

Influence of the Size of Potato Microtubers (*Solanum tuberosum* L.) on the Yield of Plants under Semi-Axenic Conditions

Abraham Diémé, Oumar Ba, Maurice Sagna, Mame Ourèye Sy*

Laboratoire Campus de Biotechnologies Végétales, Département de Biologie Végétale, Faculté des Sciences & Techniques, Université Cheikh Anta Diop, BP 5005, CP 10700, Dakar-Fann, Sénégal

Email: *oureye.sy@ucad.edu.sn

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Abstract

The potato (*Solanum tuberosum* L.) is a vegetable that ranks fifth in the world for human consumption. Its importance is growing more and more in the Senegalese diet. However, the potato production in Senegal does not meet the needs of the market, which maintains dependence on the outside for the supply of quality seeds. In addition, these imported seeds do not often have phytosanitary qualities required for local production in the Sahelian zone. The *in vitro* production of microtubers, used as seed, has been shown to be one of the most efficient means for propagation of basic material. To overcome the constraints linked to the supply and availability of potato seeds, with high germination capacity, the impact of the microtuber size on the yield of the plants under semi-controlled conditions was examined. The pre-germinated microtubers were produced *in vitro* from vitroplants of 3 different varieties (*Aida*, *Atlas*, *Odessa*) adapted to the edaphic-climatic conditions of Senegal. The effects of the seed sizes of microtubers, greater than 4 mm, sown under semi-controlled conditions, on the yield of the plants, result in an increase in the ratio, in the vegetative development of the plants, but also in the number and size of the microtubers harvested. The yield of the plants also depends on the variety. It can therefore be envisaged to produce local potato seeds from microtubers and microtubers.

Keywords

Solanum tuberosum, Microtubers, Germination, Seed Size, Yield, Microtubers

1. Introduction

Senegal is a Sahelo-Sudanese country whose major climatic features result from

the conjunction of geographic and aerological factors. Due to its geographical position on the African continent and its western limit by the Atlantic Ocean, it has a North Atlantic coastal fringe. This one extends from the Cape Verde peninsula to the delta of the Senegal River for approximately 195 km long. This coastal zone benefits from a flow under particular climatic conditions. This is represented by the maritime trade wind from the Azores high pressure north to northeast. The maritime trade winds are constantly humid, cool and even cold at night in the dry season, and marked by low diurnal thermal amplitude. It is at this coastal fringe that the cultivation of potatoes is mainly done in a sandy interdune area called the “Niayes”, from November to June [1].

The potato sector is booming in Senegal. In 2011, production was 12,000 tons of potatoes and 118,000 tons in 2017. Officially, potato production in 2019/2020 is 178,175 tons while in 2016, production is assessed to 67,485 tons; which would correspond to 4 and a half months of consumption. This positive increase in the areas sown for this horticultural speculation, places the country among the 5 largest suppliers of starch in the West African sub-region. The two main production basins, in Senegal, seem to be Kayar and Mbane with potentials estimated at 80,000 and 60,000 tons, respectively. The good results of the harvests of recent years can be explained by the increase in the annual national subsidy for the acquisition of potato seeds, which rose from 130 million CFA francs in 2013 to 3.4 billion 400 million in 2018, *i.e.* 500 tons of seeds subsidized by the State in 2013 against 10,444 t in 2018. The main constraint for producers is the lack of a tuber storage and conservation store for a long time. This is a problem that persists in the horticultural sector. The other major problem to be solved for the potato sector concerns the availability of local certified seeds. Thus, the FAO confirms that Senegal imported 43,417 t of potatoes in 2018 (8th importing country in Africa) and the state constantly subsidizes the purchase of imported seeds.

