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Original Article

Effect of Substrates and Draining Time on the Propagation of Seedlings from Stem Fragments (PIF): Case of *Musa acuminata* (Cavendish)

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Abstract *- Banana plays a very important role in people's diets. It is a source of income for producers. This study aimed to investigate the effect of substrate and explant drainage time on the mass propagation of banana plantlets. The production technique of seedlings from stem fragments has been adopted. This method consists of trimming, dehulling, and excising the explant before growing it in two types of substrate, sawdust and rice bran, with draining times of 48h, 72h and 96h. The experimental design is a split plot. The experimental unit consisted of 10 explants with three repetitions. The substrate represents the primary treatment, and the draining time is the secondary treatment. A total of 10x3x3x2=180 explants are used. It emerges from this study that the sawdust substrate is best for budding with 52.22±13.94%. Explants drain at 48h also revealed the best aptitude for budding with a rate of 51.66±17.22%. Concerning rooting, the highest rate is recorded in sawdust at 48.88±20.8%. Similarly, explants drain at 72h show a good aptitude for this parameter with 38.3±21.3%. Seedlings resulting from stem fragments make mass-producing high-quality plants possible, which could constitute an important pillar for food security in Cameroon.*

Keywords *- Seedlings, Stem fragment, Cavendish banana, Substrates, Draining time.*

1. Introduction

Bananas are one of the world's most widely produced, marketed and consumed fruits. Around 85% of production is consumed and/or sold locally in various countries in Africa, Latin America and Asia [1]. It is particularly important in some less developed countries and low-income food-deficit countries, where it can contribute not only to household food security as a staple food but also to income generation as a cash crop [2]. Bananas are rich in energy, mineral salts (Potassium, Calcium, Phosphorus) and Vitamins A, B and C [3,4]. The global banana trade has reached unprecedented levels recently, with an estimated export volume of 21 million tonnes in 2019. The main drivers of the trade are strong supply growth in exporting countries and a significant increase in import demand in particular [2].

In Cameroon, annual production reached around 300,000t in 2016, almost all destined for export. However, export volumes contracted in 2017 as a result of lower production due to poor weather conditions and the suspension of some exports by the Cameroon Development Corporation (CDC) in the South-West region following the intensification of tensions there. The situation deteriorated further, and production fell significantly in 2018. Despite this situation, some players believe that a plan to revive the sector is possible, which would enable significant production to be achieved in the coming years. This would require the creation of a cooperative between the public and private sectors to improve productivity and extend cultivation to other regions of the country.

In the high Guinean savannahs of Cameroon, as in other agroecological zones of Cameroon, growers need to be provided with large quantities of healthy, high-quality germplasm. In addition, several banana production techniques already exist, including rejection, which is a natural propagation method for plantain, but unfortunately, it is not very productive (production of a small number of daughter plants), and the risk of propagating plant material attacked by nematodes and weevils is high [5]; In vitro

propagation is costly and out of the reach of traditional growers [6]. Several authors have tested different banana propagation techniques, particularly [4, 7, 8, 9, 10, 11, 12, 13].

The technique of producing seedlings from stem fragments makes it possible to produce large quantities of healthy plant material quickly and on the surface [13]. This technique is easy to implement and is one of the possible solutions for obtaining banana plants. In the high Guinean savannahs of Cameroon, no author has discussed the technique of using seedlings from stem fragments.

This study aimed to investigate the effect of substrates and draining time on the propagation of seedlings from stem fragments of *Musa acuminata* (Cavendish).

2. Materials and Methods

2.1. Study Site

The study was carried out in the high Guinean savannahs of Adamaoua (located between 6° 00‘ and 8° 00’ North latitude and between 11° 30’ and 15° 30’ East longitude). This area is characterised by two distinct seasons with varying degrees of contrast. It has a Sudano-Guinean climate with two seasons. The rainy season runs from April to October, and the dry season from November to March. Annual rainfall varies from 900 to 1500 mm depending on the locality.

The temperature varies between 9.9°C and 34.6°C, with an annual average of 23°C. The soil in the region consists mainly of red ferralitic structures developed on old basalt [14]. The vegetation ranges from shrub to tree savannas dominated by *Daniellia oliveri* and *Lophira lanceolata* [15]. This vegetation is under considerable threat from human activity. The region is made up of several ethnic groups, including the Foulbés, Mboums, Pérés, Koutines, Haoussas, Niza'as and Dourous, who are the most common ethnic groups.

