Propagation of Black Plum (*Vitex donania* Sweet) Using Stem and Root Cuttings in the Ecological Conditions of South Benin

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Summary

The black plum is a multi-purpose tree well known to the rural and urban populations of tropical Africa. Its uses are well documented, but its regeneration possibilities have not yet been studied, despite the importance of this species. The objective of this study is to ensure that this tree can be propagated from other organs instead of seeds. In order to achieve this, a trial propagation, using stem and root cuttings, was conducted on two types of substrate: topsoil at the planting site (sand mixed with poultry manure) and soil taken from undergrowth at the forest research station in Pobe, which focuses on the oil palm tree. The trial was based on a randomised complete block, two factors and four treatments. The results show that it is possible to propagate black plum trees from stem *cuttings (46.62% of developed cuttings) but especially from root cuttings (96.25% of developed cuttings). The compared treatments significantly influenced the recovery rate of the cuttings. The first leaf produced by the stem cuttings is multi-lobed (3-5 lobes), while that produced by root cuttings is generally single-lobed. One year after transplanting, the substrate (2/3 sand and 1/3 chicken manure in volume) produced the best plant growth from cuttings.*

Résumé

Bouturage de racines et de tiges du prunier des savanes (*Vitex donania* Sweet) dans les conditions écologiques du Sud-Bénin

Le prunier des savanes est un arbre à usages multiples bien connu des populations rurales et urbaines d'Afrique tropicale. Ses usages sont bien documentés mais ses possibilités de régénération ne sont pas encore étudiées malgré l'importance de l'espèce. L'objectif du travail est d'assurer la propagation de cette espèce à partir d'autres organes que les graines. Pour ce faire, un essai de bouturage à partir de fragments de tige et de racine a été installé sur deux types de substrats: la terre de surface du lieu de l'implantation (sable) mélangée à la fiente de volaille et le terreau de sous-bois prélevé dans la forêt de la station de recherche sur le palmier à huile de Pobé. Le dispositif utilisé pour cet essai est un bloc aléatoire complet avec deux facteurs et quatre traitements. Les résultats montrent qu'il est possible d'obtenir non seulement des plants à partir de tiges (46,62% de reprise) mais surtout à partir de racines (96,25% de reprise). Les traitements comparés ont significativement influé (P< 0,0001) le taux de reprise des boutures. La première feuille émise par les boutures de tige est polylobée (3 à 5 lobes) tandis que celle produite par les boutures de racine est généralement unilobée. Un an après le repiquage, le substrat composé en volume de 2/3 de sable et de 1/3 de fiente de volaille a assuré la meilleure croissance des plants issus du bouturage.

Introduction

The black plum (*Vitex doniana* Sweet) is a tree with thick five-lobed leaves, which is found in all Benin's regions. This tree is used for its fruit, whose pulp is consumed when ripe and used to make a refreshing drink. It is, however, the immature leaves that are most used and consumed as a leafy vegetable. This type of use is equally valued in rural and urban areas, where this leafy vegetable is called "fon-man", which means 'local spinach' in order to distinguish it from leafy vegetables introduced from the West. Apart from its leaves, which are greatly in demand, the black plum tree also has many other domestic uses. For example, it is reported that the bark and root are used in traditional pharmacopoeia. In the same way, an infusion of its leaves is said to cure arterial hypertension (5). Highly valued as a food and commodity, this pan-tropical tree essence is consumed and sold in all parts of Benin (8). It belongs to the Verbenaceae family and the *Vitex* genus. The black plum tree can actually be found all over Africa's tropical regions. Okafor (22) cites it as being one of Nigeria's nutritionally important savannah trees. Leafy

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vegetables represent an important source of nutrients and provide the essential vitamins for a balanced diet, especially vitamins A, B and C, as well as vital minerals, fibres, carbohydrates and proteins (20). As they are accessible to low-income communities in rural and urban areas, traditional leafy vegetables provide an opportunity to improve the nutritional status of poor families. In addition to their nutritional importance, they play an important socio-economic role. Women are the main players in the spread, use, preparation and marketing of these vegetables. Many vegetable cultivation projects have shown that the production of traditional leafy vegetables by small businesses may represent an important source of income for poor populations and especially for women with little capital and limited access to land (19). The intensive and repeated picking of young leaves for the preparation of sauces, systematic picking of fruit, collection of firewood and 'slash and burn' represent the main anthropological factors threatening the survival of the plum tree in its biotope (12). The strong anthropic pressure affecting this species has caused its numbers to fall increasingly in its natural environment. In addition, the seeds of this tree have a very weak germinative capacity (29). In order to get around this problem (2) and ensure the adequate regeneration of this plant, it is necessary to identify other methods of propagation. In order to meet this need, this study aims to evaluate the possibilities of growing black plum tree plants from stem and root cuttings.

