

Vegetative propagation by root segment cuttings of *Securidaca longepedunculata* Fresen: effect of diameter and insertion mode of root segment cuttings in the substrate

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Abstract

The Guinean Savannah Highlands of Adamawa are full of many species of great socio-economic interests, among which figures Securidaca longepedunculata. Despite its importance, it is subjected to anthropic pressures which quicken its disappearance in this area. The main purpose of the work is to contribute to the domestication of this Polygalaceae with a view to its conservation and introduction into existing peasant production systems. The effect of the diameter of the root segment cuttings and the mode of insertion of the cuttings in the substrate on budding and rooting were evaluated. The experimental design adopted was a split-plot with three replications. The results reveal those cuttings with a diameter of 1.1-2cm showed good budding ($84.84\pm17.4\%$) and rooting ($38.88\pm26.19\%$) ability. This trend was similar for cuttings with a diameter of 0.5-1 cm ($38.88\pm25.22\%$ rooting). Regarding the mode of insertion of the cuttings inserted horizontally into the substrate to 91.11±12.69% for those incubated obliquely. The rooting rate fluctuated significantly (0.03 < 0.05) from $20\pm16.58\%$ in cuttings inserted horizontally in the substrate to $44.44\pm22.97\%$ in those inserted obliquely in the substrate. These results are useful in the development of the propagation datasheet for this species.

Keywords: Adamawa, Domestication, diameter, insertion mode, root segment cuttings, Securidaca longepedunculata.

Introduction

Securidaca longepedunculata is one of the species of socioeconomic interest highly valued by the populations of the Guinean Savannah Highlands (GSH) of Adamawa in Cameroon¹. It is highly sollicited species in traditional african medicine^{2,3}. Differents parts of the species are used in the treatments of various illness^{4,5}. The harvesting method consists of pulling out the whole plant and harvesting all organs⁶. This mode of exploitation of the species by the population does not take into account its regeneration. It is in a regressive dynamic in the GSH. It becomes necessary to contribute to its domestication. Reproduction by seeds, although the most suited to guarantee genetic diversity, remains a problem for this species because it has a low germination rate⁷. Vegetative propagation, which is faster and cheaper, appears to be an alternative⁸. It copies the parental traits and has the following advantages: rapid production of plants, early fruiting and reducing size of individuals 9,10 . It is an option for short-term mass production of plants. One of the greatest advantages of vegetative propagation is the speed with which the genetic qualities of the selected genotype can be reproduced, differing

from germination¹¹. *In-vitro* micropropagation of *Securidaca longepedunculata* has been tested⁷. However, this technique is very expensive for farmers. In the GSH of Cameroon, there is few works on vegetative propagation of *Securidaca longepedunculata* except the recent one of Oumarou *et al.*⁶. These authors evaluated the effect of substrate and length of cuttings on the rooting of this Polygalaceae. However, the suitability for cutting depends on several factors, including the diameter and the method of insertion of the cuttings in the substrate^{11,12}. Large diameter cuttings contain a high amount of carbohydrates¹³. These carbohydrates are the main sources of energy during the rooting process of the cuttings^{14,15}. At the same time, light has a positive impact on the rooting of root segment cuttings^{16,17}.

The main purpose of the work is to contribute to the domestication of *Securidaca longepedundulata* by root segment cuttings in the Guinean Savannah Highlands of Adamawa Cameroon. More specifically, the effect of the diameter and insertion mode of root segment cuttings into the substrate on the aptitude of this Polygalaceae to produce advantious buds and roots are evaluated.

Materials and methods

Study site: The work took place in the GHS of Adamawa located between 6° 00' and 8° 00' North latitude and 11°30' and 15°30' East longitude. The climate is of the guinean type with two seasons: a rainy season from April to October and a dry season from November to March¹⁸. The average monthly temperature varies from 20°C to 26°C. The average annual rainfall is 1500 mm/year¹⁹. The vegetation ranges from shrubby to wooded savannahs dominated by *Daniellia oliveri* and *Lophira lanceolata*²⁰. This flora is highly degraded by human activities so that the density of these species has regressed²¹.

