



Ex-Situ Induction of Sprouting on *Euadenia trifoliolata* Cuttings Harvested from Four Pedoclimatic Areas of Côte d'Ivoire

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Abstract

Euadenia trifoliolata (Capparaceae) is a tropical forest plant. It is used in the pharmacopoeia for its medicinal properties and for that it is overexploited. Information on propagation techniques of *E. trifoliolata* domestication and its wild management are still missing. The study conducted on nurseries aimed to obtain some young plants from cutting stems. For this purpose, cuttings harvested in different areas (Adzopé, Banco, Ehotilé Islands and Marahoué) were used and some parameters like viability, time of emission of shoot and the first leaf, number of leaves, length and width of leaves, length of stems and diameter of stems were evaluated according to the origin and treatment of the cuttings and the growing medium (unheated soil, heated soil (45 °C), soil treated with extracts of *Ricinus communis* (Euphorbiaceae) without heating and heated soil (45 °C) and treated with extracts of *R. communis*). Results showed that the viability varied according locations and treatments. Shoot emission time varied from 13.32 ± 1.12 days to 12.94 ± 1.38 days. The emission time of the first leaf ranged from 22.65 ± 3.82 to 24 ± 6.32 days. There was also variation in the number of leaves (from 5.32 ± 0.19 to 5.13 ± 0.27), the leaf length (from 50.74 ± 1.50 to 53.20 ± 2.47 cm) and leaf width (from 25.96 ± 1.37 to 39.14 ± 2.26 cm). The stem length oscillated between 25.68 ± 1.28 and 29.01 ± 1.50 cm and the stem diameter fluctuated from 12.52 ± 0.51 to 12.06 ± 0.57 cm. *E. trifoliolata* can be regenerated from cuttings and maintained its therapeutic potential. Cuttings treated with newsprint gave the best results whatever the culture medium.

Keywords

Induction, Ex-situ, Sprouting, Cutting, *Euadenia trifoliolata*, Areas Pedoclimatic

Introduction

Medicinal plants are valuable resources for the vast majority of rural populations in Africa. More than 80% of this population use these resources for human and animal health care [1,2]. In addition, non-timber products have attracted considerable interest in Africa in recent years for their contribution to the household economy and the conservation of plant biodiversity [3]. Thus, the strong demand for medicinal plants becomes a threat to the plant species. According to Mehdioui and Kahouadji [4], intensive plant exposure can become harmful if it exceeds the tolerable threshold of renewal of regeneration of the resources used. Regeneration is the process by which the plant gives a new individual by artificial induction. There are several methods of regeneration including cuttings that are the removal of a stem or root fragment from which rhizogenesis is induced [5-7]. Several works have been done on cuttings of plants such as *Garcinia kola* [7], *Vitex doniana* [8] and *Lawsonia inermis* [9]. The success of cuttings depends on endogenous factors (age, size

and appearance) and exogenous factors (soil (substrates), cultural practices, light and temperature [10]. The combined action of these factors allows to patrol some stresses in order to carry out the vegetative multiplication. In Côte d'Ivoire, investigations on medicinal plants are mainly focused on floristic diversity [11], ethnobotany and phytochemical potential [12-15], biological and ethnopharmacological activity [16,17] and biofungicide effects [18]. However, studies on the regen-

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eration of medicinal plants are still limited. This is the case of *Euadenia trifoliolata* (Capparaceae), a medicinal plant used in the pharmacopoeia to treat otitis, anemia, respiratory diseases, male and female fertility disorders and especially as an aphrodisiac [19-21]. In fact, *E. trifoliolata* is overexploited for its medical properties and is becoming more and more rare. Despite this threat, literature on regeneration of *E. trifoliolata* is still missing. This study aimed at investigating the efficacy of "cutting method" in the regeneration of *E. trifoliolata*. We hypothesized that *E. trifoliolata* can be regenerated through cutting to ensure its survive and good management.

Material and Methods

Study areas

Cutting samples of *Euadenia trifoliolata* were harvested in Adzopé, Banco, Ehotilé Islands and Marahoué. Adzopé is situated between latitudes 6°06 N - 6°25 N and longitudes 3°51 W - 3°36 W. Banco is located between latitudes 5°23 N - 5°40 N and longitudes 4°03 W - 4°07 W. Éhotilé Islands are situated between latitudes 5°10 N - 5°37 N and longitudes 3°13 W - 3°44 W. Marahoué is between latitudes 7°05 N - 7°49 N and longitudes 6°01 W - 6°32 W and the UNA is located between latitudes 5°23 N - 5°43 N and longitudes 4°17 W - 5°43 W. The

climate of the harvested and regeneration zones is equatorial. Rainfall oscillated between 1800 and 2000 mm. The relative humidity was 85%. Soils of harvest areas were ferruginous and hydromorphic with acidic pH in general [22]. The samples harvested were authenticated at the "Centre National Floristique de l'Université Felix Houphouët Boigny de Cocody". Figure 1 shows the different harvesting and growing areas of cuttings of *E. trifoliolata*.