Materials and methods

Study environment

The experiment was conducted at the research station at Seme-Podji (longitude 06^0 22' 40" north; latitude 02^0 37' 08" east and altitude 13m), which lies 20 km east of Cotonou. The soil at the site consists of a fairly narrow band of highly humus-rich coastal quartz sand. It becomes less rich in humus further inland, before becoming snow-white at the most northerly point of the concession, along the Cotonou-Porto-Novo road (6). The ground water is very close to the surface. The climatic conditions are as follows: an average rainfall of 1308 mm per year, a long rainy season (April-July) and short rainy season (September-October) and 2075 hours of sunshine per year.

Plant material

The plant material used was collected within a radius of 7 km of the experiment site. The material consists essentially of stem and root cuttings harvested from young *V. donania* plants, which have probably grown from seeds disseminated by frugivorous animals, and particularly bats. These young plants are uprooted from the soil, after digging with a hoe and machete around their bases, in order to avoid damaging their roots. Stem and root cuttings are taken from the young plants obtained in this way, using a pair of secateurs. The lengths and diameters of a sample of 30 cuttings of each type were measured. The average stem length was 62.25 $\pm$ 9.13 cm, while average root length was 21.1 $\pm$ 1.9 cm. The average stem diameter was 13.75 $\pm$ 4.07 mm and 7.3 $\pm$ 5.33 mm for roots.

Cutting cultivation method

The cuttings thus obtained were planted out in 30 cm x 199 cm^2 perforated black polythene bags. Two types of substrate were used to fill the bags. The topsoil at the planting site (sand) was mixed with poultry manure (2/3 sand to 1/3 poultry manure). The second substrate was made from soil taken from the forest undergrowth at the research station in Pobe, which is the substrate generally used for young nursery plants.

After the bags were filled, they were placed in the shade of a cashew tree (*Anacardium occidentale* L.). Watering was conducted 2-3 times per week in order to keep the substrate moist. The cuttings were planted out according to their polarity (with the part closest to the meristem of the terminal stem pointing upwards) in the bags, which were arranged according to a randomised complete block design, with four treatments per block and four repeats. Each experimental unit included 20 plant pots, each of which contained a cutting. The test was conducted from 9 July 2010 - 4 July 2011. The various treatments were as follows:

B1S1: stem cuttings planted out in sand substrate containing poultry manure;

B1S2: stem cuttings planted out in undergrowth soil substrate;

B2S1: Root cuttings planted out in sand substrate containing poultry manure;

B2S2: Root cuttings planted out in undergrowth soil substrate.

Observations

Every week for three months, the number of cuttings that had taken root in each type of substrate was recorded, together with the number of buds produced and morphology of the first leaf as soon as it developed. A final count was taken after three months for each type of substrate. Three months after planting out, the plants that had taken root were first acclimatised, before their height was measured, based on a sample of 10 plants per treatment between the point of sprouting and the terminal bud, one year after planting out.

Statistical analysis

The average values for the different parameters studied were calculated. The significance or insignificance of the differences observed was evaluated by means of a variance analysis (ANOVA) and the Newman-Keuls test for the classification of averages. The variance analysis model used was the 3-factor crossed model: a random factor (block) and two fixed factors: substrate and cutting type. The variance analyse was conducted with SAS software (version 9.1).

Results

Budding capacity and recovery of cutting types

In general, budding and recovery varied according to the types of organ used for plant propagation (Table 1). One week after planting out, the stem cuttings produced buds, which only appeared on the root cuttings during the third week. For stem cuttings, the cumulative recovery rate from the fourth to the sixth week represented the highest percentage and 50.7% of the total percentage of cuttings that budded after planting out. In terms of root cuttings, the cumulative recovery rate from the 3rd to 5th week was the highest and represented 80.5% of the total percentage of cuttings that recovered after planting out. During the first two weeks of planting, the cumulative recovery rate was 19.16% for stem cuttings, compared to 0.6% for root cuttings. This result shows that recovery begins very early for stem cuttings, even if their final recovery rate is low compared to that of roots.