Methods: The cutting test has been carried out in a polypropagator installed in the nursery of Laboratory of Biodiversity and Sustainable Development of the University of Ngaoundéré located near the Bini river. The polypropagator is placed under an aluminium sheet shed that provides shade. Six sheets of transparent sheet are inserted into the roof to filter the light. It is made of local materials and has the shape of a parallelepiped, subdivided into three compartments. The frame is made of wood and covered with transparent polyethylene, which ensures favourable conditions for the development of cuttings. The relative humidity in the polypropagator is between 80 and 100% while the temperature varies from 23 to $28^{\circ}C^{22}$. From the bottom to top, the following layers were arranged inside the polypropagator: a layer of fine sand, large pebbles, medium pebbles, gravel, sand and rooting substrates¹. Inside the polypropagator a pipe is fixed and allows checking the water level regularly.

Careful excavation of the root system of 24 healthy mothers trees was done. The root fragments were removed and transported to the nursery using a cooler containing ice blocks. At the nursery, the root fragments were fragmented into 20cm root segment cuttings and then grouped into three diameter classes: 5-1cm; 1.1-2cm; 2.1-3cm. The root segment cuttings thus described are placed in the homogeneous sand/sawdust mixture in a vertical, oblique (cuttings at an angle of about 30° above the substrate) and horizontal position. The proximal end of each cutting is slightly bevelled¹⁶; cuttings inserted vertically and obliquely into the substrate have about 1 cm of their proximal end exposed outside the substrate.

The experimental design was a split-plot with 03 replicates. The diameter of the cuttings was the primary factor and the insertion mode of the root segment cuttings in the substrate was the secondary factor. The experimental unit was 10 cuttings. The cuttings were watered every day morning and evening with a sprayer delivering water in thin droplets.

Data were collected weekly for budding (from the date of appearance of the first bud) and at the end of the experiment for rooting. These data were: the number of cuttings that budded, the number of leafy shoots, the height of the leafy shoots, the number of leaves per leafy shoots, the number of rooted cuttings, the number of roots per cutting and the roots length of cuttings. A root segment cutting is rooted when the root length is greater than or equal to 1 cm, otherwise, it is put back into the substrate²³. At the end of the experiment, the rooted cuttings are transferred to pots for acclimatization. The location of the buds on the cutting (proximal, medial or distal poles) was also noted during the evaluation of the experiment.

The data collected were subjected to an analysis of variance. Significant means were speared by the Duncan Multiple Range Test. The statistical program used for the analysis of variance was Statgraphic plus 5.0. The Excel spreadsheet was used to draw the graphs.

Results and discussion

Effect of diameter and inert ion mode of cuttings on budding: The cuttings developed the first aerial shoots five weeks after being planted. Following the appearance of buds, the leafy axes developed (Figue-1).



Figure-1: Budded *Securidaca longepedunculata* root segment cutting.

Twenty four weeks after cultivation of the cuttings, the budding rate varied from $67.77\pm31.54\%$ in the 0.5-1cm diameter cuttings to $84.84\pm17.4\%$ in the 1.1-2cm diameter cuttings (Figure-2). No significant difference was observed (0.09 > 0.05).



Figure-2: Percentage of budding of cuttings according to the diameter of the cuttings.

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Regarding the mode of insertion of the root segment cuttings, at the end of the experiment, the budding rate ranged from $52.22\pm27.28\%$ in root segment cuttings grown horizontally in the substrate to $91.11\pm12.69\%$ in those grown obliquely in the substrate (Figure-3). A significant difference was observed between the insertion modes of the cuttings (0.000 < 0.001).



Figure-3: Rate of budding of cuttings according to their insertion mode in the substrate.

Concerning the interaction diameter* insertion mode of cuttings at the end of the experiment, the budding rate varied from $26.66\pm5.77\%$ for root segment cuttings with a diameter of 0.5-1cm inserted horizontally into the substrate to $96.66\pm5.77\%$ for those with a diameter of 2.1-3cm obliquely cultivating (Figure-4). No significant difference was observed (0.06 > 0.05).



Figure-4: Percentage of budding of cuttings according to the interaction diameter* insertion mode of root segment cuttings.