The potato (*Solanum tuberosum* L.) is a perennial herbaceous plant with underground stem tubers rich in starch. It is demanding in water, the control of irrigation is at the base of the profitability of the culture in hot regions. From an agronomic point of view, its cultivation is easy and its yield potential is high, *i.e.* 20 to 30 t/ha [2]. Its culture is practiced in the dry and cold season, which allows a good tuberization of the plant because it needs a pronounced daily thermoperiod that is to say significant temperature variations between day and night, *i.e.* thermal amplitudes of 10°C to 15°C difference. The choice of suitable varieties and quality seeds, both resistant and healthy, is also essential to obtain a profitable crop. Indeed, crops grown from uncertified seeds have low or even minimal yields, and are more susceptible to diseases and insects throughout their growth, as well as after harvest.

Potato production in Senegal faces many difficulties related to the supply of quality seeds. Indeed, the production of vitrotubers is easily carried out and does not require any special infrastructure since the *in vitro* tuberization can be carried out in a dark culture chamber at room temperature of 19°C to 22°C and in

media supplemented with high concentration of carbohydrates and a cytokinin. Vitrotubers have a quality advantage conferred by a production carried out in the laboratory, under aseptic conditions, without the possibility of external contamination [3] [4].

Potato microtubers (*S. tuberosum* L.) produced *in vitro* are currently used in many sectors of agriculture as research material but also as quality seeds [5]. Indeed, the microtuber is considered as one of the most effective means of spreading basic materials, as well as transporting and preserving potato germplasm. In practice, large microtubers are preferred because they are easy to handle and produce vigorous plants [6]. They are also less prone to drying out in storage, have a short dormancy period compared to larger conventional tubers and a high survival rate when transferred directly to the soil [7]. These microtubers can be used as seeds and can be easily transported from one country to another or even from one continent to another by air. Moreover, microtubers and minitubers as well are a possible alternative for the production of potato seeds and to overcome phytosanitary contaminations [3].

To make the “potato” sector more attractive in Senegal, it would be necessary to improve the performance of Family Farming Farms and the competitiveness of their products to help strengthen food security and improve the living conditions of farmers and producers. For this, it is possible to set up an improved and shortened field multiplication scheme that requires intensification of the production of pre-basic material from *in vitro* laboratory. This is possible in tropical areas and would be profitable for the production of local seeds because the cost of local labor is lower and would have a low impact on production costs.

Given its economic importance in the Senegalese agri-food sector, the tree potato varieties (*Aida*, *Atlas* and *Odessa*) developed in the North have been chosen. They have proven to be well-adapted to Senegalese agro-climatic conditions. Furthermore, they give a good yield per hectare to the field, with large tubers and present culinary qualities appreciated by local consumers. These varieties also exhibit good storage capacity over a long period.

The aim of this study was to examine the effect of microtuber size of three potato varieties, produced *in vitro*, on the yield and vigor of plants and minitubers resulting from the germination of these vitrotubers, under semi-controlled conditions.

2. Material and Methods

2.1. Biological Material

The plant material used represents the microtubers of the 3 potato varieties (*Aida*, *Atlas* and *Odessa*) harvested aseptically from different *in vitro* tuberization media [8] and kept in Petri dishes hermetically sealed with parafilm. They were stored in darkness, in a cold room at $4^{\circ}\text{C} \pm 1^{\circ}\text{C}$, for at least 3 months, a period which corresponds to dormancy.

The microtubers of each variety were previously sorted and classified into two sizes (less than 4 mm and greater than or equal to 4 mm). Then, they were

pre-germinated under diffused light at room temperature ($25^{\circ}\text{C} \pm 1^{\circ}\text{C}$) for 2 - 3 weeks.

The germinated microtubers were maintained at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 3 weeks, at a physiological stage of “multiple sprouts”, as previously described by [9], and were sown in black polyethylene bags. These ones were filled with a sterilized soil (120°C , 2 h) aerated with perlite granules (2v/v). They were incubated in a glasshouse and sown at the rate of one microtuber per bag and 30 per size and per variety. Watering is done every two days with tapwater. At the end of the plant’s development cycle (3 - 4 months), one harvest was made per variety and per size (Table 1). The harvested minitubers were washed in deionized water, surface-wiped with blotting paper, weighed and then calibrated.