Plant material and its regeneration tests

The plant material consisted of cuttings from four areas (Adzopé, Banco, Ehotilé Island and Marahoué), which constituted four populations of *Euadenia trifoliolata* (Schum & Thonn) Oliv (Capparaceae). Four hundred young and old cuttings with external and internal diameters respectively between intervals [1,22] cm and [1,20] cm were used. Their length was 10.5 cm. These cuttings consisted of large and small caliber. The cuttings were taken from end branches of wild plants from June 2015 to September 2015. For the experiment, cuttings were soaked in water from the harvested site in coolers. These cuttings were divided into three groups. The first group had its upper surface in contact with ambient air when the upper surface of the second group and the

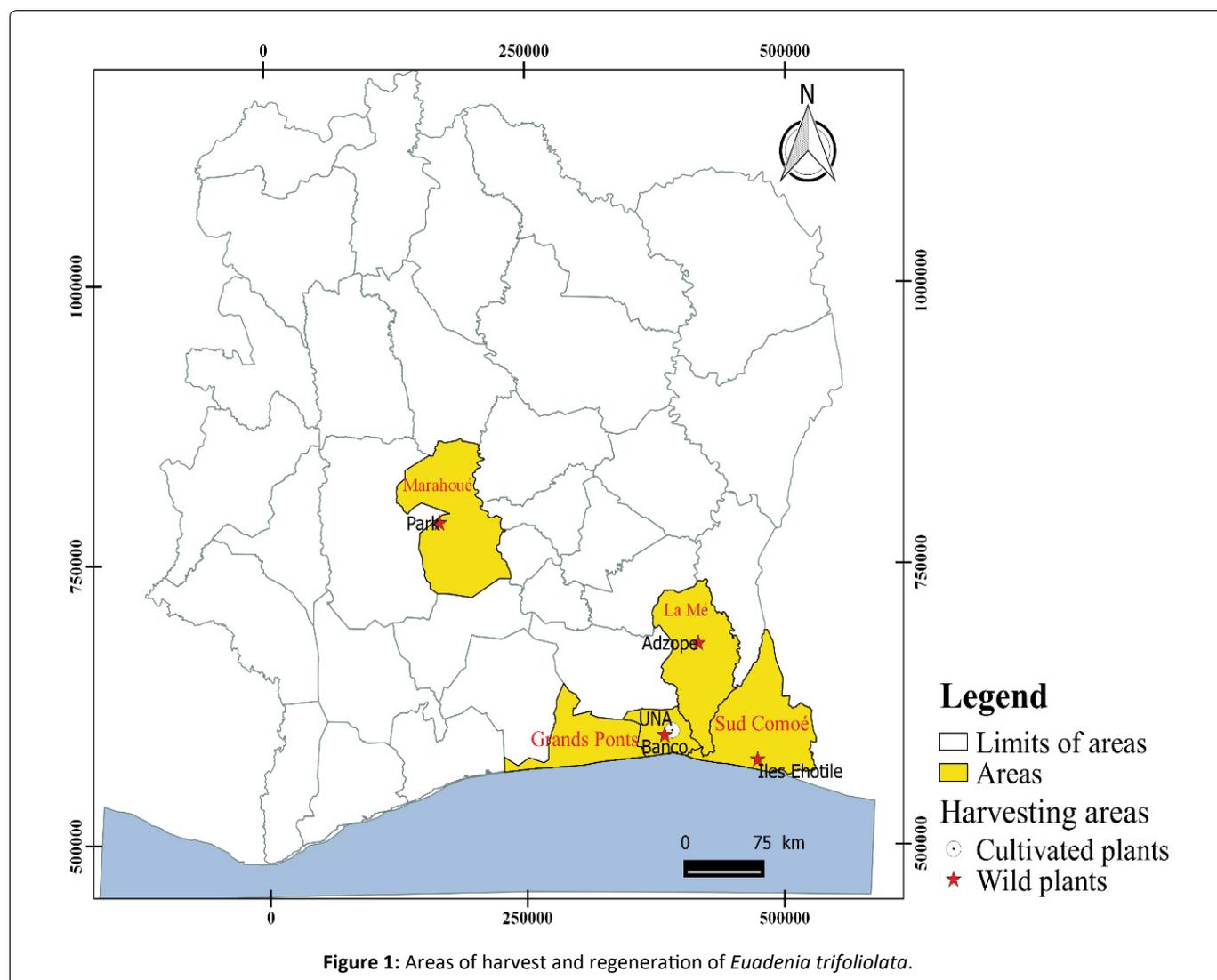


Figure 1: Areas of harvest and regeneration of *Euadenia trifoliolata*.

Table 1: Viability rate of cuttings of *Euadenia trifoliolata* (Capparaceae) (Schum & Thonn) Oliv. according to the period of experiment.

Origins	Rate of average viability		Statistical parameters	
	45 days	90 days	F	P
Adzopé	91.43 ± 0.04	82.43 ± 0.49	0.5	0.67
Banco	75.43 ± 0.04	58.43 ± 0.49		
Ehotilé Islands	98.39 ± 0.04	95.39 ± 0.49		
Marahoué	89.43 ± 0.02	80.47 ± 0.50		

Note: Values are mean ± SEM (n = 5). P < 0.05 compared to group in the same line (One-way ANOVA followed by Turkey's post hoc test).

third group was protected with newspaper and transparent plastic bag on 2 cm length respectively. Cuttings treated were grouped into 4 batches and were sown obliquely in black nursery bags containing untreated soil, heated soil (45 °C), soil treated with extracts of *Ricinus communis* (Euphorbiaceae). The combination of soil treatments and cuttings also generated the following groups of individuals:

- Cuttings that had upper surface free and planted in the soil without treatment (BLS),
- Cuttings that had upper surface free and planted in the heated soil (BLC),
- Cuttings that had upper surface free and planted in the soil treated with extract of ricin (BLR),
- Cuttings that had upper surface free and planted in the heated soil and treated with extract of ricin (BLCR),
- Cuttings that had upper surface protected with newspaper and planted in the soil without treatment (BJS),
- Cuttings that had upper surface protected with newspaper and planted in the heated soil (BJC),
- Cuttings that had upper surface protected with newspaper and planted in the soil treated with extract of ricin (BJR),
- Cuttings that had upper surface protected with newspaper and planted in the heated soil and treated with extract of ricin (BJCR). The treatment of cuttings with transparent plastic bag gave also four groups. They were BTS, BTC, BTR and BTCR.

The bags were placed in the shade. Bio-pesticides applied at the time of cuttings were extracts of *R. communis* and *Azadirachta indica* (Anacardiaceae) [23,24]. Each day, castor and neem extracts were applied on plants at a dose of 1 kg/L of fresh crushed leaf to protect them against pests. The nursery was watered daily to keep the humidity at 85% and weed control was regular. For humidity measurement, the "Extech model RH210" manual device was used. Droppings of guinea fowl were used as compost (fertilizer of the culture medium). The experiment lasted 90 days and Parameters measured were the viability of the cuttings (VC), the time of shoot emission (TSE), time of emission of first leaf (TEFL), number of leaves (NL), the length of leaf (LeL) and width of leaf (WiL), the length of stems (LeS) and the neck diameter of stems (ND). The regenerated plants were subjected to qualitative phytochemical analysis and compared to wild plants. In fact, sam-

Table 2: Averages of viability according to the neck diameters of stem for the period of experimentation.

Origins	Diameters (cm)	
	NDe [1,22]	NDi [1,20]
Adzopé	9.55 ± 4.20 ^a	7.02 ± 3.80 ^a
Banco	9.72 ± 4.43 ^a	6.73 ± 0.49 ^a
Ehotilé Island	8.90 ± 3.37 ^c	6.16 ± 2.87 ^c
Marahoué	11.81 ± 3.97 ^b	8.69 ± 3.75 ^b

Note: In column, averages assigned different letters show significant differences according to Turkey's 5% threshold comparison test.

ples of leaves and roots of *Euadenia trifoliolata* harvested in the different environments were dried at room temperature and then crushed with a grinder "Culatti typ MFC" brand. The different powders obtained were macerated in distilled water using 400 g of powder for 1 L during 24 hours and then filtered. The residues were macerated again for 24 hours twice always in solvent. After filtration, the filtrates were dried in an oven at 40 °C to obtain aqueous extracts that were subjected to phytochemical tests according to methods described by Boua, et al. [25].

Statistical analysis

Data obtained were analyzed using Statistical software 10.0228.2. GLM statistical tests (generalized linear model) ANOVA was used to determine the differences between modalities and Turkey test was applied for averages comparison. Significant differences between means were determined by P-values < 0.05 (i.e. α = 0.05).

Results

Viability rate of cuttings

Cuttings viability rate at 45 and 90 days after burial is summarized in Table 1. There was no significant difference between viabilities at 45 days and 90 days for the same origin. The cuttings without plastic remain viable whatever their origin during the period of experimentation according to Turkey test. The duration of the experiment had no effect on the cuttings. They kept their appearance on the 90 days experimentations with a rate remaining higher than 50%.

Average viability according to diameters

There was a variation in viability according to cutting diameters (Table 2). For cuttings with diameter from 1 to 22 cm,