Percentage of stem and root cuttings that produced 1 or 2 buds per cutting and substrate type

The number of buds produced varied very little according to the cutting and substrate type. The percentage of cuttings that produced a bud was 56.2% for stem cuttings, compared to 63.6% for root cuttings. The percentage was practically the same (30%) for stem and root cuttings that produced

Table 1
Number and percentage of stem and root cuttings that recovered, according to the number of weeks

Parameters observed	Weeks												
	1	2	3	4	5	6	7	8	9	10	11	12	Total
Number (%) of stem cuttings that recovered	10 (6.25)	4 (2.50)	1 (0.62)	13 (8.12)	9 (5.62)	15 (9.37)	7 (4.37)	4 (2.5)	1 (0.62)	5 (3.12)	2 (1.25)	2 (1.25)	73 (45.62)
Cumulative recovery % for stem cuttings	6 .25	8.75	9.37	17.50	23.12	32.50	36.87	39.37	40.00	43.12	44.37	45.62	45.62
Number (%) of root cuttings that recovered	0 (0)	1 (0.62)	27 (16.87)	57 (35.62)	40 (25)	13 (8.12)	6 (3.75)	6 (3.75)	2 (1.25)	1 (0.62)	0 (0)	1 (0.62)	154 (96.25)
Cumulative recovery % for root cuttings	0	0.62	17.50	53.12	78.12	86.25	90.00	93.75	95.00	95.62	95.62	96.25	96.25

Table 2
Number and percentage of stem and root cuttings that produced 1-2 buds per cutting and substrate type

Cutting and substrate type	Number of buds				
	1	2	3	4	Total
Stem cuttings in sand mixed with poultry manure	17 (23.3)	12 (16.4)	5 (6.9)	0 (0)	34 (46.6)
Stem cuttings in undergrowth soil	24 (33)	10 (14)	4 (5)	1 (1.4)	39 (53.4)
Total stem cuttings	41 (56.2)	22 (30.1)	9 (12.3)	1 (1.4)	73 (100)
Root cuttings in sand mixed with poultry manure	47 (30.5)	22 (14.3)	6 (3.9)	2 (1.3)	77 (50)
Root cuttings in sand mixed with poultry manure	51 (33.1)	25 (16.2)	1 (0.6)	0 (0)	77 (50)
Total root cuttings	98 (63.6)	47 (30.5)	7 (4.5)	2 (1.3)	154 (100)

The figures without brackets represent the number of stem and root cuttings that produced 1, 2, 3 or 4 buds per cutting and substrate type. The figures in brackets represent the number of stem and root cuttings that produced 1, 2, 3 or 4 buds per cutting and substrate type, compared to the total number of stem or root cuttings that recovered.

Table 3
Average number of stems and roots that recovered per treatment

Treatments	Average number of stem and root cuttings that recovered
Stem cuttings in sand mixed with poultry manure	8,500 b
Stem cuttings in undergrowth soil	9,750 b
Root cuttings in sand mixed with poultry manure	19,000 a
Root cuttings in undergrowth soil	19,250 a
P	0,0001***
CV %	14,78

*** Highly significant difference with a 0.1% threshold; averages followed by the same letter are not statistically different.

Photo 1: *Vitex donania* plant produced from a stem cutting with 1st multi-lobed leaf.

two buds (Table 2). The percentage of cuttings that produced 1-2 buds was 86.3% for stem cuttings and 94.1% for root cuttings.

In terms of substrates, the percentage of stem cuttings that produced one bud per plant in sand was 23.3% compared to 33% for undergrowth soil. However, the percentage of root cuttings that produced a bud in sand was 30.5% compared to 33.1% for undergrowth soil (Table 3). The percentage of stem cuttings that produced two buds was 16.4% in sand compared to 14% for undergrowth soil. For root cuttings, it was 14.3% in sand compared to 16.2% in undergrowth soil.

Recovery rate according to treatment

The average number of stems and roots that recovered per treatment after three months ranges from 8.5 (42.5%) to 19.25 (96.25%). The variance analysis shows highly significant differences between treatments (Table 3). After 12 weeks in the nursery, the best recovery rates were observed for root cuttings, whether the latter were planted out in sand containing poultry manure or in undergrowth soil. In general, the two substrates compared did not influence the recovery rate of the two types of cutting in a different way.

Morphology of the first leaf

It is generally believed that the morphology of the first leaf varies according to the type of material being propagated. The first leaf on the stems was multi-lobed (3-5 lobes, Photo 1), while those on the roots were generally single-lobed (1 lobe, Photo 2). The different percentages obtained for the cutting types are as follows: 55% 3-lobed and 30% 5-lobed for stem cuttings; 88% single-lobed and 10% three-lobed for root cuttings.

Photo 2: *Vitex donania* plant produced from a root cutting with 1st single-lobed leaf.

Height of plants one year after planting out

One year after planting out, the growth in height of plants produced from different cutting types varied according to the treatment. Statistical analysis shows a highly significant difference between treatments (data not provided). The treatment that produces plants with the highest growth (53.4 cm) is that applied to root cuttings in sand mixed with poultry manure. Stem cuttings in undergrowth soil produced the weakest growth (29.6 cm).