2.2. Biomass Determination

After 3 - 4 months of culture, the fresh and dry weight of the aerial and root parts (data not shown) and minitubers were measured. The parameters were evaluated (Table 1) using a Sartorius precision scale (accuracy: 0.0001 g) for fresh and dry biomass determination. Following these measurements, each part was separated from each other. Then, these latter parts are dried in an oven (BINDER brand) for 72 h for the aerial parts and for 10 days for the minitubers samples at a temperature of $70^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Then, the dry biomass of the aerial parts and the minitubers were weighed again, respectively.

2.3. Statistical Analysis

A statistical study comprising an analysis of the variance and the calculation of the mean, are carried out by the software SPSS version 17.0. The statistical analysis focused on the comparison of the different calibers using an analysis of variance (ANOVA) at a threshold of 5%, followed by a comparison of the means (Student, Newman and Keuls’s test). The results obtained are then represented in graphs using the EXCEL software.

3. Results

3.1. Influence of Microtuber Size on the Vegetative Development

By testing three varieties, we found that the vegetative development of plants from microtubers, with a size greater than 4 mm, is more vigorous than that ones with a caliber less than 4 mm, for all varieties. Thus, the fresh weight of the

Table 1. Agro-morphological traits characterized for the 3 varieties of potato maintained growing under shade for 3 - 4 months.

Varieties	Microtubers sown	Microtubers sown	Agro-morphological parameters studied for each size of Microtubers sown					
	Size < 4 mm	Size \geq 4 mm	Fresh Biomass of Aerial parts (g)	Dry Biomass of Aerial parts (g)	Number of minitubers collected/Variety	Average Size of minitubers (mm)	Average fresh weight of minitubers (g)	Dry Biomass of minitubers (g)
<i>Aida</i>	30	30						
<i>Atlas</i>	30	30						
<i>Odessa</i>	30	30						

aerial part of the plants resulting from microtubers, with a size greater than 4 mm, is also higher (Figure 1, Plate 1 and Table 3). This result is more significant for the *Aïda* and *Odessa* varieties. However, in the *Atlas* variety, there is no significant difference between the plants obtained from these two weights.

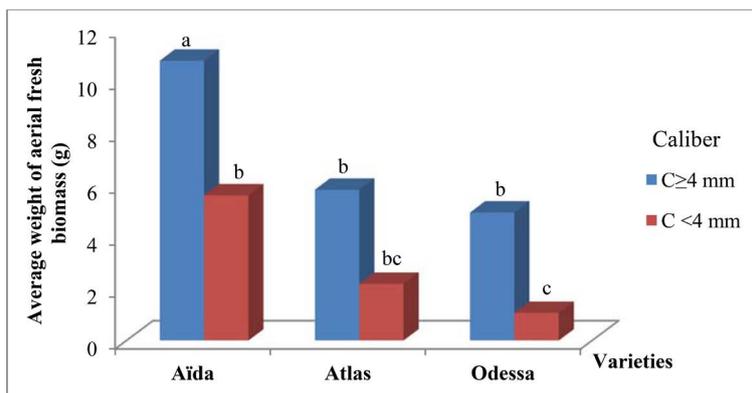


Figure 1. Effects of microtuber size on the vegetative development of plants of the *Aïda*, *Atlas* and *Odessa* varieties. For each variety and caliber, values followed by the same letter do not differ significantly at a p value of 5%.



Plate 1. Morphological appearance of minitubers at the start of formation (A1, A2) in alveolar sowing trays and potato plants obtained from microtubers of 3 varieties (B, C, D and F) sown in polyethylene bags and placed under shade, after 3 to 4 months of culture.