2.2. Methodology

The experiment was carried out in a polypropagator installed in the Laboratory of Biodiversity and Sustainable Development nursery at the University of Ngaoundéré, located near the River Bini. The polypropagator is housed in a shed that provides shade. It is made from local materials, is shaped like a parallelepiped and is subdivided into 03 compartments. The framework is made of wood and covered with transparent polyethylene, which provides favourable conditions for developing the explants. The relative humidity in the polypropagator is between 80 and 100%, while the temperature varies between 23 and 28°C. From bottom to top, the following layers are arranged inside the polypropagator: a thin layer of fine sand, large boulders, medium boulders, gravel, sand, and finally rooting substrates.

The shoots at the bayonet stage used in this trial were carefully collected from the adult plants in the plantation to avoid injuring them. These shoots were then decorticated. This phase consists firstly of carefully removing the layers of sheaths, starting by crossing them in a ‘V’ shape and cutting 2 mm above the insertion line on the stem each time, up to the stage where there are three layers of sheaths. Then remove the last leaf sheaths, which may harbour eggs or adult weevils. Finally, cut the remaining pseudostem 2 cm above the last shelled level [9]. The bulbs are cleaned using a sterilised, sharp knife to remove all the roots and necrotic parts of the bulb [12]. The bulb explants are then obtained and placed on a support to avoid contamination by soil-borne diseases. After identifying the cauline apical meristem (central point inside the last of the concentric circles of the pseudostem), a 3 cm deep cross-shaped incision was made using a sterilised knife and latex gloves (Figure 1). The first incision was made in line with the point of attachment of the shoot to the mother plant. The cauline apical meristem was touched to disorganise it and remove apical dominance [16]. The excised explants were placed in the shade for 48h, 76h and 96h.

Fig. 1 Explant trimmed and incised

The explants were grown in two substrates (sawdust and rice bran) previously installed in the polypropagator. The explants were separated by 15 cm between the rows and columns and spaced 6 to 10 cm apart. The trial was watered every morning using a watering can.

2.3. Weaning and Acclimatisation of Seedlings

This stage consisted of harvesting 20 cm or taller seedlings 60 days after the explants were planted. The polypropagator was watered before weaning the seedlings. The explants were harvested using a small, sharp knife sterilised beforehand to avoid contamination. The explants were removed from the substrate, and the seedlings were cut 3 cm above their point of attachment to the explant and placed in bags containing a mixture of sand, sawdust, soil and previously decomposed droppings. The explant was returned to the substrate for the rest of the trial. This removal triggered the activation of new buds on the explant, which produced new seedlings. The transplanted seedlings were

placed under a shade canopy to continue the acclimatisation process. The seedlings were watered twice a day.

The substrate was the primary treatment, and the drainage time was the secondary treatment. The experimental unit consisted of 10 explants with three repetitions. The experimental design used was a split plot.

2.4. Data Collection and Analysis

Data collection began from the date of appearance of the first bud and covered the following growth parameters: the number of explants that had budded, the number of seedlings, the height of the seedling, the number of leaves, and the length and width of the leaves. Rooting was assessed at the end (60 days after the explants were placed in culture) and concerned the number of rooted seedlings and the length and number of roots of the seedlings.

The data collected were subjected to an analysis of variance. The means of the different treatments were separated by Duncan's test. STATGRAPHIC plus 5.0 was used for this purpose. The curves and graphs were produced using a Microsoft Office Excel 2010 spreadsheet.

3. Results

Two weeks after planting, the first shoot was observed in the sawdust substrate, and 4 weeks later, shoots were observed in both substrates.

3.1. Budding

3.1.1. Budding Rate and Growth Parameters According to Substrates

Explant Bud Break According to Substrate

This evolution shows that the bud burst rate of explants, depending on the substrate, varies from 40 ± 21.94% for explants cultured in rice bran to 52.22 ± 13.94% for those cultured in sawdust (Figure 2). Generally speaking, observation of this figure shows that the bud break rate of explants in sawdust from the 2nd to the 5th week is higher than that of explants introduced into rice bran and that at the 1st week, the bud break rate is the same. From the 5th to the 6th week, the bud burst rate stopped increasing, and a plateau was reached. Analysis of variance showed no significant difference between the different substrates (0.17>0.05).