Discussion

This preliminary study on stem and root cuttings taken from black plum trees shows that it is possible to regenerate the whole plant from other organs than the seeds. The recovery rate obtained for roots is very high (96.25%), but significantly lower for stems (45.62). The less positive regeneration rates obtained from stem cuttings may be due to several factors: age, sampling period, origin (samples from young or old parent plants), cutting type (basal, intermediate and terminal cuttings) and environmental conditions (1, 3, 9, 10, 11, 16). The good recovery of cuttings is also linked to the internal factors of the latter. According to various authors (1, 14, 26), the following internal factors may influence the recovery rate: carbohydrate content of plants, growth hormones, nitrogen, etc. The significant difference in recovery observed between roots and stems may be due to these internal factors. It has also been observed that the morphology of the first leaf on stems is different from that observed on roots. The first leaf on the stems that appeared after recovery was multi-lobed, while that on roots was single-lobed. Branches on stems develop from axillary buds at the leaf axils, whereas branches form on roots from the *inner initial cell mass* produced by the dedifferentiation of pericambial cells, which return to their meristematic state, then redifferentiate and begin to split again, in the same way as cells in the apical meristem (15). This mechanism forms the basis for the appearance of the first single-lobed leaves on the roots. In all cases (stems and roots), according to Heller (15), the caulogenesis (development of the stem and branches) is followed by the organisation of a caulinary meristem, of the same structure as the apical meristem in the main stem, and comparable tissue elongation and differentiation processes (histogenesis). This shows that the internal composition of the cuttings (stem and root) varies from one place to another and the action of their components is also different. The strong recovery rate observed at root level can be explained by the collection period of the latter. In fact, according to various authors (1, 9, 24), carbohydrate reserves in the roots are high at the end of the rainy season, but low when the plants begin to grow again. Our roots were collected in July, which marks the end of the long rainy season in South Benin. In relation to the substrates that were compared, no difference was observed in the recovery rate for the cutting types used. Studies by several authors on the use of cuttings have shown that physical characteristics, such as particle size, are less decisive for rhizogenesis than the air and humidity of the substrate (27, 30).

The addition of growth substances in the rooting medium used for black plum stem cuttings could improve their recovery rate. Several studies have shown that soaking cuttings in an auxin solution at a concentration of between 300-500 mg/l or adding this hormone to the rooting medium makes it possible to stimulate rhizogenesis and increase the proportion of newly formed roots, with a marked improvement in the recovery rate of some forest trees (4, 13, 17, 21, 23, 27, 28). The new root formation process is intensified by the action of indole butyric acid (IBA), which hydrolyses the polysaccharides, thus increasing the physiological activity of the sugars required to provide vital energy for the formation of the meristematic tissues, which will later produce roots (17, 18). In addition, the interactions between auxin and carbohydrates have been recognised as vital for new root formation (17, 18, 25).

Regeneration techniques, using either stem or root cuttings, make it possible to obtain huge quantities of clones from the selected genotypes, if this propagation method can be used. The cultivation of plants from root cuttings is rare, as they are naturally without buds. Danthu *et al*. (9) have regenerated plants from the roots of species, such as *Ficus platyphylla* Del. and *Ficus elasticoides* De Wild. No comparable studies are currently available on plant propagation using tree roots from the *Verbenaceae* family from the tropical African savannah. This study is therefore the first of its kind to show that it is possible to regenerate a forest species from the *Verbenaceae* family, using organs other than stems and seeds. Clément (7) states that it is possible to propagate catalpas and paulownias from roots. Our study has proven that the plant propagation technique using roots can be used for the propagation of black plum plants, which may have originated from a genetic improvement programme, without this damaging the resulting clones. This propagation method is an important tool for the conservation of the genetic resources of the black plum tree and also for the implementation of domestication programmes focusing on this species. A considerable number of plum tree clones could be produced using simple root cuttings from elite trees selected for their qualities. The nursery and materials used in this study were appropriate, inexpensive and accessible to growers in developing countries. The use of root cuttings is a mass propagation method for *V. doniana*, which makes it possible to overcome the limitations linked to low germination power of seeds of this species.

The improved growth observed one year after planting out in sand mixed with poultry manure can probably be explained by this substrate’s richness in nutrients (particularly nitrogen).

Conclusion

This study has shown that the root cutting technique is efficient and can be used for rapid, large-scale propagation of *V. doniana*. However, regeneration from stem cuttings is less efficient than that obtained from root cuttings. The best recovery rate is obtained from root cuttings planted in substrates containing poultry manure. This underlines the good response of saplings obtained from this type of cutting. The development of an efficient propagation method for black plum trees represents the first vital stage, in terms of promoting the cultivation of this plant as a source of leafy vegetables. Additional investigations are necessary in order to determine the most suitable cropping practices for this new crop (planting density, frequency and height of cutting, response to the application of organic and mineral fertilisers).

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