3.2. Influence of Microtuber Size on the Average Number of Minitubers

The influence of the size of the microtubers on the yield of the plants obtained is examined. Thus, plants from microtubers, larger than 4 mm in size, gave a higher yield. The number of minitubers, harvested from plants grown from microtubers with a size greater than 4 mm, for the *Atlas*, *Aïda* and *Odessa* varieties is, respectively equal to 18, 32 and 16 minitubers. The ratio is also, respectively, equal to 1.8, 3.5 and 2 minitubers harvested per microtuber planted (Figure 2). However, for the *Aïda* variety, the Student-Newman-Keuls test indicates that there is no significant difference in the yield of plants from microtubers larger or smaller than 4 mm.

The yield of minituber plants also depends on the variety (Table 2). A higher yield was found in *Aïda* (58 minitubers) than in other varieties (Plate 2). The following is *Atlas* variety (26) which, despite the lack of germination of two microtubers, had a higher yield than that of *Odessa* (23).

3.3. Influence of Microtuber Size on the Average Weight of the Minitubers

This study revealed that the average weight of minitubers harvested per plant is higher in plants produced from microtubers with a size greater than 4 mm for all varieties (Figure 3 and Table 3). This weight is not significantly different for all

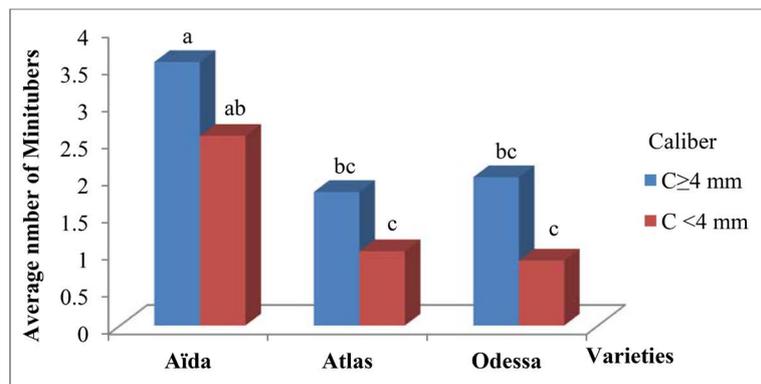


Figure 2. Effects of microtuber size on the mean number of minitubers of the *Aïda*, *Atlas* and *Odessa* varieties. For each variety and caliber, values followed by the same letter do not differ significantly at a p value of 5%.

Table 2. Number of minitubers harvested per size and variety.

Varieties	Number of minitubers per Size		Total
	≥ 4 mm	< 4 mm	
<i>Aïda</i>	32	26	58
<i>Atlas</i>	18	8	26
<i>Odessa</i>	16	7	23