Pole of appearance of buds: The pole of appearance of the buds is variable. 98.15% of the root segment cuttings produced buds on the proximal end compared to 1.85 % on the median end (Figure-5).



Figure-5: Cuttings of *Securidaca longepedunculata* that have formed leafy shoots on the proximal pole (a) and on the median part (b).

Effect of the diameter of root segment cuttings on the growth parameters of at the end of the experiment (24 weeks): The number of leafy shoots fluctuated between 1.19 ± 0.49 in root segment cuttings with a diameter of 1.1 - 2cm and 2.57 ± 1.14 in those with a diameter of 2.1 - 3cm (Table-1). No significant difference between the diameter of cuttings was observed (0.09 > 0.05).

The height of leafy shoots ranged from 25.22 ± 7.49 cm for cuttings with a diameter of 0.5-1cm to 31.77 ± 12.09 cm for those with a diameter of 2.1-3cm (Table-1). No significant difference was noted (0.33 > 0.05).

The number of leaves varies significantly (0.00 < 0.05) from 25.88±9.39 in cuttings of 0.5-1cm diameter to 51.33±24.34 in those of 2.1-3cm diameter (Table-1).

Table-1: Growth parameters according to the diameter of cuttings.

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Diameter of	Number of	Height of leafy	Number of
cuttings (cm)	leafy shoots	shoots (cm)	leaves
0.5 - 1	1.71±0.48a	25.22±7.49a	25.88±9.39a
1.1 - 2	1.9±0.49a	26.23±7.72a	31.72±12.62b
2.1 - 3	2.57±1.14a	31.77±12.09a	51.33±24.34c
Average	2.06±0.71	27.74±9.1	36.31±15.45

Means followed by the same letter are statically identical (P \geq 0.05).

Effect of insertion mode of root segment cuttings in the substrate on the growth characteristics at the end of the experiment (24 weeks): The number of leafy shoots ranged from 1.82 ± 0.63 in vertically inserted cuttings to 2.23 ± 0.95 in those placed obliquely in the substrate (Table-2). There was no significant difference between the insertion modes of the cuttings (0.55 > 0.05).

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The height of the leafy shoots fluctuated between 23.42 ± 6.12 cm in root segment cuttings grown horizontally in the substrate and 30.48 ± 12.75 cm in those inserted obliquely (Table-2). No significant difference was observed (0.28>0.05).

The number of leaves ranged from 23.84 ± 13.48 in cuttings grown horizontally in the substrate to 44.41 ± 24.93 in those inserted obliquely (Table-2). A significant difference was observed (0.01 < 0.05).

Table-2: Growth parameters according to the insertion mode of root segment cuttings.

Insertion mode of cuttings	Number of leafy shoots	Height of leafy shoots (cm)	Number of leaves
Vertical	1.82±0.63a	29.32±7.58a	40.69±12.87b
Horizontal	2.13±0.92a	23.42±6.12a	23.84±13.48a
Oblique	2.23±0.95a	30.48±12.75a	44.41±24.93b
Average	2.06±8.83	27.74±8.82	36.32±17.09

Means followed by the same letter are statically identical (P \geq 0.05).

Interaction diameter*insertion mode of cuttings on the growth parameters at the end of the experiment (24 weeks) : The number of leafy shoots ranged from 1.67 ± 0.13 in cuttings with a diameter of 0.5 - 1 cm grown obliquely in the substrate to 3.06 ± 1.21 in cuttings with a diameter of 2.1 - 3 cm grown in the same position (Table-3). No significant difference was observed (0.82 > 0.05).

Table-3: Number of leafy shoots according to the interaction diameter* insertion mode of cuttings.

Diameter (cm)/ insertion mode of cuttings	Vertical	Horizontal	Oblique	Means
0.5 - 1	1.73±0.58	1.72±0.75	1.67±0.13	1.71±0.48
1.1 – 2	1.73±0.37	2±0.5	1.96±0.73	1.89±0.54
2.1 - 3	1.99±1.01	2.67±1.36	3.06±1.21	2.57±0.19
Means	1.82±0.65	2.13±0.87	2.23±0.68	2.06±0.74

The height of the leafy shoots varied from 19.1 ± 5.58 cm in cuttings with a diameter of 1.1 - 2 cm inserted horizontally in the substrate to 35.22 ± 34.39 cm in those with a diameter of 2.1 - 3 cm grown obliquely (Table-4). No significant difference was observed (0.91> 0.05). The combined effect of diameter* insertion mode of the cuttings did not influence the height growth of the leafy shoots of the cuttings.

