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Evaluation of Different Substrates Compositions for Acclimatization of Tissue Culture Taro Plantlets in a Propagator

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Abstract

Taro is cultivated in most Regions of Cameroon and it is affected by taro leaf blight disease since 2010 which has decreased its production. Lack of disease-free planting materials has been a main problem to farmers. This study was carried out at International Institute of Tropical Agriculture (IITA) Yaounde and Institute of Agricultural Research for Development (IRAD) Bambui to assess different substrates for acclimatization of tissue culture taro plantlets in apropagator. No information is available on acclimatization of Cameroonian taro plantlets in different substrates. Taro plantlets from tissue culture were acclimatised in a propagator for six weeks under different substrates, the first substrate consisted of sterile three parts of soil and one part of river sand mixed together (3:1), the second substrate consisted of sterile two parts of soil and two parts of river sand mixed together (2:2), the third substrate consisted of sterile two parts of soil, one part of rice husk and one part of river sand mixed together (2:1:1) and the fourth substrate consisted of sterile one part of soil and three parts of river sand mixed together (1:3). After acclimatisation of the different taroplantlets (Dark green petiole with small leaves (L1), Red petiole with small leaves (L2), Light green petiole with large leaves (L3) and Light green petiole with small leaves (L4) in these four substrates, it was observed that the best growth rate of plant was recorded on substrate sand + soil (1:3). The other substrates showed moderate growth of plants. Substrate sand + soil (1:3) can be recommended for acclimatization of Cameroonian taro plantlets.

Keywords

Taro Tissue Culture Plantlets, Substrates, Acclimatization, Propagator

1. Introduction

Taro (*Colocasia esculenta* (L) Schott) is an annual herbaceous plant that originated from South East Asia and later spread into other parts of the continent, and Africa [1]. Taro corms are used as food for man and animal feed [2]. It is eaten in many forms: roasted, boiled, fried, baked, pounded and the leaves are eaten as a preferred vegetable, representing an important source of vitamins [3] [4]. Mineral content and medicinal values of taro are higher compared to other tuber crops such as cassava, potatoes, and yams [5] [6] [7]. It is also rich in proteins, carbohydrates, sugars, and minerals such as calcium, manganese, phosphorus, potassium, and zinc [8]. The petioles and flowers are consumed in Cameroon and certain parts of the world. From an ethno medicinal point of view, the uncooked taro roots are applied to fresh wounds to stop bleeding and the washed fresh leaves are used to treat tooth ache [9]. The crop is a good source of income to the local producers, to the extent that some subsistence farmers generate enough revenue from taro production to take care of basic family needs [10].

The crop is susceptible to a wide range of pests and diseases such as viral, bacterial, nematode and fungal diseases [11] [12] amongst these diseases taro leaf blight caused by Phytophthora *colocasiae* Racib has reduced taro production in Cameroon since 2010 [13]. Prominent small, brown, water-soaked lesions that enlarge and coalesce into large lesions with yellow exudates, are the visual symptoms of taro leaf blight disease which leads to defoliation of leaves and decay of the plants within a few weeks [14] [15]. Reports from FAO [16] revealed that from 2016 to 2020 the production of taro in Cameroon has maintained a constant value of 1, 8 metric tons due to the lack of high-quality seeds and reduction in planting materials.

In an attempt to solve these problems, tissue culture plantlets have been produced in the laboratory. Meristem culture techniques are used to produce plantlets free from viruses, bacteria and fungi mostly in vegetative propagated plants [17]. Cameroonian taro landraces were used to produce disease free planting materials from tissue culture and the last stage of this process is acclimatization which needs serious attention since plantlets are removed from the laboratory to a screenhouse under different environmental conditions and media [18]. Media provides nutrients and water for plant growth. A good media for acclimatization of tissue culture plantlets should be disease free, aerated and easily binds water [19]. Although *in-vitro* propagation of taro has been done in Cameroon [20], no information is available on the acclimatization of taro landraces in different substrates in a screenhouse. Comprehensive details of acclimatization media will help researchers to produce more disease-free plantlets that will be handed to farmers to plant and the disease-free plantlets serve as a cultural control measure for the diseases. This work was designed to assess different substrates for acclimatization of tissue culture taro plantlets in a screen house.