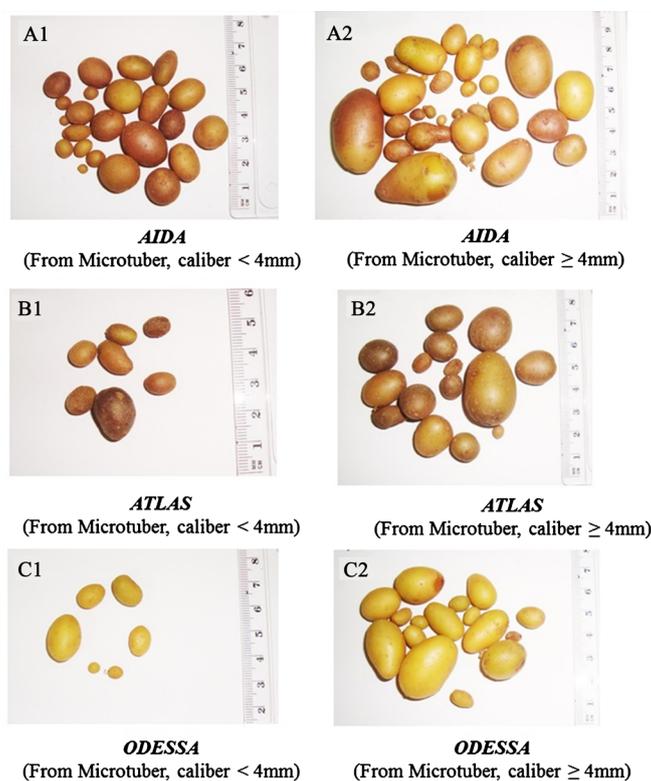


Plate 2. Morphological appearance of minitubers of the three potato varieties (Aida, Atlas, Odessa) harvested from plants, at the end of the development cycle, and issued from microtubers of size < 4 mm and ≥ 4 mm (A2, B2, C2).

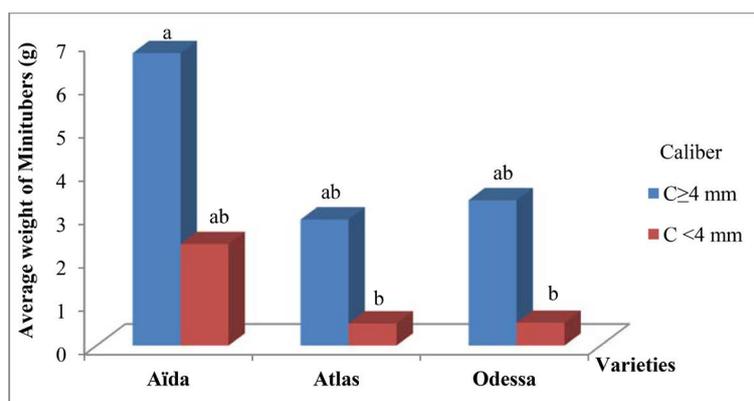


Figure 3. Effects of microtuber size on the fresh biomass of the harvested minitubers. For each variety and caliber, values followed by the same letter do not differ significantly at a p value of 5%.

varieties. Within each variety, there is no significant difference between the average weights of the minitubers from plants of the two sizes. However, the *Aida* variety appeared to be the best as it gave the highest average weight per plant.

3.4. Effects of Microtuber Size on the Average Size of the Minitubers

The experiment revealed that for all varieties, the average minituber size of

plants, from microtubers larger than 4 mm, is higher than that of minitubers from plants grown from microtubers smaller than 4 mm. The average size of minitubers of the *Odessa* variety is bigger than that of other varieties. However, this size is not significantly different from other varieties according to the Student-Newman-Keuls test. For the minitubers of plants produced from microtubers, with a caliber less than 4 mm, the variety *Aida* produced the larger minitubers. However, this result, as previously, is not significantly different from that of the other varieties (Figure 4 and Table 3).

3.5. Effects of Microtuber Size on the Above-Ground Dry Biomass

The Student-Newman-Keuls's test revealed that the above-ground dry biomass of these three varieties depends on the size of the microtubers sown. Thus, plants from microtubers, with a size greater than 4 mm, gave a higher dry biomass comparatively to plants produced from microtubers, with a size less than 4 mm. The *Aida* variety, with a higher yield in number of minitubers formed (32), gave a higher dry biomass than that of other varieties for the same size. This result is