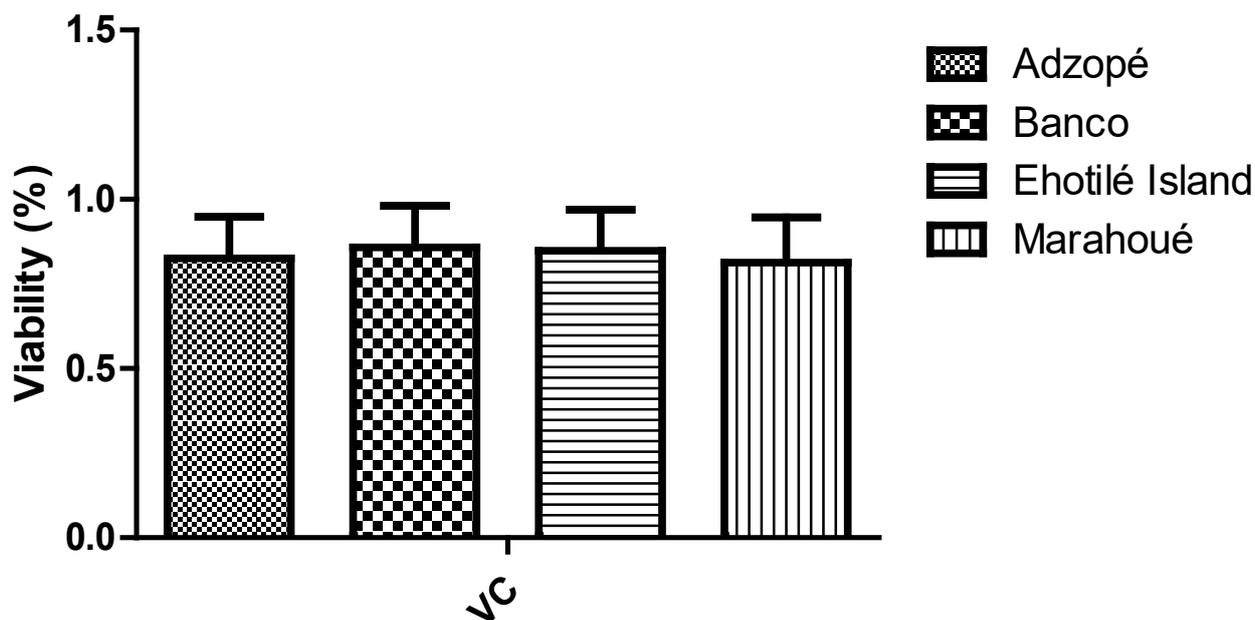


Figure 2a. Viability of the cuttings according to the origins

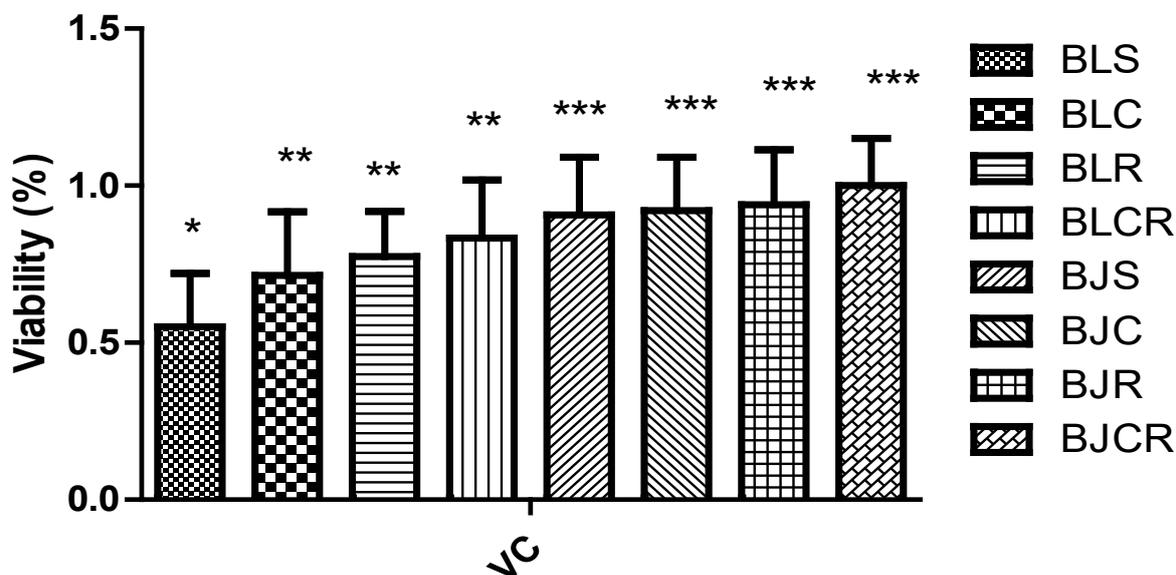


Figure 2b. Viability of the cuttings according to the treatment

Figure 2: Viability of cuttings.

the viability ranged from 8.9 to 11.81. The highest viability was obtained with cuttings from Marahoué and the lowest from Ehotilé Islands. However, there was no significant difference between the viability of cuttings from Adzopé and that of cuttings from Banco. Similar observations were made while using cuttings of diameter varying from 1 to 20 cm.

Viability of cuttings during their growing

The influence of cuttings origin on viability is represented on Figure 2 and shows that the origin of cuttings provenance

does not influence their viability. Cuttings remained viable whatever their origin (Figure 2a). This viability differed according to the origin and varied from 0.81% to 0.85%. The highest viability was obtained with cuttings from Banco and the lowest with cuttings from Marahoué. Viability was 0.82 ± 0.12 for Adzopé, 0.85 ± 0.12 for Banco, 0.84 ± 0.12 for Ehotilé Island and 0.81 ± 0.13 for Marahoué. The treatment influenced the viability of the cuttings (Figure 2b), with a rate ranging from 0.55% to 100%. The averages for the treatments were respectively 0.55 ± 0.17 for BLS, 0.71 ± 0.20 for BLC, 0.77

