

Propagation, development phases and domestication of *Cyperus rotundus* L. in the Western Highlands of Cameroon

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Abstract

Cyperus rotundus Linn. is a potentially valuable aromatic plant given its ability to synthesise essential oil and the variety of traditional uses associated with it. The valorisation of this plant would imply the exploitation of a significant quantity of its biomass, which cannot be obtained from the wild. The aim of this study was to determine the best methods for regeneration and the development phases of *C. rotundus*, in order to ensure the continuous availability of its biomass. The mode of natural regeneration and the state of domestication of *C. rotundus* by local populations were investigated through surveys of traditional healers and herbalists. In addition, *ex situ* regeneration trials were carried out using vegetative propagation from bulbs and seed germination. The vegetative propagation was assessed by estimating the lag time and percentage regeneration of the bulbs, while the seed germination percentage was evaluated. The development phases of the plant were identified following the BBCH (Biologische Bundesanstalt Bundessortenamt and Chemische Industrie) scale. The yield was calculated from the underground biomass of the bulbs produced at the end of the plant life cycle.

The results showed that *C. rotundus* regenerates naturally from bulbs and rarely from seeds. In the Western Highlands of Cameroon, traditional practitioners and herbalists have begun domesticating *C. rotundus* on a small scale. Propagation attempts from seed were unsuccessful in the present study. However, from bulbs, a lag time of 10 days was observed and a regeneration percentage of 88% obtained after 47 days. Five phases of development were identified including emergence (0), bolting (1), flowering (2), maturation (3) and senescence (4). The production of bulbs, which are organs containing the essential oils, was observed from the bolting phase. The biomasses of about 383, 493 and 520 kg/ha were obtained during phase 1, 2 and 3 respectively.

The best propagation method and the development phases of *Cyperus rotundus* investigated in this work could contribute to an advance in the domestication process in order to ensure its availability and sustainability.

Historic

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1. Introduction

Cameroon has a significant biodiversity, enriched with indigenous aromatic and medicinal plants that produce essential oils [1]. These oils serve as raw material for a variety of uses notably in the perfumery, cosmetics, agri-food and pharmaceutical industries [2, 3, 4]. However, most of the plants are irrationally and anarchically exploited, leading to mismanagement of biomass, which in most cases is harvested from the wild. The need for large quantities of this biomass is of great concern, as its large-scale exploitation favours its depletion and even the extinction of certain species. This is the case of *Prunus africana* which has been classified in Cameroon by the International Union for Conservation of Nature (IUCN) as a rare and vulnerable species due to the high pressure on the resource [5]. For a sustainable exploitation, the use of these plants must reconcile meeting population demands and their well-

being with the conservation of their biological diversity [6, 7]. Domestication appears to be an alternative for the sustainable management of these plant resources insofar as it allows the production and assurance of an available, continuous and valuable biomass for traditional and industrial uses.

Essential oils extracted from plants play an important role in the economy. In 2015, world production of these oils was estimated at 110,000 tonnes for a value of \$1300 million and was expected to grow by 9.6% between 2017 and 2022. This production is dominated mainly by Brazil (28.6%) followed by India (26.6%). Africa represents only 0.1% with Egypt being the main exporting country [8, 9]. Despite this low representation, Africa abounds in many aromatic plants, the exploitation of which could generate income and improve the living conditions of its population. In Cameroon, the bioprospection of aromatic plants in the western highlands has identified potentially valuable species. Taking into account the traditional uses of these species and the importance of the essential oils produced for the perfume industries, several plants, especially *Cyperus rotundus* was prioritized [10].