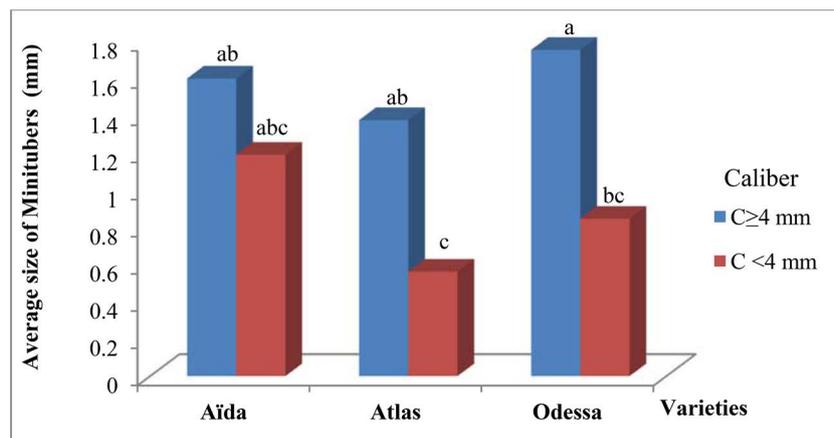


Figure 4. Effects of microtuber size on the average size of harvested minitubers. For each variety and caliber, values followed by the same letter do not differ significantly at a p value of 5%.

Table 3. Effects of microtuber size on the yield of the plants under semi-axenic conditions.

Varieties	Size (mm)	Yield of plants from microtubers					
		Fresh Biomass of Aerial parts (g)	Dry Biomass of Aerial parts (g)	Ratio	Average weight of minitubers (g)	Mean Size of minitubers (mm)	Dry Biomass of minitubers (g)
<i>Aida</i>	C < 4	5.61 ^b	0.94 ^b	2.55 ^{ab}	2.355 ^{ab}	11.9 ^{abc}	0.31 ^b
	C ≥ 4	10.78 ^a	1.69 ^a	3.55 ^a	6.749 ^a	16.1 ^{ab}	0.82 ^a
<i>Atlas</i>	C < 4	2.21 ^{bc}	0.29 ^c	1 ^c	0.514 ^b	5.7 ^c	0.08 ^c
	C ≥ 4	5.83 ^b	0.86 ^b	1.8 ^{bc}	2.912 ^{ab}	13.8 ^{abc}	0.45 ^b
<i>Odessa</i>	C < 4	1.07 ^c	0.24 ^c	0.87 ^c	0.531 ^b	8.5 ^{bc}	0.06 ^c
	C ≥ 4	4.95 ^b	0.82 ^b	2 ^{bc}	3.364 ^b	17.6 ^a	0.38 ^b

Ratio: Number of minitubers per plant; For each variety, values in the same column followed by the same letter do not differ significantly at 5% level.

significantly different from that of the other varieties. *Atlas* and *Odessa* varieties have the same behavior with respect to biomass. So, they were not significantly different (Figure 5 and Table 3).

3.6. Effects of Microtuber Size on the Dry Biomass of the Minitubers

The dry biomass of the minitubers of plants derived from microtubers, with a size greater than 4 mm, is higher than that of the minitubers of plants from microtubers with a caliber less than 4 mm, for all varieties (Figure 6 and Table 3). The minitubers harvested from the *Aïda* variety plants resulting from the microtubers, with a size greater than 4 mm, gave the highest dry biomass with 0.82 g. The latter is significantly different from that of all other varieties. The dry biomass of minitubers collected from plants, derived from microtubers smaller than 4 mm in size, was not significantly different for all varieties.

4. Discussion

Our results clearly established that vegetative development of plants, diameter,

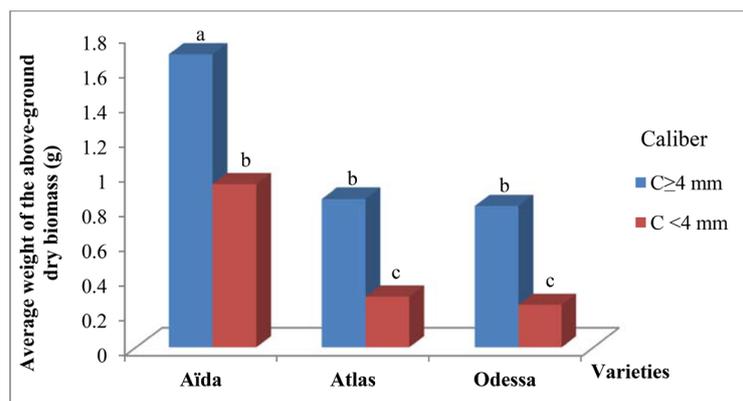


Figure 5. Effects of microtuber size on the above-ground dry biomass. For each variety and caliber, values followed by the same letter do not differ significantly at a p value of 5%.