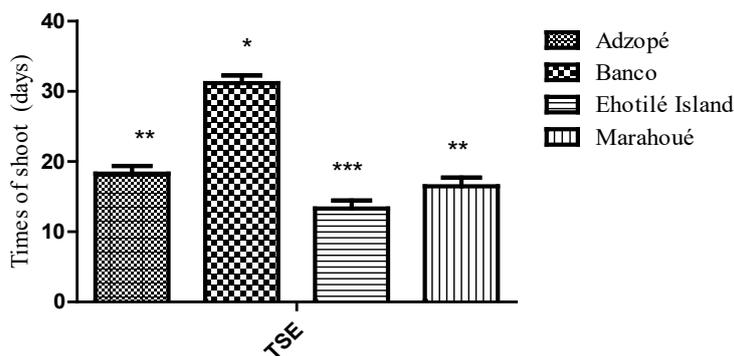


Figure 3a. Time of emission of the growths according to the origin

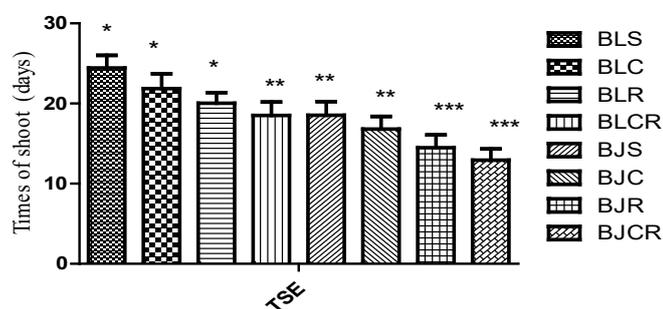


Figure 3b. Time of emission of the growths according to the treatment

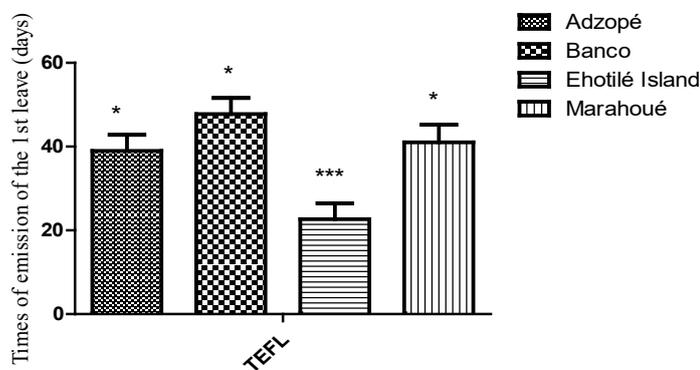


Figure 3c. Time of emission of the 1st leaf according to the origin

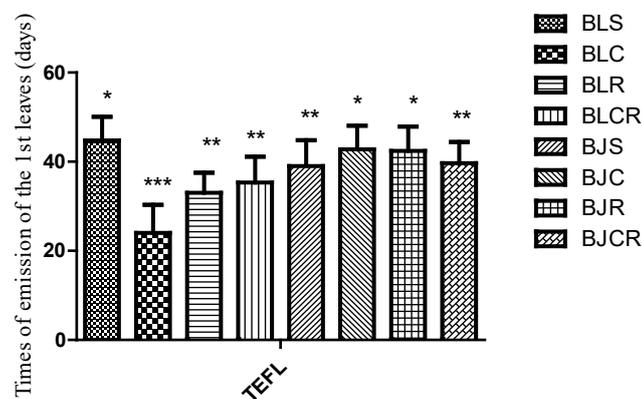


Figure 3d. Times of emission of the 1st leaf according to the treatment

Figure 3: Shoot and first leaf emission time.

± 0.14 for BLR, 0.83 ± 0.18 for BLCR, 0.90 ± 0.18 for BJS, 0.92 ± 0.17 for BJC, 0.94 ± 0.17 for BJR, and 1 ± 0.15 for BJCR. There was no viability for the culture media as BTS, BTC, BTR and BTCR. Globally, it appears that the viability of the cuttings depends on the treatment and not on the origin.

Timing of shoot and first leave emission

Figure 3 shows the emission timing of shoots and first leave in function of cuttings origin and treatments. Plants from Banco had the longest shoot time (31.17 ± 1.12 days) compared to other localities. Plants from Ehotilé Islands had the shortest shoot time (13.32 ± 1.12 days) (Figure 3a). The timing for shoots emission in function of treatments varied from one culture medium to another. The longest time was recorded for BLS treatment (24.42 ± 1.57 days) while the BJCR treatment got the shortest shoot time emission (12.94 ± 1.38 days) (Figure 3b). The emission of the first shoot and leaf on the cuttings from Ehotilé Island was faster than in those of other origins. Cuttings from Banco took the longest time for the emission of the shoot according to Turkey's test. The emission times of the first leaf of the cuttings differed significantly (Figure 3c and Figure 3d). Emission time of the first leaf of the cuttings from Ehotilé Island (22.65 ± 3.82 days) was shorter than that of the cuttings from Banco (47.77 ± 3.82 days) (Figure 3c). Concerning the cuttings from Adzopé and Marahoué, the emission time of the first leaf was the same statistically. The emission time of the first leaf of the BLC treatment (24.00 ± 6.32 days) was shorter compared to those of other treatments. BLS treatment recorded the longest time (44.75 ± 5.34 days) (Figure 3d). Also, the emission of the first shoot was done in a different way and gave three groups of plants. Group 1 or early group was constituted of BJCR and BJR, and group 2 or medium group gathered BJC, BJS and BLCR, when group 3 or group that lasts consisted of BLR, BLC and BLS.

Number, length and width of leaves

The variation in the number of leaves, the length of leaves, the width in function of the origin of cuttings and treatments are shown on Figure 4. The plants of Adzopé had fewer leaves (3.99 ± 0.19) than those of Banco, Ehotilé Island and Marahoué following Turkey test. However, the plants of Ehotilé Islands had the highest number of leaves (5.32 ± 0.19) (Figure 4a). The average number of leaves per cutting ranged from 4 to 5. Plants from BLS, BJS and BJC treatments produced fewer leaves than the other treatments (BLC, BLR, BLCR, BJR and BJCR) (Figure 4b). The leaves of Adzopé (50.74 ± 1.50 mm) and Banco (50.87 ± 1.50) were longer than they were wide while those of the Ehotilé Islands (47.14 ± 1.37 mm) and Marahoué (45.80 ± 1.50 mm) were wider than they were long (Figure 4c). The leaves from the plants of the BLCR and BJCR treatments were the widest while the plants of the other treatments produced the longest leaves (Figure 4d).

