional crossovers during recovery and regeneration in the natural environment.

The *Bothriocline auriculata* species specimens were collected and rescued from their fragile ecosystems along the slopes of Mount Elgon for multiplication into many seedlings for propagation and conservation. The collected specimens were put into specialized aerated specimen sampling bags. The bags containing the species specimens were in a cool box and taken to the Makerere University Tissue Culture laboratory for processing. In the lab, the specimens were cleaned with tap water, disinfected with ethanol and cut into replicate 25 smaller vegetative portions of length 2–3 cm. The specimens were regenerated and multiplied into many seedlings using plant tissue culture and micropropagation protocols. The vegetative species specimens were successfully regenerated and multiplied into at least 150 juvenile seedlings under aseptic conditions, artificial lighting and growth hormones. The seedlings produced are identical to the parent donor *Bothriocline auriculata* plants. **Figure 4** shows some of the pictures for species tissue cultured *Bothriocline auriculata* seedlings.

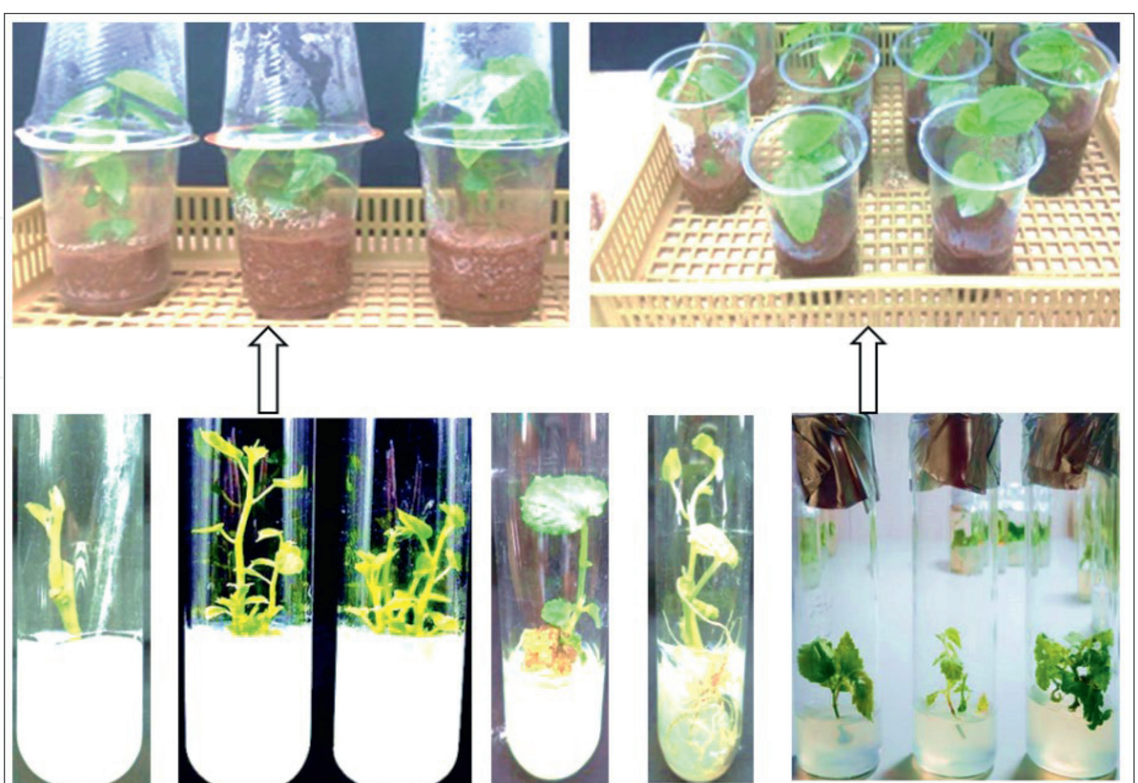

Figure 4.
Photos of the Bothriocline auriculata *seedlings tissue cultured in situ.*

Over three tissue cultures, seedling propagation cycles were performed during the generation of BAT specimens. A total of 150 seedlings were propagated and regenerated during tissue culture propagation cycles.