Table-4: Height of leafy shoots according to the interaction diameter* insertion mode of cuttings.

Diameter (cm)/ insertion mode	Vertical	Horizontal	Oblique	Means
0.5 – 1	24.63±6.13	23.13±6.08	27.89±5.69	25.22±5.96
1.1 – 2	31.25±2.94	19.1±5.58	28.35±8.97	26.23±5.83
2.1 – 3	32.07±15.17	28.02±16.13	35.22±34.39	31.77±21.89
Means	29.32±8.08	23.42±9.26	30.48±16.35	27.74±11.23

The number of leaves fluctuated between 16 ± 6.08 in cuttings of 0.5 - 1 cm diameter inserted horizontally and 67.38 ± 34.39 in those of 2.1 - 3 cm inserted obliquely in the substrate (Table-5). No significant difference was observed (0.48 > 0.05).

Table-5: Number of leaves per leafy shoot according to the interaction diameter* inertion mode of cuttings.

Diameter of cuttings (cm)/ insertion mode	Vertical	Horizontal	Oblique	Means
0.5 – 1	27.88±6.13	16±6.08	33.77±5.69	25.88±5.96
1.1 - 2	44.66±2.94	18.43±5.58	32.06±8.97	31.72±5.83
2.1 - 3	49.54±15.17	37.07±16.12	67.38±34.39	51.33±21.89
Means	40.69±8.08	23.83±9.26	44.41±16.35	36.31±11.23

Effect of diameter and insertion mode of cuttings on roots formation: Several cuttings of different diameters and inserted vertically, obliquely and horizontally rooted (Figure-6) as a result of the development of leafy shoots.



Figure-6: Rooted cuttings of *Securidaca longepedunculata* from vertical (a), oblique (b) and horizontal (c) positions.

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The rooting rate of the cuttings ranged from $30\pm15.81\%$ in cuttings of 2.1-3cm diameter to $38.88\pm25.22\%$ and $38.88\pm26.19\%$ in those of 0.5-1 cm and 1.1-2 cm diameter respectively (Table-6). The analysis of variance did not reveal any significant difference between the diameters of cuttings (0.58>0.05).

Table-6: Rooting percentage according to the diameter of the root segment cuttings.

Diameter of cuttings (cm)	0.5 - 1	1.1 - 2	2.1 - 3	Means
Pooting (%)	$38.88\pm$	38.88±	30±	35.92±
Kooting (%)	25.22	26.19	15.81	22.41

Concerning the mode of insertion of the cuttings into the substrate, the rooting rate fluctuated significantly (0.03 < 0.05) from $20\pm16.58\%$ in cuttings inserted horizontally into the substrate to $44.44\pm22.97\%$ in those inserted obliquely (Table-7).

Table-7: Rooting percentage according to the insertion mode of the root segment cuttings.

Insertion				
mode of	Vertical	Horizontal	Oblique	Means
cuttings				
Rooting	43.33±	20±	$44.44 \pm$	35.93±
(%)	20b	16.58a	22.97b	19.85
		-		

Means followed by the same letter are statically identical (P \geq 0.05).

Regarding the interaction diameter* insertion mode of root segment cuttings into the substrate, rooting ranged from $16.66\pm7.07\%$ in cuttings of diameter 0.5 - 1 cm inserted horizontally into the substrate to $56.66\pm20.82\%$ in cuttings of diameter 0.5 - 1 cm and 1.1 - 2 cm inserted vertically and obliquely respectively (Figure-7). No significant difference was observed (0.55 > 0.05).



Figure-7: Rooting percentage according to the interaction diameter* insertion mode of the cuttings.

