

# Effect of Planting Bed Materials on Plantlet Acclimatization and Mini Tubers Production in Four Varieties of Potatoes (*Solanum tuberosum*) Cultivated in Niger

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**Abstract:** Tissue culture is currently used by many seed potato (*Solanum tuberosum* L.) programs enabling the production of high quality, disease free seed potatoes. However, the choice of the ideal substrate is essential for the acclimatization and seedlings growth of the propagated plantlets derived from *in vitro* micropropagation, since some substrates can increase the seedling mortality and/or limit the seedling growth due to its physical and chemical characteristics. Thus, the aim of this study was to evaluate the performance of three weeks old *in vitro* plantlets of potato variety ATLAS, weaned on different locally sourced substrates. The study took place in the culture room of tissue culture laboratory of Radio-Isotopes Institute, University ABDOU MOUMOUNI, Niamey, Niger. The following substrates mixes (volume/volume) were tested: T01 Sand top soil, T02 Sand-Clay (1:1), T03 Sand-Clay (2:1), T04 Sand-Sawmill waste (1:1), T05 Sand-Sawmill waste (2:1), T06 Sand-Manure (1:1), T07 Sand-Manure (2:1), T08 Sand-Clay-Manure (1,1,1), T09 Sand-Clay-Manure (2,1,1), T10 Sand-Commercial potting soil-Sawmill waste (2,1,1). Completely randomized design was used with four replications. According to the results of the analysis of variance, the differences between substrates were statistically highly significant for the number of leaves, the height of the plants and the shoot fresh biomass. No significant difference was found for root length and biomass. Highest shoot fresh weight was obtained with the commercial potting soil substrate. Seven days after transplanting, recovery rate was 100% for all treatments. However, at 12 DAT, mortality reached 100% for treatment T6 with manure, joined later by treatments T8 and T9 (sand-clay and manure) with 75% mortality after 15 DAT. Plantlets transplanted on T2 and T3 substrates performed well in comparison with commercial soil potting. It could be a potential substitute for the conventional substrate in weaning potato plantlets for pre-base seeds production.

**Keywords:** Potato, In Vitro, Local Soil Substrates, Niger

## 1. Introduction

Potato (*Solanum tuberosum* L.) is grown in more than 150 countries all over the world and plays an eminently important role in global food security [1]. Its role in eradicating poverty

and achieving food self-sufficiency was well documented [2-3]. Potato production has increased dramatically in developed countries over the past two decades [4]. The potato

has a very significant production potential in Niger. Its production is only increasing, from 1,500 tons in 1990 to 195,000 tons in 2017 [5]. The main constraints to potato production in Niger are abiotic constraints (hot and dry climate) and the availability of quality seeds [6]. Added to all this the climate change, which intensifies the exposure of potatoes to these stressful conditions [7]. This work is part of the development of a local production scheme for good quality potato seeds from the biotechnology and plant improvement laboratory of IRI, in Niger. Thus, this stage of the work consists in determining among ten different local substrates, the one that is the most suitable for the most efficient transplantation of vitro-plants and a higher yield in the production of potato mini-tubers.

## 2. Material and Methods

### 2.1. Substrates and Mini-tubers Production

The objective of this study is to determine the best growing substrate for the production of mini tubers, as potato pre-base seed in greenhouses. The variety Atlas has been used for this test. Ten types of substrates corresponding to combinations of various soil materials were tested (Table 1). The sand corresponds to local fine earth taken from the surface horizon (0-20 cm) and sieved with a 2 mm sieve. The clay comes from the rice fields of the first terraces of the Niger River. Commercial potting soil is an imported compost from Europe and distributed by local trade was used as promoted substrate for horticulture. The sawmill waste comes from a local sawmill. The manure is cattle manure. The 4-week-old vitro-plants were used in the study. The test took place in a refrigerated greenhouse at 20°C. Before planting, the vitro-plants are exposed on the bench at room temperature for 8 to 10 hours, without opening the caps of the test tubes. The vitro-plants thus acclimatized were then removed from the test tubes and washed to remove excess MS culture medium, then transferred to 2-liter plastic pots containing the different substrates tested as described in Table 1. Watering was done daily. The test lasted 5 weeks. The data collected relate to the survival rate, the plant height, the number of leaves, the shoots biomass and the roots biomass.

### 2.2. Experimental Design

The test was conducted according to an experimental

in complete randomized blocks with four repetitions for each treatment. Each pot containing a substrate receiving a is considered a repetition.