3.5 Assessing the growth performance of the *Bothriocline auriculata* species, and its response to abiotic stress under different conservation management schemes and environments

Availability of high-quality seedlings that are disease-free, fast-growing, biotic and abiotic stress-tolerant and are genetically identical to the parent species is one of the primary challenges facing the conservation biologists and forest nursery operators in Uganda. As such, the lack of a reliable supply of quality seedlings for the threatened and most critically endangered species such as *Bothriocline auriculata* species makes them get excluded from the local species restoration, conservation and adaptive management programs such as agroforestry, forest nurseries, parks, plant genetic resources, gene banks and forest ecosystems.

In response, quality seedlings of the critically-endangered *Bothriocline auriculata* species were produced in an aseptic (disease-free environment in the lab) using micro-propagation and tissue culture protocols at Makerere University (**Figure 4**). After tissue culture and micropropagation, the juvenile *Bothriocline auriculata* species seedlings were transplanted into PE-plastic pots for hardening. During hardening, the potted seedlings were irrigated with distilled water for about 5 weeks under culture room conditions in a screen house. The growth performance parameters were root collar diameter and shoot height.

The growth performance of the seedlings was studied in the screen house under artificial conditions namely, lighting, watering and growth media. **Table 2** shows a summary of the growth performance of the seedlings in response to the abiotic conditions of the growth media.

The growth performance parameters, namely shoot height, root collar diameter and number of leaves per seedlings increase with increasing duration as well as bulk density of the soil media and soil moisture level (**Table 2**).

The results suggest a normal seedling growth rate of the *Bothriocline auriculata* species under favorable growth media soil biophysical conditions; as represented by soil moisture and artificial lighting) in the screen house. Out planting success of plant

Time/ Treatment parameters	Root collar diameter (mm)	Shoot height (cm)	Bulk density of soil media ($g \times cm - 3$)	Moisture content (%)	Mean number of leaves/plants
Week 1	2.12 ± 0.01	10.4 ± 0.3	0–5	0–5	1
Week 2	3.23 ± 0.02	21.3 ± 0.4	6–10	6–10	2
Week 3	5.75 ± 0.02	25.2 ± 0.2	11–15	11–15	3
Week 4	7.53 ± 0.03	43.8 ± 0.3	16–20	16–20	4
Week 5	6.85 ± 0.04	45.6 ± 0.2	21–25	21–25	4
SD	*0.48*	*15.12*	—	—	
CV	*0.07*	*0.52*	—	—	