Stems length and diameters evaluation of regenerated plants

Figure 5 presents the influence of origin and treatments on stems length and diameters of regenerated plants. The stems of the Adzopé cuttings were shorter than those of Banco, Ehotilé Islands and Marahoué (Figure 5a). However, the stems of the plants of the Ehotilé Islands were the longest (25.68 ± 1.28 cm). The stems of the plants from the BLC, BLR treatments were larger than those of the BJS and BJC treatments. BLS, BLCR, BJR and BJCR treatments resulted in medium size of stems (Figure 5b). The external neck diameter of the plants varied from 9 to 12 cm and the internal diameter from 5.89 to 9.93 cm (Figure 5c and Figure 5d). Plants from Marahoué cuttings had the largest diameter, followed respectively by those from Banco, Adzopé and Ehotilé Islands cuttings. Thus, the BJR and BJCR treatments had the

Table 3: Qualitative contents of extracts of regenerated and wild plants of *Euadenia trifoliolata*.

Phytochemical compounds	Extracts									
	Adzopé		Banco		Iles Ehotilé		Marahoué		UNA	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
Polyphenols	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Quinones	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	+
Anthracenic	+	+	+	+	+	+	+	+	+	+
Compounds										
Reducing compounds	+	+	+	+	+	+	+	+	+	+
Sterols and polyterpenes	+	+	+	+	+	+	+	+	+	+

+: Presence; - : Absence.

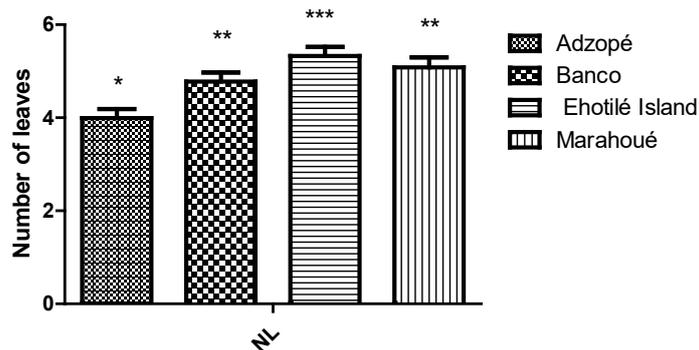


Figure 4a. Nombre of leaves according to origin of cutting

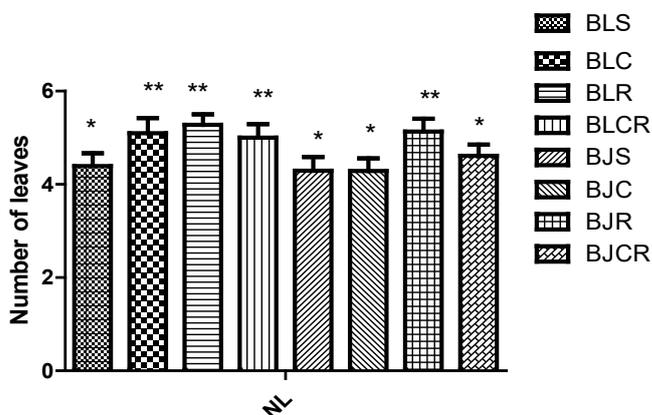


Figure 4b. Number of leaves according to treatment

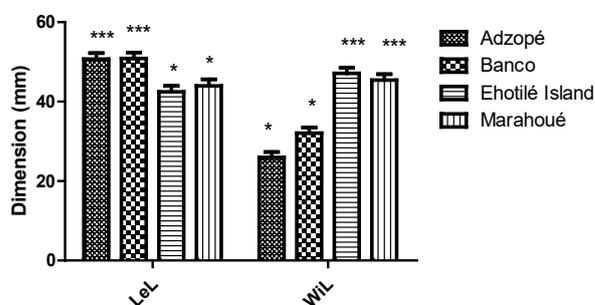


Figure 4c. Length, width of the leaves according to the origin of the cuttings

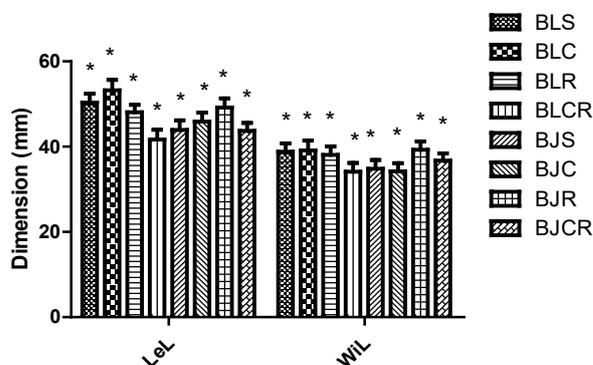


Figure 4d. Length and width of the leaves of the cuttings according to the treatments

Figure 4: Number, length and width of leaves.