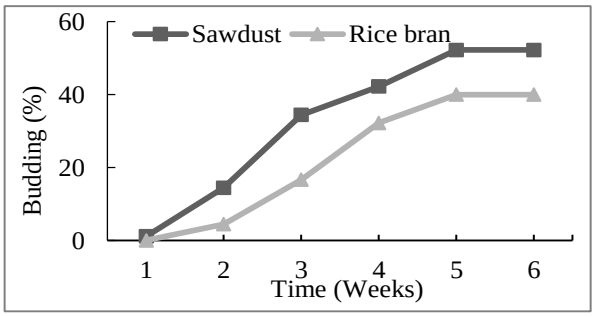

Fig. 2 Evolution of budburst rate according to substrate

Growth Parameters According to Substrates

The number of seedlings varied from 9.77±7.99 in the rice bran substrate to 13.33±4.41 in the sawdust substrate (Table 1). Despite the superiority of seedlings in sawdust, the analysis of variance did not show a significant difference (0.26>0.05). Using rice bran or sawdust as a growing medium gave a satisfactory result. In fact, using either of these substrates resulted in more than 23 seedlings per Cavendish bulb. The height of seedlings ranged from 12.78±3.56 cm for seedlings grown in rice bran to 15.09±3.73 cm for those grown in sawdust (Table 1). Despite the superior height of seedlings in sawdust, analysis of variance showed no significant difference (0.06>0.05).

The number of leaves varied from 12.44±7.92 in seedlings grown in rice bran to 22.55±8.07 in those grown in sawdust (Table 1). Analysis of variance indicated a significant difference (0.01<0.05). This result indicates that the substrate containing sawdust has better porosity and a better water retention capacity and is, therefore, better supplied with organic matter.

Leaves length varied from 13.65±6.10 cm for explants grown in rice bran to 17.82±4.45 cm for those grown in sawdust (Table 1). Despite the superiority of leave length in the sawdust substrate, analysis of variance did not show a significant difference (0.11>0.05).

The width of leaves varied from 8.14±3.33 cm for seedlings grown in rice bran to 9.58±2.07 cm for those grown in sawdust (Table 1). Analysis of variance did not show a significant difference (0.29>0.05). The width of the leaves reacted favourably to our two substrates.

Table 1. Growth parameters according to substrate

Substrates	Growth parameters				
	Number of seedlings	Height of seedlings (cm)	Number of leaves	Length of leaves (cm)	Width of leaves (cm)
Sawdust	13.33±4.41a	15.09±3.73a	22.55±8.07a	17.82±4.45a	9.58±2.07a
Rice bran	9.77±7.99a	12.78±3.56a	12.44±7.92b	13.65±6.10a	8.14±3.33a
Mean	11.55±6.20	13.93±3.64	17.49±7.99	15.73±5.27	8.86±2.70

This means the same column with different letters is statistically different at the 5% threshold.

3.1.2. Budding Rate and Growth Parameters According to Drainage Time

Explant Bud Break According to Drainage Time

Figure 3 shows that the budding rate at the end of the trials varies from 41.66±20% for explants cultured with a 72h draining time to 51.66±17.22% for explants cultured with a 48h draining time. The observation indicates that the bud break of explants cultured after draining for 48h is higher than that of explants drained for 96h and 72h. Explants cultured for 96 hours showed a rapid increase in bud burst between the 3rd and 4th weeks until they surpassed those cultured for 72 hours ahead of them. From the 5th week onwards, the bud burst rate stopped increasing. It reached an optimum and remained constant until the 6th week. There was a plateau until the 6th week. The analysis of variance did not show a significant difference between drainage times (0.67>0.05). Latent time does not influence explant budbreak, and it could be that other endogenous than exogenous factors need to be taken into consideration.

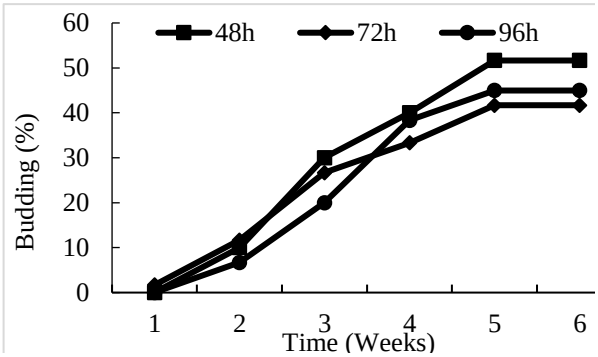

Fig. 3 Change in bud break rate as a function of drainage time

Growth Parameters According to Draining Time

Seedling numbers ranged from 9.83±5.67 in explants drained for 48 h to 14.5±7.79 in explants drained for 96 h (Table 2). This result suggests that the longer the time, the greater the number of seedlings. Analysis of variance does not indicate a significant difference despite this variation (0.42>0.05).