Table 2.
Growth rate of the Bothriocline auriculata *species seedlings.*

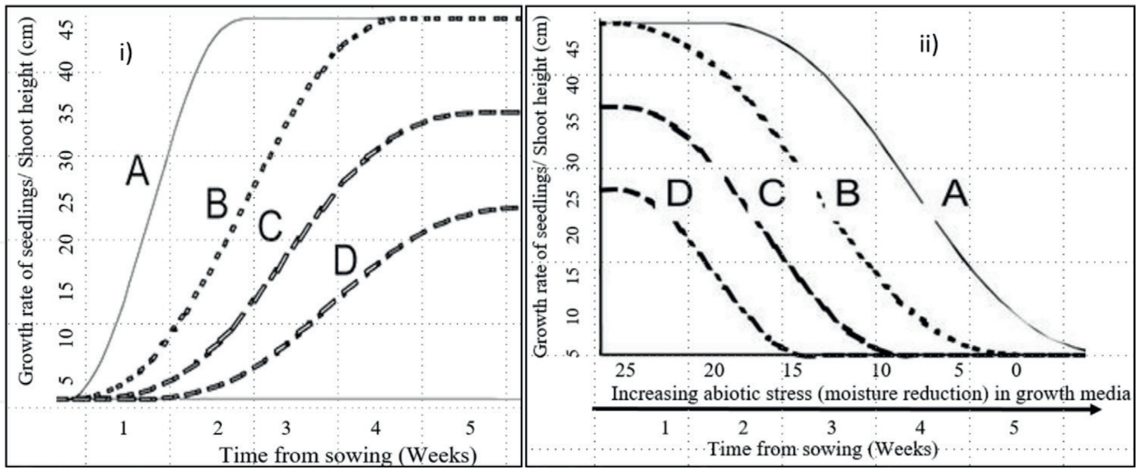

Figure 5.
Variations in the growth rate of the seedlings (shoot height) for the Bothriocline auriculata species seedlings under screen house conditions with: i) normal soil moisture and ii) moisture deficient.

species depends on several species' interaction between light, soil moisture other ecological conditions [13]. It has also been argued that the phenotypes of different plant species are characterized by morphological and physiological attributes, and are hence more important in predicting species-specific survival and growth during ex-situ conservation [15].

Conversely, the *Bothriocline auriculata* species seedlings were also subjected to stressful conditions to ascertain their growth response under soil moisture deficient conditions. The growth response of the species seedlings under both normal soil moisture and soil moisture deficient conditions were analyzed. The results describing the growth of the seedlings in terms of shoot length and root collar diameter are given, by a comparative approach (**Figure 5**).

Under optimum light and soil moisture conditions in a screen house, the growth rate (shoot height) of the *Bothriocline auriculata* species seedlings exponentially increases with time from the 1st week and attains a peak growth rate after the 5th week. The results suggest that the *Bothriocline auriculata* species complete the juvenile seedling growth cycle just after 1 month from sowing in appropriate soil media. On the contrary, the growth rate of the *Bothriocline auriculata* species seedlings declines rapidly with increasing deficiency of the soil moisture in the growth media. The data/results further confirm the high susceptibility of the *Bothriocline auriculata* species seedlings to abiotic stress conditions in the soil ecosystems. The results are consistent with the findings of IUCN [7] and [17]; who also reported positive correlational relationships between growth rate, soil moisture, temperature and other abiotic ecological conditions.

Nonetheless, the growth performance of the *Bothriocline auriculata* species was only assessed at the screen house level. Performance at the greenhouse and field level in the different ecosystems was NOT done due to a gap in funding. To this end, only 150 seedlings were regenerated enough for only replicate trials in the screen house. The data collected for the species growth performance was inconclusive because it did NOT include greenhouse conditions and under natural ambient weather and other environmental conditions in the fields. Therefore, future studies and efforts should focus on assessing the growth performance of the *Bothriocline auriculata* species seedlings beyond the artificial screen house conditions but rather under the natural ecological conditions; where the species will undergo ex-situ conservation in protected ecosystems and/or adaptive management in the agro-ecosystems.

4. Conclusions and recommendations

The *Bothriocline auriculata* species is endemic along the slopes of Mount Elgon The species has a limited ecological range despite its native ecosystem being at stake because of massive deforestation, arable farming and climate change. As a result, the *Bothriocline auriculata* species are among the critically endangered species and are currently threatened with extinction.

In this project, a few species specimens were rescued from their fragile native ecosystem and multiplied into massive juvenile quality (fast-growing and disease-free) seedlings that are genetically identical to the parent *Bothriocline auriculata* species. The species seedling exhibited a faster growth rate when subjected to artificial lighting and soil media under aseptic conditions in a screen house.

The most important lesson we learnt was that, in searching for any critically endangered plant species, the involvement of local farming communities and leadership is very important and should never be underestimated. Future efforts should be directed toward building capacity for a robust and sustainable supply of quality seedlings for the *Bothriocline auriculata* species in Uganda and beyond. If so, the seedlings should be supplied to local actors (farmers, conservationists and forestry bodies) for it to be streamlined, and included in the ongoing species conservation and restoration programs. Future programs should also be directed toward building the capacity of the local actors in conservation and adaptive management of the *Bothriocline auriculata* species to ensure full species recovery and restoration.

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