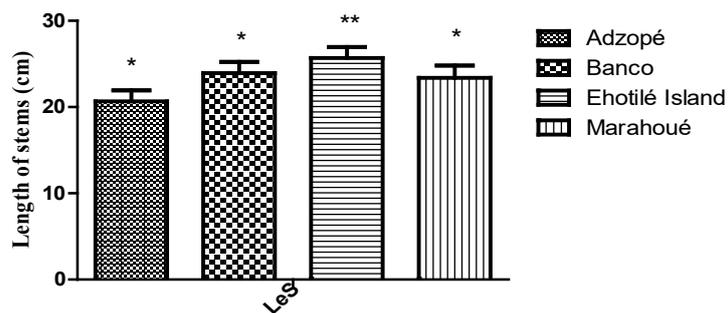


Figure 5a. Length of stems according to origin

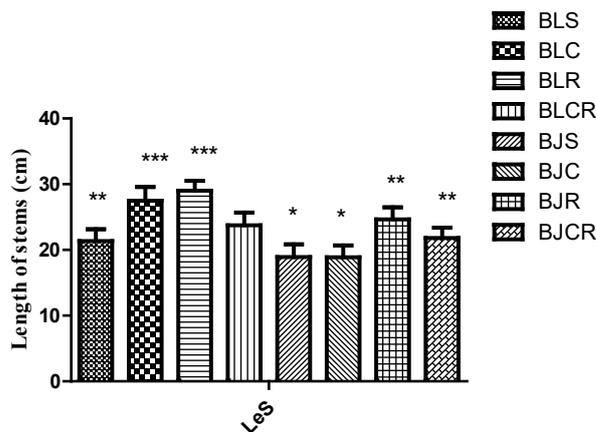


Figure 5b. Length of the stems according to the treatment

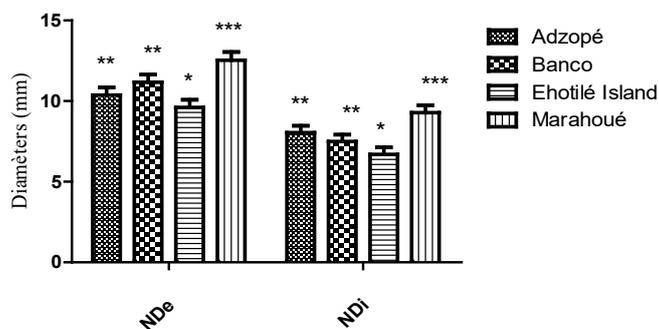


Figure 5c. Evaluation of the diameters according to the origin

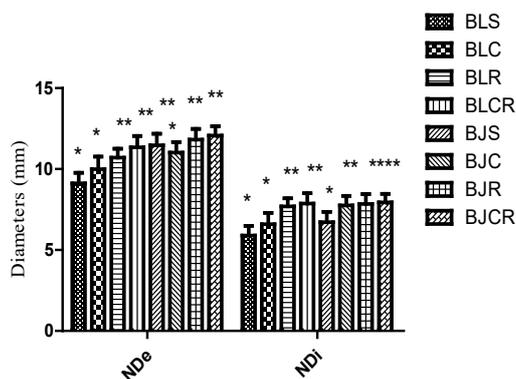


Figure 5d. Evaluation of the diameters of the cuttings according to the treatment

Figure 5: Stems length and diameter evolution of regenerated plants.

largest external diameter followed by BLCR, BJS, BLR and BJC respectively. BLS treatment produced the smallest external diameter. Concerning the internal diameters, the BLR, BLCR, BJC, BJR and BJCR treatments gave the largest diameters. The BLC and BJS treatments produced medium diameters, and BLS treatment gave the smallest diameters (9.11 ± 0.65 , 5.89 ± 0.59 mm) (Figure 5d).

Phytochemicals

Table 3 presents the qualitative composition of *Euadenia trifoliolata*. These results showed that regenerated and wild plants contain polyphenols, tannins, flavonoids, coumarins, saponins, quinones, alkaloids, cardiac glycosides, anthracene compounds, reducing compounds, sterols and polyterpenes. From this study, it appeared that the regenerated and wild plants had the same phytochemicals composition.

Discussion

Good management of genetic resources of medicinal plants is an alternative to their disappearance, erosion and threat. Thus, vegetative propagation has been undertaken to facilitate the reproduction of plants. The multiplication or regeneration of *Euadenia trifoliolata* has been studied through viability, shoot emission time, first leaf emission time, leaf number, leaf length and width, stem length and neck diameter of stem. A quality control of regenerated and wild plants was carried out through phytochemical tests.

The viability of the cuttings during handling showed that this parameter was similar despite the difference in cuttings origin. This analysis showed that the cuttings remained viable at 45 and at 90 days after burial, although the viability at 45 were greater than that at 90 days. These results could be explained by the adaptation of the plant to its new environment which may have allowed it to resist during the regeneration period. The viability expressed with respect to cuttings' diameters showed a significant difference between the parameters. This difference could be attributed to geographical and genetic factors. Indeed, the Ehotilé Islands contain island populations. This geographical position may favor the establishment of plants of small diameters. The study of the viability according to the origin of the cuttings indicated that there was no significant difference but it varied significantly according to treatments. This difference could be due to the treatment of cuttings, biotic and abiotic factors. Regarding the treatments, the use of transparent bag would have prevented the cuttings from breathing. Remaining free or wrapped in newspaper might allow plants to breathe and therefore increase their viability.