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C. rotundus (Cyperaceae) is a perennial rhizome herb that grows in small clumps, from 30 to 100 cm high. The plant produces a dense network of rhizomes, suitable for vegetative propagation [11]. It is widely used in traditional medicine as an analgesic, sedative, antispasmodic and antimalarial drug. In addition, it is used against gastric disorders, diarrhoea, cough, bronchitis, skin diseases, memory loss, food poisoning, indigestion and lactation deficiency [12, 13]. The rotundone, one of the most widely used sesquiterpenes in the food industry, perfumery and medicine, was first discovered in the bulbs of *C. rotundus* [14]. Moreover, characterisation of essential oils of *C. rotundus* collected in the Western Highlands of Cameroon revealed the presence of cyperotundone, a species-specific substance of particular interest to the perfume manufacturing industry.

The exploitation of the essential oil of *C. rotundus* could make the species vulnerable, since a yield of 0.37% was obtained during essential oil extraction trials from dried bulbs [15]. Moreover, harvesting the entire plant makes *C. rotundus* a species of medium vulnerability [16]. For industrial uses, the species could be subjected to numerous problems, particularly that of its sustainability due to the unavailability of its biomass. Similarly, the lack of knowledge of its phenology and its optimal production phase for essential oils are also factors that can hinder its exploitation. The objective of this work was therefore to determine the best methods for regeneration and the development phases of *C. rotundus*, in order to facilitate its domestication and ensure the continuous availability of its biomass.

2. Materials and methods

2.1. Study area

The Western Highlands of Cameroon is an agroecological zone located between 05°20' and 7°00' North latitude and between 10°03' and 12°00' East longitude. It is characterised by very complex geomorphological features, consisting of volcanic mountains as well as collapsed plains [17]. The relief is very diverse with altitudes ranging from 800 to 2740 m. The temperature varies from 16 to 27°C and the relative humidity between 40 and 100%. The average rainfall, spread over a single season from March to November, is 1900 mm/year, with a minimum of 1179 mm and a maximum of 2630 mm. The climate is Sudano-Guinean with a very large proportion of the land covered by grass [18].

2.2. Assessment of natural regeneration of *C. rotundus* and local practices on its domestication

Surveys were conducted among 20 traditional practitioners and 10 herbalists in 10 localities namely Bantoum II, Tonga, Makenene, Balessing, Fotomena, Massangam, Malentouenn Baboutcheu-Ngaleu, Baboutcha-Nintcheu and Balatchi (Figure 1). The choice of the collection areas was made on the basis of an ethnobotanical survey previously conducted in collaboration with the association of traditional healers of West Cameroon (OSEMETO) where people with good knowledge of the species had been identified. In each locality, the questionnaire focused on the state of knowledge of the species. Information was collected on the

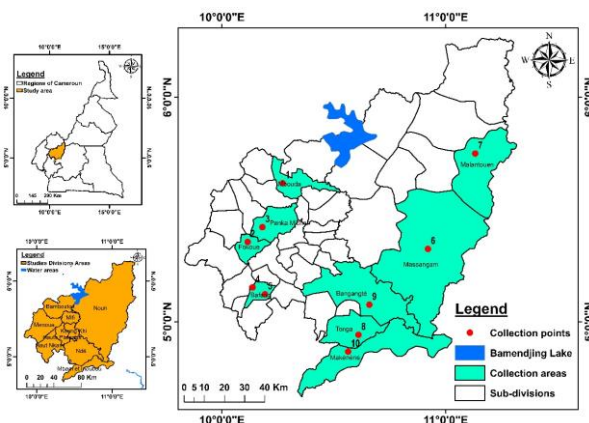


Figure 1 : Location of the collection areas of *C. rotundus* in the West region of Cameroon

presence or absence of the species in the locality, the mode of natural regeneration, the abundance, the local know-how and practices in terms of conservation and propagation of the species.

2.3. Ex-situ regeneration trials of *C. rotundus* from seed and bulbs

Matured seeds were collected from Bantoum II locality and dried for two weeks in the shade at room temperature. A viability test was carried out using seeds sown in Petri dishes containing moistened blotting paper in one hand, or nursed on soil watered daily for 2 months in the other hand. The seed germination percentage was evaluated.