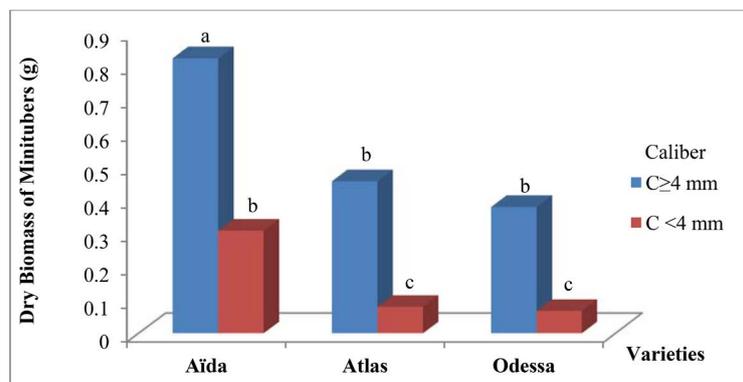


Figure 6. Effects of microtuber size on the dry biomass of the harvested minitubers. For each variety and caliber, values followed by the same letter do not differ significantly at a p value of 5%.

weight and number of minitubers harvested in the greenhouse are influenced by the size of the microtubers sown.

4.1. Effects of Microtuber Size on Vegetative Development

The vegetative development of plants is influenced by the size of the microtubers planted but also by the genotype. Indeed, the larger the size of the microtuber, the more the plant resulting from the latter has a vigorous vegetative development. Thus, for all varieties, plants produced from microtubers, with a size greater than 4 mm, are more vigorous than those from microtubers with a size less than 4 mm. Thus, the larger the diameter of the microtubers planted, the greater the yield. It, thus, emerges that the size effect responsible for a large variation in the yield is a consequence of the physiological state of the microtubers at the time of planting. In addition, the size of microtubers also positively influences their germination capacity and vigor. Therefore, the yield of plants is dependent to these factors.

This result can be explained by the amount of reserves contained in the microtuber and intended for the development of germs but also of the future plant. Indeed, the more the size of the microtubers increases, the more the quantity of carbohydrate reserves is important and is beneficial to the vegetative development of the plants and to the yield.

However, for microtubers, with a size greater than 10 mm, we notice the opposite, namely that the germination capacity is lower as for the classic tubers for which, it is known that those of large sizes have a reduced germination capacity and a vigorous less important plants. This negatively influences the yield of the plants and the number of sprouts that develop after the dormant period. The size of microtubers, therefore, has a significant importance and influence on their physiological and agronomic characteristics [10].

This study confirms previous works carried out by [6] and [10] who show that large microtubers produce vigorous plants. However, plants of the *Aida* variety are much more vigorous than those of other varieties. This remark confirms that vegetative development is also a function of the genotype.

4.2. Effects of Microtuber Size on the Average Number of Minitubers

The production of potato minitubers is the classic intermediate step in making possible the field use of the plant material originated from *in vitro* tissue culture. The techniques used for the production of minitubers are diverse but are most often based on the transplanting of vitroplants or *in vitro* cuttings in a disinfected organic substrate or not. In this study, a production of minitubers of superior sanitary quality has been considered using pre-germinated microtubers obtained previously under axenic conditions.