The shoot emission timing data on cuttings indicated that there was a significant difference in origin and treatments. From this analysis, it appeared that the shoot growth of the cuttings depended on their origin and treatment. The time of shoots emission of plants from Ehotilé Islands were shorter than others. This result suggested that plants in this locality have adapted to the new environment. The time of shoots emission from Banco cuttings were the longest. These results could be explained by the effect of the environment that, through biotic and abiotic factors, favors the emergence of

shoots. Similar observations were made by Ky-Dembele, et al. [26] during the vegetative propagation of *Detarium microcarpum*. According to these authors, the humid environment of the greenhouse favors the emission of shoots compared to outdoor. In this study, the emission time of shoots from cuttings according to the treatments varied significantly. The BLS treatment took the longest time when that of the BJCR treatment were the shortest. These results could be assigned to the fact that the cuttings from the first medium did not receive any treatment. In the BJCR treatment, protection of upper surface by the newspaper would have created a microclimate that would have kept humidity constant and favored the rapid emission of shoots. Thus, culture conditions may affect shoot release on the organ used, the phenological stage, the level of growth hormone contained in the substrate, and the growing season [27-30]. Regarding the emission time of the first cuttings leaf, there was a significant difference in function of origins on one hand and in function of treatments on the other hand. This difference could be explained by the nature of the cuttings. The cuttings from island populations would have acquired the capacities necessary to support the stress related to the cultural practices. These cultural practices would have stimulated the rapid appearance of leaves in the individuals of the Ehotilé Islands. However, BLC treatments were not promptly for the expression of this parameter. The late reaction of the BLC treatment could be explained by heat that inhibited certain components involved in the rapid appearance of the leaves.

The number of leaves, the length and width of leaves varied in function of the origin and the soil treatment. These results could result from the effect of the environment and genetic factors. Indeed, the synergistic action of these two factors would have stimulated the massive production of leaves. Also, in plants from Ehotilé islands, these environmental and genetic factors would have an optimal effect which would explain a high number of leaves. Our results do not corroborate with those of Kshatri, et al. [31] obtained with the species *Ficus benjamina*. In fact, Kshatri, et al. [31] observed an average number of leaves varying between 1 and 3 with *F. benjamina*. The observed difference in leaves number could be assigned to the composition of growing medium. The results concerning the length and width of the leaves indicated no significant difference between treatments. However, leaf shape varied significantly in function of origin from one treatment to another. This result could be explained by the edaphic and genetic factors. These factors would have favored long-leaf growth in the Adzopé, Banco, BLS, BLC, BLR, BJS, BJC and BJR plants as they were allowed for growth in width for BLCR and BJCR treatments. In addition, the cultivation methods by using compost would have brought nutriment (nitrates) and increased the production of proteins implied in the synthesis of genes that determine differentiation of leaves. Those practices favored a new architecture to the plants in different growth media. The architecture of a plant is the whole of the shapes structural observable at one time. It results from the simultaneous activity of the apical and underground air meristems of the plants. That would have explained the different length of leaves within the regenerated plants. Stems length and diameters

changed significantly from one origin to another. These observations could be explained by the application of compost, the ability of the roots to draw nutrients from the soil and the bioavailability of these nutrients. Indeed, the application of compost may have fertilized the substrate, which allowed the roots to absorb the nutrients necessary for the vertical growth of plants. Since the plants were from different origin and cultivated in different environments, this could explain the difference in size. The use of compost as fertilizer would have increased the level of nitrogen which is the limiting factor of growth and production of plants [32]. The large size of the plants of the Ehotilé Islands and treatments BLC and BLR would be due to threshold content of nitrogen that brought compost to these environments from which the plants derived. This content of nitrogen would have favored rapid growth, hence the production of large plants [33].

Concerning the diameter, it differed significantly in function of origin of treatment. This difference could be due to genetic, physiological factors (photosynthesis and root sucking) and nitrogen in the culture medium [32,34]. The growth in thickness could result from the activity of the genes involved in photosynthesis which allowed gas exchange between the environments necessary for the synthesis of nutrients. In addition, by the phenomena of root sucking, plants would draw nutrients that would have favored the growth in diameters. The nitrogen supply by composting may have contributed to the increase of the diameter of plants.

According to the qualitative analysis, phytochemical elements were detected in all the parts of the regenerated plants as wild plants. This result explains the capacity of cuttings regeneration to conserve its properties. In fact, the regenerated plants are clones of wild plants, and consequently have expressed the same phytochemical constituents. Our results are different from those reported by Rathnayake, et al. [35] when working on *Momordica charantia*. According to these authors, the wild species contains more phytochemical compounds than the cultivated one. This difference could result in the regeneration medium that allow the maintenance of these compounds in the regenerated plants.

Conclusion

From this study, it appears that the regeneration of *Euadenia trifoliolata* can be performed using stem cuttings. But the viability and diameter parameters of the cuttings remain important factors for achieving this regeneration. Thus, the cuttings of big caliber produced shoots in order to have plants. Cuttings from Ehotilé Islands had a better regenerative ability. BLS, BLC, BLR, BLCR, BJS, BJC, BJR and BJCR treatments were favorable for vegetative propagation by cuttings. No important changes have been observed in phytochemical compounds of regenerated plants compared to wild plants.

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