One-year-old bulbs bearing at least one bud were equally collected from the same locality and dried in the shade for 2 weeks at room temperature (Figure 2). Then, 70 holes of 3 cm deep spaced at 50 cm were filled with 100 g of hen droppings and watered. Two days after, 70 bulbs were sown. The experimental plot was maintained by daily watering and weeding until the end of the experiment. The vegetative propagation was assessed by estimating the lag time (period between the sowing of the bulbs and the day of the first emergence) and percentage regeneration of the bulbs. The regeneration percentage was obtained from the following formula:

$$RP (\%) = \frac{\text{number of bulbs planted}}{\text{number of budding bulbs}} \times 100$$

RP = Regeneration percentage



Figure 2 : Bulbs of *C. rotundus* used for regeneration

2.4. Growth monitoring and identification of developmental phases of *C. rotundus*

As soon as the first buds appeared, the developmental phases of *C. rotundus* were identified by following up the growth of the « mother plants », which were labelled to differentiate them from

the secondary plants that rapidly emerged around them through their underground rhizome systems. The aerial parts were monitored by measuring growth parameters such as the height of each mother plant, the number of young shoots or "daughter plants" produced per mother plant, and the number of leaves of each mother plant. The number of leaves and the number of daughter plants were counted every 4 days for 3 months. The BBCH scale was used to identify the different developmental phases [19].

The underground parameters monitored included the changes in colour of the bulb, and the changes in the central pith and the epidermal layer. The underground biomass was measured and the yield estimated by weighing the bulbs produced at the end of the experiment.

3. Results

3.1. Distribution and abundance of *C. rotundus* in the Western Highlands of Cameroon

During the survey, *C. rotundus* was found in 03 localities out of 10 visited in the western highlands of Cameroon. These included Bantoum II in the Nde division, Balessing in the Menoua Division and Massangan in the Noun division. The species is found in a domesticated state near the huts and in the medicinal gardens of traditional practitioners. It is cultivated on very small areas of about 1m² and found in continuous stands covering 3/4 of these areas.

3.2. Local know-how on the domestication of *C. rotundus*

C. rotundus reproduces naturally from bulbs and rarely from seeds. Although traditional practitioners and herbalists have already undertaken the cultivation of *C. rotundus*, it is done on very small areas. The harvested part, the bulb, requires the destruction of the entire plant, which could have a negative impact on the conservation of the species.

3.3. Regeneration from seeds and bulbs of *C. rotundus*

In this study, the regeneration trials from *C. rotundus* seeds were unsuccessful. No seed germinated in the nursery and in Petri dishes after 02 months of sowing.

Regeneration trials from *C. rotundus* bulbs produced 1% budding percentage ten days after sowing. These 10 days represent the lag time of *C. rotundus*. This percentage increased to 78% from day 17th to 24th. From the 25th to 35th day, the percentage of budding increased to 87%. From day 45 the bulbs of *C. rotundus* stopped budding with no further change in percentage (Figure 3).

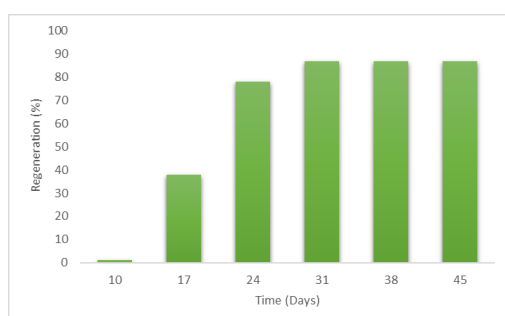


Figure 3 : Evolution of the budding percentage of *C. rotundus* bulbs with time

3.4. Growth features of *C. rotundus*

3.4.1. Evolution of the height of the mother plant with time

Figure 4 shows the evolution of the relative growth rate of *C. rotundus* plants. This rate evolved continuously to reach its peak on day 28 with a value of 1.85 cm.cm⁻¹.d⁻¹. From day 29 to day 60, this rate gradually decreases to reach a value of 1.21 cm.cm⁻¹.d⁻¹. From day 61 onwards, the growth rate remains constant until the experiment is stopped.