Effect of root segment cuttings diameter on root growth characteristics: The number of adventitious roots ranged from

 1.86 ± 1.12 in root segment cuttings with a diameter of 1.1 - 2 cm to 2.18 ± 1.01 in those with a diameter of 2.1 - 3 cm (Table-8). The analysis of variance revealed no significant difference between the diameters of the cuttings (0.79 > 0.05).

The length of the adventitious roots varied from 4.15 ± 2.65 cm in cuttings with a diameter of 0.5 - 1 cm to 5.71 ± 4.05 cm in those with a diameter of 2.1 - 3 cm (Table-8). No significant difference was observed (0.67 > 0.05).

Diameter of cuttings (cm)	Number of roots	Roots length (cm)
0.5 - 1	2.11±0.95	4.15±2.65
1.1 – 2	1.86±1.12	4.67±3.91
2.1 – 3	2.18±1.01	5.71±4.05
Means	2.05±1.03	4.84±3.54

Table-8: Effect of cuttings diameter on growth characteristics.

Effect of insertion mode of cuttings on the growth characteristics of adventitious roots: The number of adventitious roots ranged from 1.55 ± 1.24 in cuttings placed horizontally in the substrate to 2.43 ± 0.98 in those grown in an oblique position (Table-9). Despite the disparity observed, the analysis of variance did not reveal any significant difference between the modes of insertion of the root segment cuttings (0.21>0.05).

The length of the roots emitted varied from 3.62 ± 3.22 cm in cuttings grown horizontally in the substrate to 5.94 ± 3.94 cm in those grown vertically (Table-9). There was no significant difference between insertion modes of the root segment cuttings (0.43 > 0.05).

Effect of interaction diameter* insertion mode of cuttings on adventitious roots growth characteristics: The number of roots emitted ranged from 1.42 ± 0.88 in cuttings of 2.1 - 3 cm diameter grown horizontally to 2.58 ± 0.57 in those of 0.5 - 1.1 cm inserted obliquely (Table-10). No significant difference was observed (0.82 > 0.05)

Table-9: Influence of insertion modes of cuttings on growth characteristics.

characteristics.		
Insertion mode of cuttings	Number of roots	Roots length (cm)
Vertical	2.18±0.53	5.94±3.94
Horizontal	1.55±1.24	3.62±3.22
Oblique	2.43±0.98	4.97±3.35
Means	2.05±0.92	4.84±3.51

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Table-10	Number	of roots	emitted	according	to the	interaction
diameter*	insertion	mode of	cuttings	5.		

Diameter (cm) / insertion mode of cuttings	Vertical	Horizontal	Oblique	Means
0.5-1.1	2.07±0.61	1.66±0.71	2.58±0.57	2.12±0.63
1.2 - 2	2.25±0.5	1.55±0.46	1.77±0.47	1.86±0.48
2.1 - 3	2.22±0.69	1.42±0.88	2.92±0.14	2.18±0.57
Means	2.18±0.61	1.54±0.68	2.43±0.39	2.05±0.56

The length of the roots emitted varied from 2.87 ± 0.26 cm in the 1.1-2cm diameter cuttings grown obliquely to 7.61 ± 4.08 cm in the 1.1-2cm cuttings grown vertically in the substrate (Table-11). The analysis of variance indicated no significant difference (0.68 > 0.05).

Table-11: Length of roots emitted according to the interaction diameter * insertion mode of cuttings.

Diameter (cm) /insertion mode of cuttings	Vertical	Horizontal	Oblique	Means
0.5 – 1	4.11±1.51	3.08±3.72	5.28±2.86	4.16±2.69
1.1 – 2	7.61±4.08	3.53±4.29	2.87±0.26	4.67±2.88
2.1 - 3	6.11±4.09	4.25±5.48	6.76±3.91	5.71±4.49
Means	5.94±3.23	3.62±4.49	4.97±2.34	4.84±3.36

Discussion: Root segment cuttings have developed the first aerial shoots five weeks after being placed in culture. Indeed, the earliness of the appearance of the first bud depends on the diameter of the cutting, the genotype, the mode of insertion of the cuttings, the application of hormones^{16,24,25}. Carbohydrates in the tissues also play an important role. This is because they are the main sources of energy during the budding process of cuttings^{14,26}. In Burkina Faso, the first bud appeared in *Detarium microcarpum* five weeks after planting¹⁶.