The height of seedlings varied from 12.25±2.87 cm for seedlings with a draining time of 96 h to 14.85±3.98 cm for those with a draining time of 72 h (Table 2).

Observation of this table shows that the height of seedlings at the 48h and 72h draining times is greater than at the 96h draining time. Analysis of variance does not indicate a significant difference (0.42>0.05).

The number of leaves according to draining time ranged from 17±8.94 for explants cultured at 72h draining time to 17.83±12.54 for explants drained for 48h (Table 2). Analysis of variance showed no significant difference (0.98>0.05).

The length of leaves according to draining time varied from 13.55±4.89 cm for explants cultured at 96h draining time to 17.71±6.47 cm for those cultured at 72h draining time (Table 2). Analysis of variance showed no significant difference (0.46>0.05).

The width of leaves varied from 8.03±1.97 cm for explants cultured at 96h draining time to 9.38±2.61 cm for those cultured at 48h draining time (Table 2). Analysis of variance showed no significant difference (0.69>0.05).

Table 2. Seedling growth parameters as a function of drainage time

Draining time	Growth parameters				
	Number of seedlings	Height of seedlings (cm)	Number of leaves	Length of leaves (cm)	Width of leaves (cm)
48h	9.83±5.67	14.70±4.27	17.83±12.54	15.95±5.53	9.38±2.61
72h	10.33±6.01	14.85±3.98	17±8.94	17.71±6.47	9.17±3.81
96h	14.5±7.79	12.25±2.87	17.66±7.68	13.55±4.89	8.03±1.97
Mean	11.55±6.49	13.93±3.70	17.49±9.72	15.73±5.63	8.86±2.79

3.2. Rooting of Seedlings

During the evaluation of the trial, some seedlings showed a dense root system and considerable root lengths in the different substrates and at different draining times. Figures 4 a and 4b show the root systems of seedlings on the different substrates.

(a) **(b)**

Fig. 4 Seedlings rooted in rice bran (a) and sawdust (b)

3.2.1. Rooting According to Substrate

Rooting Rate According to Substrate

The rooting rate ranged from 22.2±9.7% for seedlings grown in rice bran to 48.88 ± 20.8% for those grown in sawdust (Table 3). In fact, the rooting rate of seedlings in the sawdust substrate was higher than those in the rice bran. The analysis of variance shows a significant difference (0.00<0.01).

Rooting Parameters According to Substrates

The number of roots varied from 13.90±2.61 in seedlings grown in rice bran to 15.97±2.40 in those grown in sawdust (Table 4). Roots length varied from 9.47±3.41 cm in seedlings grown in rice bran to 11.22±2.58 cm in those grown in sawdust (Table 4). Analysis of variance showed no significant difference in either root number (0.09>0.05) or root length (0.23>0.05).

3.2.2. Rooting of Seedlings According to Draining Time

Rooting Rate of Seedlings According to Draining Time

The rooting rate fluctuated from 31.6 ± 18.3% in seedlings with a 48 h drainage time to 38.3 ± 21.3% in those with a 72 h drainage time (Table 5). However, the rooting rate was 36.6±25.8% for seedlings with a draining time of 96h. Analysis of variance showed no significant difference (0.86>0.05).

Rooting Parameters of Seedlings According to Draining Time

The number of roots varied from 14.41±2.37 in seedlings with a 72 h drainage time to 15.35±3.53 in those with a 48 h drainage time (Table 6). Analysis of variance did not reveal any significant difference (0.84>0.05). Roots length ranged from 9.33±3.19 cm in seedlings with a draining time of 96 h to 12.02±2.90 cm in those with a draining time of 48 h (Table 6). Analysis of variance showed no significant difference (0.27>0.05).

3.3. Weaning and Acclimatisation of Seedlings

After 60 days of observation, the seedlings with a height of 20 cm or more were weaned (Figure 6) into plastic pots. These seedlings were acclimatised at the nursery.