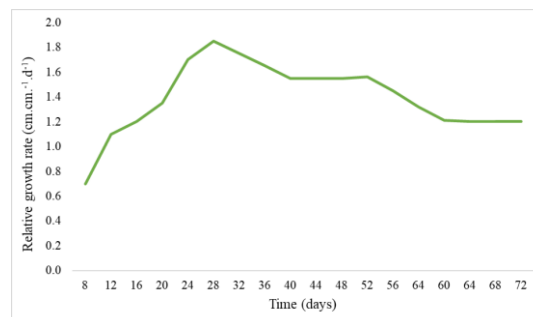


Figure 4 : Evolution of the relative growth rate of the mother plants of *C. rotundus* with time

3.4.2. Shoots production rate and underground biomass

C. rotundus plant regenerates naturally from underground rhizomes to produce lateral shoots or "daughter plants". A single mother plant could multiply to form a dense network or clump. Figures 5 and 6 show changes in the number of lateral shoots produced during the development of *C. rotundus* mother plants and their vegetative aspect, respectively. After budding, the production of lateral shoots started from the 4th week. One shoot was produced per week until the 7th week. A peak production of the daughter plants was observed from the

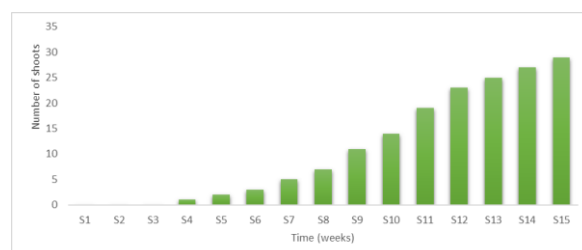


Figure 5 : Evolution of the number of young shoots produced by *C. rotundus* mother plants with time

7th to the 12th week with an average 03 shoots produced per week. A decrease in production to 2 shoots/week was then observed from week 12 to 15. After the 15th week, no shoot production was observed.



Figure 6 : Aspects of the vegetative apparatus of *C. rotundus*: 4 weeks after sowing (a), 12 weeks after sowing (b) and 15 weeks after sowing (c)

3.4.5. Development phases of *C. rotundus*

Changes were observed in the morphology of the plants and in the internal structure of the bulbs, which made it possible to identify

the growth phases of *C. rotundus*. These variations made it possible to identify 5 phases of growth in *C. rotundus*, namely: emergence, bolting, flowering, maturation and senescence.

Emergence, which corresponds to phase 0 on the BBCH scale, was the first phase of development (Figure 8). It was materialised by the appearance of a shoot from the bud that developed at the soil surface. When it appeared, the shoot was whitish in colour and 2 cm high. The period between the sowing of the bulb and the appearance of this shoot was 10 days. At this phase, the new underground bulb had not yet appeared.



Figure 7: Aspect of the *C. rotundus* plant at phase 0 of its growth

The bolting phase is the vegetative growth phase that represented one of the longest phases during the development cycle of *C. rotundus*. It lasted for about 7 weeks after the emergence phase. The bolting period was marked by the development of the vegetative organs, especially the growth of the main stalk that reached a height of 50 cm. A change in its colour was observed: its gradually apex turned from white to green while the colour of its base remained white.

Bulb production was effective at the end of the bolting phase. From its aspect, it consisted of a small central pith, which was white in colour at the beginning, and then evolved to a light brown colour at the end of the phase. This central pith was surrounded by four thin, light-coloured epidermal layers that became increasingly visible (Figure 9). The main bulb regenerated young bulbs that gave rise to seedlings through the development of rhizomes. The propagation of the seedlings was more intensive, with the mother plant producing an average of 10 to 15 seedling

The flowering phase started with appearance of flower and ended with the first seeds. This growth phase 2 lasted for about 5 weeks



Figure 8: Aspects of the internal structures of the bulb of *C. rotundus* (a) at the beginning and (b) at the end of phase 1 of growth

after the vegetative development phase. At this phase, the stalk grew to about 70 cm and its colour which were light green became dark green.