### 2.3. Statistical Analyzes

The data were mainly processed with the office-2016 software and the analysis of variance was carried out using the GENSTAT software version 12.1.0.3278 and a separation of the means by the Student-Newman-Keuls test at the threshold of 5%.

**Table 1.** Treatments and corresponding substrates.

Treatment	Composition of the substrate	Proportion (v, v, v)
T01	Sand	1
T02	Sand-Clay	1,1
T03	Sand-Clay	2,1
T04	Sand- Sawmill waste	1,1
T05	Sand- Sawmill waste	2,1
T06	Sand-Manure	1,1
T07	Sand-Manure	2,1
T08	Sand-Clay-Manure	1,1,1
T09	Sand-Clay-Manure	2,1,1
T10	Sand-Commercial potting soil-Sawmill waste	2,1,1

## 3. Results

### 3.1. Recovery of Vitro-Plants After Transplanting

At 7 days after transplanting (DAT), the recovery rate was 100% for all treatments. However, at 12 DAT, mortality reached 100% for treatment T6 (sand + manure), joined later by treatments T8 and T9 (sand-clay and manure) with 75% mortality after 15 DAT. Thereafter, the results will relate to the 7 substrates whose mortality is less than 50%.

### 3.2. Leaves Number

It emerges from the analysis of figure 1 (and table 2) that the T10 treatment, a substrate composed of commercial potting soil and sawmill waste, presents the highest number of leaves (38.7), and followed of T03 and T02, substrates with clay soil. On the contrary, treatments T5 and T7, including sawmill waste and manure, recorded the lowest number of leaves (3.6). All treatments with manure were not effective for leaf development. Statistical analysis shows a significant difference in the number of leaves ( $P = .034$ ) between the substrates (table 3).

**Table 2.** Biometric parameters of vitro-plants according to transplanting substrate.

Treatments	Number of leaves	Plant height (cm)	Shoot fresh biomass (g)	Root length (cm)	Root fresh biomass (g)
T5	3,63 <sup>a</sup>	15,47 <sup>a</sup>	0,506 <sup>a</sup>	7,49	1,007
T7	4,44 <sup>ab</sup>	18,89 <sup>a</sup>	0,323 <sup>a</sup>	6,69	0,322
T4	7,19 <sup>abc</sup>	16,25 <sup>a</sup>	0,528 <sup>a</sup>	7,75	0,935
T1	10,73 <sup>abc</sup>	21,55 <sup>ab</sup>	4,448 <sup>a</sup>	15,77	3,038
T2	24,50 <sup>abc</sup>	24,75 <sup>ab</sup>	8,408 <sup>a</sup>	18,25	6,747
T3	25,23 <sup>abc</sup>	29,33 <sup>ab</sup>	10,051 <sup>a</sup>	22,31	3,534
T10	38,73 <sup>bc</sup>	33,55 <sup>b</sup>	19,565 <sup>b</sup>	18,10	6,071
Average	16,4	22,8	6,26	14,29	3,09
LSD	22,13	9,17	8,073	14,29	5,627
F probability	0,034	0,008	0,002	0,167	0,152

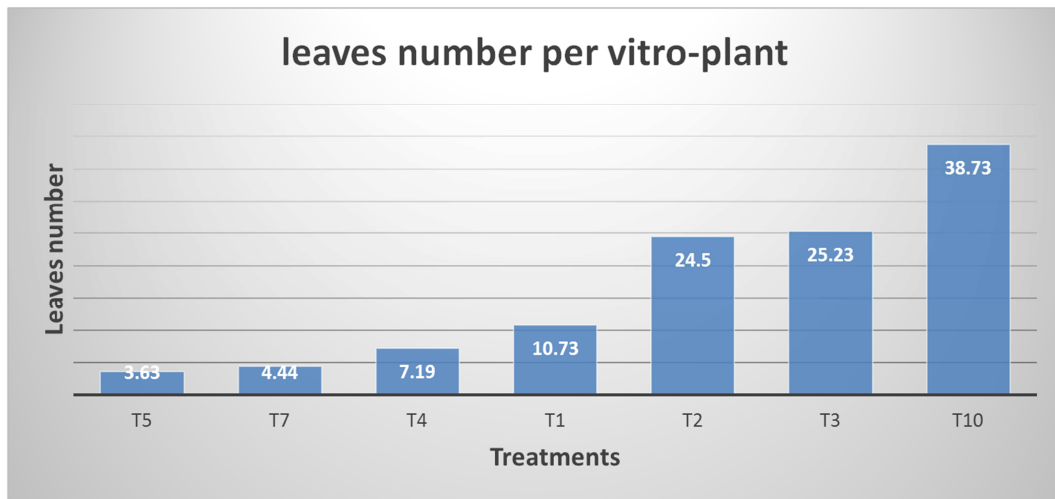


Figure 1. Potato leaves number as affected by transplant substrate.