The diameter class has no significant effect on budding. The amount of carbohydrates is not a limiting factor for budding of this species. A similar result is noted in aspen hybrids in Finland²⁷. In the Guinean Savannah Highlands of Adamawa in Cameroon, *Ximenia americana* and *Lophira lanceolata* had developed a high budding rate (57%) in root segment cuttings of 1.5-2.5cm diameter²⁸. In Benin, *Pseudocedrela kotschyi* cuttings of 1.5-3cm diameter budded better (73.91%) compared to cuttings of 0 - 1.5cm diameter (56.52%)²⁹.

Concerning insertion mode, cuttings inserted in an oblique or vertical position bud better, there is no significant difference between these two ways of insertion mode of root segment cuttings in the substrate. This means that they are similar. In fact, one centimeter of the cuttings inserted in these positions is exposed to light. Light would therefore have a positive impact on the budding of this species. Similar results are noted in Burkina Faso¹⁶. These authors noted a high budding rate in D. microcarpum cuttings grown in a vertical position (22±6%). In the same area, all Bombax costatum root segment cuttings inserted horizontally in the substrate died¹⁷. The response of these species is due to their specificity in combination with exogenous factors. 98.15% of the root segment cuttings produced buds on the proximal end compared to 1.85% on the medial part. The same trend was observed in previous work on the same species⁶.

The large diameter cuttings showed high growth parameters. These cuttings develop numerous fast-growing leafy shoots. They would accumulate enough carbohydrates to allow a good leaves formation. Similar results were noted for *Detarium microcarpum*, *Sclerocarya birrea and Vitex doniana*^{16,22,30}. One centimeter of the Polygalaceae cuttings exposed when the cuttings were arranged in vertical and oblique position would positively affect the budding parameters^{16,17}.

Rooting begins after the development of the leafy shoots. The leafy shoots through the process of photosynthesis produce carbohydrates and phytohormones that initiate rhizogenesis³¹. A rooting rate of 35.92% was recorded. However, in the same area, this species was found to be refractory to rooting⁶. Indeed, the collection period of the cuttings also plays a significant role in this process. The production of carbohydrates responsible for budding and rooting varies seasonally^{25,32}.

The diameter of the cuttings had no significant effect on the rooting of this species. However, high rooting rates were recorded in cuttings of 0.5-1cm ($38.88\pm25.22\%$) and 1.1-2cm ($38.88\pm26.19\%$) in diameter. A high rooting rate (34%) in small diameter root segment cuttings (0.15 - 0.30cm) was recorded on *Populus tremula*²⁹. The low rooting rate of large diameter root segment cuttings of aspen (12%) is due to age. When the age of the cuttings is high, regeneration is low³³. These results are contradictory to those in the literature. Several authors reported that large diameter root segment cuttings root better^{17,30}.

The vertical and oblique arrangement of the cuttings significantly improved rooting. This behaviour would be due to the fact that carbohydrates synthesized in the leafy shoots are descended in the cuttings grown vertically and obliquely in the substrate. These carbohydrates coupled with phytohormones are the main precursors of rooting process³⁴. Furthermore, on *Detarium microcarpum* a high rooting rate were obtained both in cuttings placed horizontally and vertically in the substrates ¹⁶. The substrates used by these authors were richer and more

porous than ours. The substrate is one of the factors controlling rooting³⁵.

Conclusion

This work aimed to contribute to the domestication of Securidaca longepedunculata by root segment cuttings. Cuttings with a diameter of 1.1 - 2cm showed good budding and rooting ability; it was alike to root segment cuttings with a diameter of 0.5 - 1cm. As for the insertion mode of the root segment cuttings into the substrate, the oblique and horizontal modes of insertion were more successful for budding and rooting. These first findings demonstrate that S. longepedunculata is suitable to vegetative propagation by root segment cuttings. In the future work, it would be useful to: determine the right period for collecting root segment cuttings; evaluate the effects of diameter at breast height of mother plants; the provenance of cuttings on the aptitude of this species to produce adventitious buds and roots.

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