The colour of the bulb changed from light to dark brown. The central medulla was larger than in phase 1 and was white with a spongy centre. The thinner epidermal layers surrounding the medulla disappeared completely (Figure 10).

The production of young lateral shoots continued until it reached an average of 25 daughter plants per mother plant.



Figure 9: Aspect of the internal structure *C. rotundus* bulb at growth phase 2

The maturation phase represents phase 3 in the development cycle of *C. rotundus*. Morphological changes were almost non-existent in the main stalk. However, there were some changes in the internal structure of the bulb. Its central medulla was dotted with small red spots, the colour of its centre changed from white to brown (Figure 11). The production of lateral shoots evolved and stopped at an average of 29 shoots per mother plant. The seeds reached physiological maturity and turned light brown (Figure 12).



Figure 10: Aspect of internal structure of *C. rotundus* bulb at growth phase 3

The senescence which represents phase 5 and thus marks the end of the development of *C. rotundus* was marked by the complete cessation of growth in height of the stalks. At the same time, colour changes in the stalks were observed, which gradually



Figure 11: Seed structure of *C. rotundus* at (a) early and (b) late phase of maturation;

turned from dark green to yellow. During these changes the stalks dropped to the point of complete senescence. In the bulbs, variations were no longer visible and it entered into a quiescence phase. Figure 13 presents the diagrammatical representation of the development phases during the life cycle of *C. rotundus*.

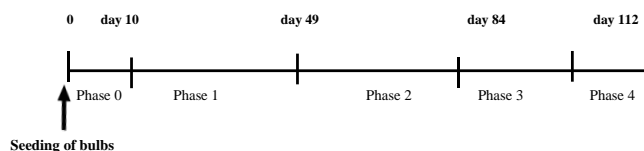


Figure 12: Diagrammatical representation of the development phases during the life cycle of *C. rotundus* (Phase 0 = Emergence; phase 1= bolting; phase 2= flowering; phase 3= maturation; phase 4= senescence)

3.4.6. Biomass production of *C. rotundus*

From one sown bulb, *C. rotundus* produced 29 bulbs at the end of its growth corresponding to a weight of 0.59 kg and produced a yield of 520.83 kg/ha. This yield increased by 383.77 493.42 and 520.83 kg/ha respectively during the vegetative growth or bolting, flowering and ripening phases.

4. Discussion

The observed distribution or range of a species corresponds to the portion of territory occupied by all the populations of this species in the studied area [20]. This is a complex concept, which encompasses the distributional limits of species that are evolving as a result of population dynamics but also human activities [21, 22, 23]. Clear distributional boundaries do not exist unless there is a physical barrier. In this study, the species *C. rotundus* was found in 3 localities and absent in 7. The absence of this species in some localities of the study area could be due to the lack of knowledge of its traditional uses by the populations. Indeed, in the same climate, knowledge of the use of a plant can lead to the domestication of a species from one locality to another. Also, the migratory movements of a population from one locality can lead to the presence of a species in the migration area. Abiotic, biotic or climatic factors can also be limiting or favouring factors for the presence of a species in a locality [24].

Within the natural range of a species, depending on its ability to reproduce naturally, it may have a preference for its mode of propagation. In the conservation process, knowledge of the natural mode of reproduction and the reproductive organs of species helps in approaches to sustainability and improvement. This knowledge includes the type of sexual and asexual reproduction, or the combination of both types [24]. In the case of *C. rotundus*, germination from seed was not successful. This would involve that *C. rotundus* is not able to reproduce generatively from seed but rather vegetatively from bulbs. These results are similar to those of Peerzada in 2017 [25] who showed that *C. rotundus* rarely reproduces by seed but, mainly by bulbs or rhizomes.