Table 3. Variance Analysis in leaves production in potato as affected by planting bed material.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Bloc stratum	3		847.2	282.4	1.40	
bloc.*Units* stratum						
Substrate	6		4261.3	710.2	3.51	0.034
Residual	11	(7)	2223.3	202.1		
Total	20	(7)	5658.0			

### 3.3. Plant Height

The plant height obtained after 4 weeks of growth varied from 15.47 cm to 33.55 cm, with an overall average of 22.8 cm (Table 4). Statistical analysis reveals a very highly significant difference ( $P=0.008$ ) in plant height between the different substrates. As with leaf production, the T10

treatment, substrate composed of commercial potting soil and sawmill waste, had the highest height (33.5 cm), followed by T03 and T02, substrates with clay soil, unlike T5 and T7, treatments with sawmill waste and manure recorded the lowest height (15.5 cm).

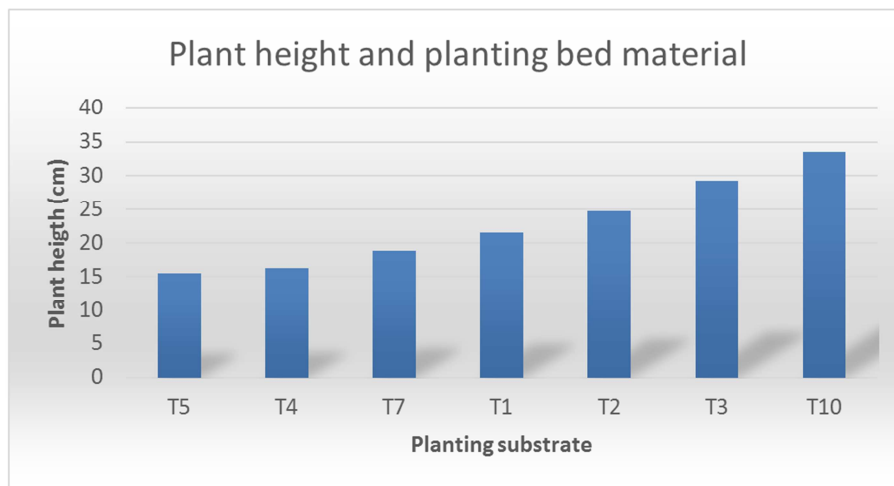


Figure 2. Plant height as affected by transplanting substrate.

Table 4. Variance Analysis in plant height of potato as affected by planting bed material.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Bloc stratum	3		108.11	36.04	1.02	
bloc.*Units* stratum						
Substrate	6		1102.06	183.68	5.18	0.008
Residual	12	(6)	425.52	35.46		
Total	21	(6)	1323.86			

### 3.4. Root Length

Root length (represented by longest root) averaged 13.8 cm. Although the statistical analysis reveals no significant difference, the T3 and T2 substrates, containing sand and clay in good proportions, produced the longest roots (figure 3 and table 5).