The vegetative development of the plants has a consequence on the minituber yield because we obtained, in the *Aida* variety, an average number of 3.55 minitubers harvested per plant from microtubers with a size greater than 4 mm compared

to 2.55 for those from microtubers with a size less than 4 mm. For *Atlas* and *Odessa* varieties, plants from microtubers, with a size greater than 4 mm, have a higher ratio than plants from small sizes. Thus, *Atlas* and *Odessa* respectively gave a ratio of 1.8 and 2 for diameters greater than 4 mm against 1 and 0.875 for smaller diameters. These facts corroborate the results of [11] which explain that the larger the diameter of the microtubers planted, the greater the number of minitubers harvested per plant. These results do not seem valid in the case of classic tubers of larger size. Indeed, [12] have demonstrated that plants produced from small tubers (12.5 ± 2.5 g) generally produce more stems and tubers and give a better yield than large tubers (37 ± 2.5 g) for an equivalent planting density.

4.3. Effects of Microtuber Size on the Weight and Average Size of the Minitubers

The influence of the size of the microtubers planted on the weight and size of the minitubers harvested is demonstrated. In the *Aïda* variety, we obtained an average weight and an average size of higher minitubers in plants produced from microtubers, with a size greater than 4 mm. These same observations were made with the *Altas* and *Odessa* varieties. The study of [13] confirms these results, by showing a positive correlation between the vigor of cultivated plants and the size of the minitubers harvested. Indeed, the most vigorous plants produce more homogeneous minitubers with a size greater than 5 mm. According to [11], the size effect responsible for a large variation in yield, can be explained by the physiological state of the material at the time of planting. Indeed, [10] have demonstrated that the duration of dormancy is correlated with the size of the microtubers. In the same way, the germination vigor is modified and the final yield is dependent on the size. In fact, when the diameter of the microtubers increases, the pool of reserves, in particular carbohydrates, is greater and could thus be more favorable to the development of the plant and to the final yield in minitubers produced [11].

4.4. Influence of Microtuber Size on the Above-Ground Parts and the Minituber Dry biomass

During the tests under semi-controlled conditions, we were able to verify importance of the size of the microtubers sown on the above-ground dry biomass. There is a correlation between vegetative development of plants and dry biomass. Indeed, the plants resulting from microtubers, with a size greater than 4 mm and having a significant vegetative development, have a higher dry biomass. This result can be explained by the pool of reserves contained in the large-caliber microtubers, allowing the harmonious development of the plants. This development is also a function of photosynthesis which allows the production of organic substances necessary for plant growth. Minitubers are potato tubers obtained from tissue culture plants or vitrotubers under controlled condition. Plants, grown from the large caliber microtubers, gave higher dry biomass of the

minitubers. This result is explained by the vigor of plants from this caliber. According to [14], the enrichment of the *in vitro* nutrient solution in phosphorus induces an increase in the number of minitubers produced later with a size greater than 15 mm (>5 g). Likewise, a basic nutrient medium containing 100 mM of nitrogen in the form of Ammonium nitrate (NH₄NO₃) allows tubers to grow better [15]. We can assume that the large caliber contains important nutrients that affect the dry biomass of harvested minitubers.

5. Conclusions

True potato seeds are botanical seeds used in seedlings for growing potatoes. It is an alternative to the use of seedlings, often incorrectly called “seeds”, which is of economic interest in hot tropical regions where the cost of supplying quality seedlings is a limiting factor in potato production. The disadvantages of true potato seeds are linked to the genetic variability which tends to produce tubers of heterogeneous quality, the cultivated varieties of potatoes having a very high degree of heterozygosity. Another alternative to the use of imported seeds is to produce minitubers under semi-axenic conditions (under shade) from microtubers produced *in vitro*. Since, the production of potato minitubers is the classic intermediate step in making possible the field use of the plant material originated from *in vitro* tissue culture.

The size effects of the microtubers sown on the yield of the plants result in an increase in the ratio, in the vegetative development of the plants, but also in the number and size of the minitubers harvested. Thus, plants produced from microtubers, with a size greater than 4 mm, were more vigorous and produced minitubers with a higher number, average weight, size and dry biomass than those from plants produced from microtubers with a size less than 4 mm.

The use of microtubers as seeds requires that the size be large to avoid drying out, but above all, to increase the yield of the plants that will be produced there.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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