A budding percentage of 88% after 45 days was obtained during regeneration trial of *C. rotundus* from the bulbs. These results are not very far from those of [26] who obtained a regeneration percentage of 92% for *C. rotundus* bulbs. The high regeneration rate would be due to the favourable condition such as suitable soil moisture and temperature. Indeed, a *C. rotundus* bulb placed under room temperature can germinate after a minimum of four days or after a maximum of 30 to 45 days [27]. *C. rotundus* bulbs planted in the rainy season regenerated 7 days after sowing [28]. In this work the first bulbs planted in the dry season regenerated from day 10. This result shows that whatever the sowing period, *C.*

rotundus bulbs have practically the same latency period if the conditions for regeneration are met.

Appearance of the young lateral shoots from the underground rhizomes took place 4 weeks after the budding of the mother plant, confirming that formation of the new bulbs that will generate new shoots takes place four to eight weeks after the budding of the mother plants [29]. This production of new shoots was more intensive during the vegetative propagation phase where each mother plant produced an average of 10-15 new shoots. According to previous investigations, a mother plant of *C. rotundus* can produce an average of 20 shoots in two months [30].

Development of a plant is the quantitative and qualitative transformations that accompany the course of the different phases of its life from seed to maturity. These are biological processes that result in an irreversible increase in the dimensions and weight of an individual or its component organs over time [31]. According to the BBCH scale, there are 9 major phases of plant development. However, in this study, 5 major phases of development were identified, including the emergence phase, the bolting or vegetative development phase, the flowering phase, the maturation phase and the senescence phase. Phases such as leaf development and secondary stalk formation were not present. Some phases were considered as secondary phases and were not considered. Many authors such as have equally defined the life phases of a plant in five main phases [32]. Observations started with the emergence of seedlings on the soil surface. The developmental phases of *C. rotundus* identified in this study were on the basis of the date of vegetation initiation, the rate of vegetative development and the transition to reproduction [33]. This model does not take into account environmental signals or biotic interactions. On the other hand, many authors have defined phenology as the set of seasonal biological events of plant life such as germination or budding, vegetative growth, flowering, maturation and senescence determined by the influence of climate variations [34, 35, 36]. Unfortunately, this definition of phenology is not easily adapted to herbaceous species.

Bulb production begins during the bolting phase. Indeed, the bulbs considered as reserve organs are the result of the photosynthetic phenomenon. During photosynthesis, molecules are produced and distributed in the plant. Some of them are made available to the plant for its growth and others are stored in reserve forms in different structures such as bulbs. The bulb was initially white in colour when formed but during its development it turned from light to dark brown at maturity. These results confirmed those of [37] who in his work on *Cyperus esculentus* found that the young, newly differentiated bulbs are white and fleshy and gradually change colour to brown as they mature. This colour change is thought to be due to an accumulation of reserve molecules such as carbohydrates and lipids. In addition, the bulbs can also store other essences such as terpenes which are the constituents of essential oils. In *C. rotundus*, essential oil extractions from senescent bulbs revealed a production of 0.37% from dried bulb. However, this percentage could be variable or impacted under certain conditions, notably the harvesting period, the phase of development of the plant during harvesting, and the climatic conditions in which the plant grows. Indeed, some authors reported that the biosynthesis of essential oils is dependent on

photosynthesis, the level of soil fertility and the climatic conditions [38, 39, 40]

Conclusion

The objective of this study was to find the best methods of multiplication and to identify the growth phases of *C. rotundus* in order to ensure the availability of its biomass for a better valorisation. *C. rotundus* is a perennial plant that naturally regenerates through rhizomes. It is easily propagated vegetatively from bulbs and has 5 growth stages: emergence, bolting, flowering, maturation and senescence. Thanks to its underground organs (rhizomes and bulbs), the plant is able to regenerate naturally when living conditions are favorable to form a dense network. In its settlement area, it is found more in the domesticated state on small plots. The harvesting method with total destruction of the plant could compromise its availability in biomass for industrial and sustainable use. Its bulbs produce essential oils, usable for the perfume industry. From the results obtained, it is advised to use the bulbs to regenerate the species, and to harvest the essential oil extraction at the maturation phase of development of the plant.

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