(a) **(b)**

Fig. 6 Seedling weaned from the explant (a) and transferred to a pot (b)

Table 3. Rooting percentage according to substrate

Rooting	Substrates		
	Sawdust	Rice bran	Mean
Rooting percentage	48.88±20.8b	22.2±9.7a	35.54±15.25

Means with different letters are statistically different at the 5% threshold

Table 4. Rooting parameters according to substrates

Substrates	Growth parameters	
	Number of roots	Roots length (cm)
Sawdust	15.97±2.40	11.22±2.58
Rice bran	13.90±2.61	9.47±3.41
Mean	14.93±2.50	10.34±2.99

Table 5. Rooting percentage according to draining time

Rooting	Draining time			
	48h	72h	96h	Mean
Rooting percentage	31.6 ± 18.3	38.3 ± 21.3	36.6±25.8	35.5±21.8

Table 6. Rooting parameters according to draining time

Rooting parameters	Draining time			
	48h	72h	96h	Mean
Number of roots	15.35±3.53	14.41±2.37	15.05±2.32	14.93±2.74
Roots length (cm)	12.02±2.90	9.69±2.89	9.33±3.19	10.34±2.99

4. Discussion

The first buds were observed in the sawdust 14 days after planting the explants. In the Central African Republic, the sand + parchment mixture favored a faster emergence, with an emergence rate of 100% on the fourteenth day [7]. [10] obtained a lag time varying from 21 to 54 days in the Democratic Republic of Congo. The lag time varies with varieties and growing conditions [12]. Substrate and drainage time do not significantly influence explant budbreak in the present study. So it may be that other endogenous than exogenous factors need to be taken into account.

According to [17], banana production is influenced by the growth of the vegetative apparatus. This growth concerns the number of leaves emitted on the one hand and the height and circumference of the pseudotruncus on the other. The number of seedlings varies from one cultivar to another. However, [18] in Gabon reports that the number of seedlings varies from 10 to 100. While [11] in Benin on plantain, obtained a variation of 6-9 seedlings. [10], report an average number of 3 seedlings per release in the Democratic Republic of Congo. [8] worked on the effect of compost based on banana and cocoa residues on the growth and development of Vivo seedlings of three plantain varieties (Corne 1, PITA 3 and FHIA 21) in Côte d'Ivoire and reported that growth in height and circumference of banana plants was greater in compost-based substrates than in the control substrate, which was a mixture of peat, sand and black soil.

Their results suggest these substrates have physical and chemical qualities favourable to plant growth. The organic matter in the compost would, therefore have improved the characteristics of these substrates, and the effect of Hydrogen potential, Cation Exchange Capacity (chemical characteristics), granulometry, and porosity (physical characteristics) are factors that influence the availability and uptake of nutrients [19, 20]. These factors also influence water mobility in the environment, allowing plants to grow in height and thickness. Moreover, [21] obtained significantly higher growth variables with 75% peat than with compost-based substrates.

In the present work, the results obtained show that the substrate containing sawdust has better porosity and a better water retention capacity and is, therefore, better supplied with organic matter. This favoured the emission and leaf growth of plants repotted on this substrate. These plants also have a good photosynthetic capacity, especially as the number of leaves is an indication of good photosynthetic activity [22]. Similar results were reported by [23] and [24], who highlighted the significant effect of substrates based on organic materials on the number of leaves and leaf area.

The bud break of explants cultured after draining for 48h (51.66±17.22%) was slightly higher than that of explants drained for 72h and 96h. [9] in the Democratic Republic of Congo, several plantain cultivars set a draining time of 48h and noted a maximum recovery rate of 92.2±1.8% in the Saba variety. Draining times vary with the author, ranging from 24 to 48 hours [9, 12, 16]. Rice bran proved to be a good substrate for seedling rooting. This result agrees with that of in Togo, who showed that rice husks produced a good percentage of budding and rooting. According to [25], root emission and growth in young banana plants were not affected by the growing substrates, showing that the plants were in the same conditions from the water point of view. Water conditions strongly influence the number of roots.

5. Conclusion

The aim was to study the mass production of good quality banana plants using the technique of production of seedlings from stem fragments in the high Guinean savannahs of Adamaoua. As far as budding is concerned, a high budding rate (52.22 ± 13.94%) was noted in explants cultured in sawdust compared with rice bran (40 ± 21.94%). Similarly, explants cultured with a draining time of 48 hours budded better (51.66±17.22%) than those cultured at 72 and 96 hours. As far as rooting was concerned, sawdust was better (48.88 ± 20.8%), as were the explants with a 72-hour draining time (38.3 ± 21.3%). In future work, it would be important to test different cultivars and treat the explants with disinfectant solutions before cultivation.

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