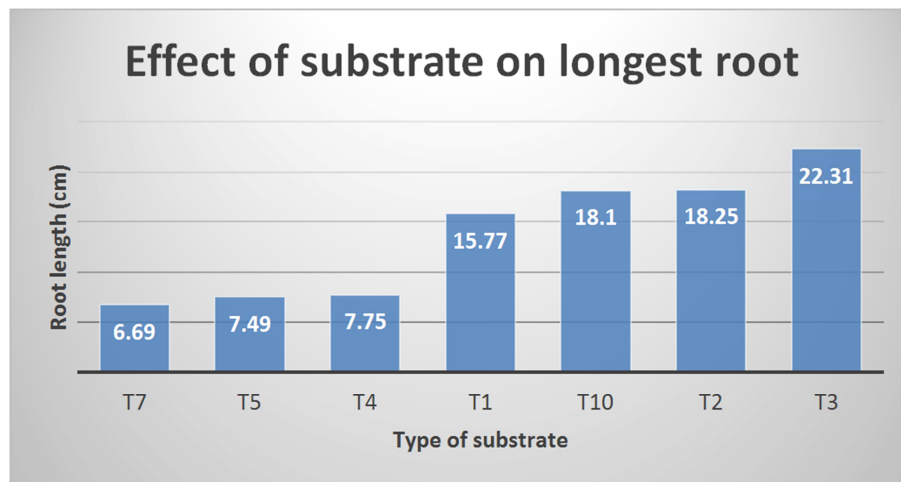


Figure 3. Root length (longest root) as affected by transplanting substrate.

### 3.5. Shoot Fresh Biomass Weight

Shoot biomass production varied from 0.323 grams to 19.565 grams per potato plant (Figure 4). The statistical analysis shows a very highly significant difference ( $P=0.002$ ) between the planting substrates (Table 5). The Commercial

Soil (T10), and the T3 and T2 substrates containing clay, showed the best shoot biomass production. Substrates with manure (T4 and T5) as well as that with sawmill waste produced low shoot biomass.

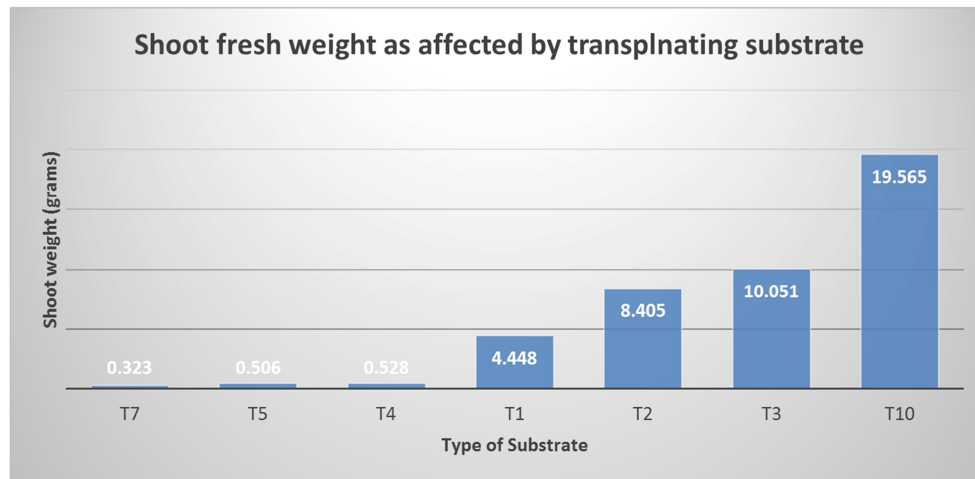


Figure 4. Potato shoot biomass according to planting substrate.

Table 5. Statistical Analysis of potato shoot biomass as affected by transplanting substrate.

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Bloc stratum	3		116.55	38.85	1.42	
bloc.*Units* stratum						
Substrate	6		1202.02	200.34	7.30	0.002
Residual	12	(6)	329.46	27.46		
Total	21	(6)	1248.90			

### 3.6. Root Biomass

Although the differences are not significant between substrates, it is still the commercial substrate and those with clay that had the greatest quantities of roots (figure 5).

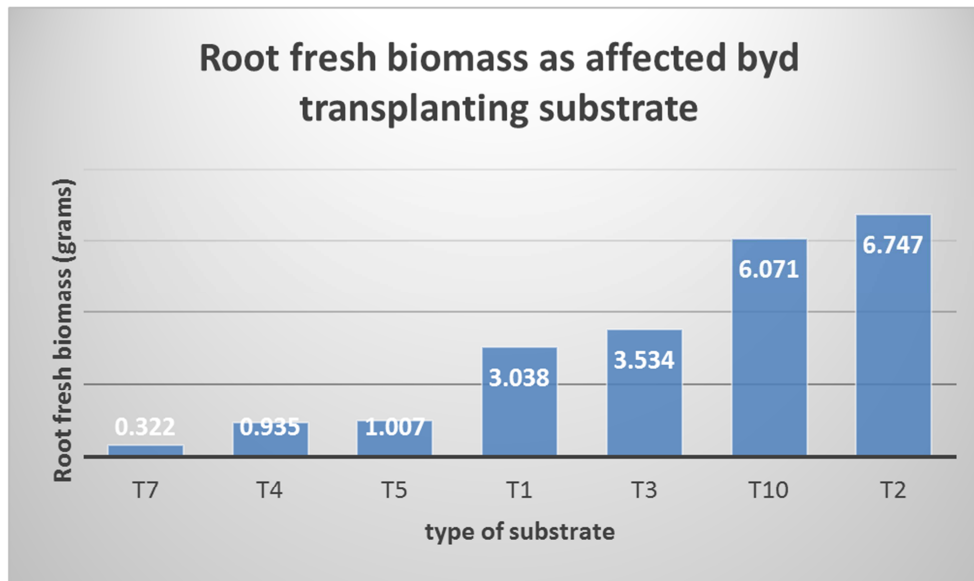


Figure 5. Root fresh biomass as affected by transplanting substrate.



Figure 6. Root fresh biomass as affected by transplanting substrate. Left: sand only; right: sand with clay (1:1, vol/vol).

## 4. Discussion

According to the results of the analysis of variance, the differences between substrates were statistically highly significant for the number of leaves, the height of the plants and the shoot fresh biomass. No significant difference was found for root length and biomass. Highest shoot fresh weight was obtained with the commercial potting soil substrate, probably related to the physical and chemical properties of the substrate facilitating the absorption of water by the plants [8]. In general, peat as a substrate induces good plant growth due to its good porosity and good water and nutrient retention capacity. This indicates very clearly that the type of substrate is of primary importance for the growth of tissue culture plantlets after transplantation. T1, a substrate comprising only

sand, is characterized by low levels of fertility linked to its coarse grain size and a low water retention capacity. T6, T7 and T8, substrates comprising organic matter, are characterized by high levels of toxic elements  $H^+$  and  $Al^{3+}$ . This explains the high level of mortality recorded. These substrates did not make it possible to provide water and mineral elements at the required rate and under satisfactory conditions for good growth of potato seedlings. This result is in agreement with several previous works. In reference [9], authors working on *in vitro* cassava plantlets (*Manihot esculenta*), found any performance with substrates with organic matter (saw dust and rice hull waste). In reference [10] authors working on pineapple (*Ananas comosus*), demonstrated a depressant effect of organic matter. However, well-composted organic matter can provided seedlings with a greater growth level [11]. In all cases, the best performance

was found with the commercial substrate which includes a balanced mixture of sand, peat and mineral addition, all things necessary for good plant growth. On the other hand, in reference [12], authors working on cassava plantlets acclimatization, found no significant difference between the substrates used as planting bed, probably because the materials used (sawdust waste and palm fibers), in low proportion, shrivel up after autoclaving and their effects weaken.

The commercial substrate was the most effective, according to the results of our study, but accessible at an expensive price, making it necessary to seek local substitute substrates. Thus, using locally available materials contribute to lower substrate associated costs as recommended by several authors [13-15].

The T2 and T3 substrates, having a relative efficiency (compared to the commercial substrate), respectively of 63.3 and 65.1% on leaf development, of 73.8 and 87.4% on the height of the plant and of 43 and 51.4% on aerial biomass, can be validly offered as a replacement for commercial soil (table 6). In addition, these two substrates have greater root development than commercial potting soil. Good plant growth in T2 and T3 can be linked to suitable conditions, including good porosity and good ventilation and good water-holding capacity, as reported by [16]. Thus, according to our results, the T2 and T3 substrates can be validly used for the efficient transplantation of vitroplants and the production of potato minitubers in Niger.

**Table 6.** Performance (in%) of local substrates compared to imported commercial soil.

Substrate	Number of leaves	Plant height	Shoot fresh biomass	Root length	Root fresh biomass
T5	9,4	46,1	2,6	41,4	16,6
T7	11,5	56,3	1,7	37,0	5,3
T4	18,6	48,4	2,7	42,8	15,4
T1	27,7	64,2	22,7	87,1	50,0
T2	63,3	73,8	43,0	100,8	111,2
T3	65,1	87,4	51,4	123,3	58,2

## 5. Conclusion

From this study, it appears that the substrate of transplantation has a capital importance for the growth and the development of the tissue culture potato vitro-plants. Substrates comprising an intimate mixture of sand and clay offer good growth conditions and can be proposed as substitute for the commercial substrate (certainly more efficient) which is very expensive to acquire. Substrates containing non-composted organic matter were shown to be depressive, with up to 100% mortality of vitro-plants.

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