2. Materials and Methods

This study was carried out at the Tissue culture laboratory of the Institute of Agricultural Research for Development (IRAD), Bambui, North-West Region and soil analysis was done in the Analytical laboratory of the International Institute of Tropical Agriculture (IITA) Nkolbisson, Yaounde, Centre Region of Cameroon. The positions of the experimental sites were recorded using GPS mark Garmin etrex 20. The Institute of Agricultural Research for Development, Bambui is situated at 32˚, 0627'N latitude, 0659'E longitude, and altitude 1262 meters above sea level and the International Institute of Tropical Agriculture is situated at latitude 32˚86'N, longitude 270'E and altitude 777 meters above sea level.

2.1. Media Preparation

Murashige and Skoog medium was prepared by putting 700 ml of sterilized distilled water in a beaker and 30 g of sugar was added to it while stirring on a magnetic stirrer until all the sugar was dissolved. Five grams of Murashige and Skoog Basalt medium that contains vitamins, macro and micro elements was measured, added to the sugar solution, and stirred until dissolved. Five millilitres of Fe-EDTA complex and 5 mls of ascorbic acid were measured, added to the solution and stirred. 1.1 ml of 6-benzylaminopurine was also added and stirred. The volume of the solution was made up to 1000 ml by adding distilled water. The pH of the medium was adjusted to 5.7 ± 1 using HCl or NaOH and 7.5 g of agar was added to the mixture and heated until the solution was clear. The clear medium was distributed (2 mls per tube) by means of a sterile pipette into cylindrical test tubes of 13 mm in diameter and 100 mm in height. The tubes were sealed with aluminium foil or corks and the medium was sterilized in an autoclave at 121˚C, 103.4 Pa for 15 minutes. The medium was allowed to cool and solidify overnight. Only one type of medium was used for all the incubation stages of *in-vitro* culture [21].

2.2. Collection and Surface Sterilization of Taro Corms

Four landraces of taro (Dark green petiole with small leaves (L1), Red petiole with small leaves (L2), Light green petiole with large leaves (L3) and Light green petiole with small leaves (L4) with young healthy offshoots of taro plants bearing rhizomes of 8 cm long were collected from (IRAD), Bambui, research farms in the month of September 2022. These landraces were 4 months old and carried to the tissue culture laboratory for surface sterilization. Cultivars were washed with tap water, roots and leaves were removed, and the plants were trimmed into smaller pieces of plant material (explants). The explants were trimmed into 8 mm height; 3 mm at the base, with some corms measuring 3 mm in thickness attached using a knife. Corms were sterilized in 20% sodium hypochlorite under an alcohol-swapped laminar air-flow chamber, the explants were immersed in 10% sodium hypochlorite containing 2 drops of tween 80 per 100 ml in a closed

vessel and were shaken for 45 minutes. The bleach was decanted and the explants rinsed three times in sterile distilled water to remove the bleach. Explants were immersed in 70% Ethanol for 2 - 3 minutes. Ethanol was decanted and the explants were rinsed with sterile distilled water. Explants were immersed in 5% sodium hypochlorite for 5 minutes, decanted and rinsed in sterile distilled water 3 times. Third and second leaf sheaths were trimmed and explants were placed directly on a solidified Murashige and Skoog medium in sterile test tubes. Tubes were labelled with landraces' names and placed in a growth room under light intensity (white fluorescent lamps) at 18˚C. This was the initiation stage.

2.3. Shoot Proliferation and Root Initiation Stage

Buds produced 4 weeks after incubation in Murashige and Skoog medium were sub-cultured twice in the same media to obtain *in-vitro* plantlets. The concentration of 6-benzylaminopurine (BAP) was increased to 2.2 ml during the third subculture in order to initiate bud formation for 8 weeks. Plantlets were removed from the test tubes and sub cultured on rooting media to stimulate rhizogenesis. This media consists of the same media as above with 2.2 ml of 6-benzylaminopurine being replaced by 10 ml of 0.1 mg/ml naphthalene acetic-acid (NAA). The *in-vitro* plantlets were removed after 60 days of shoot tip culture for acclimatization in a screen house [22].

2.4. Analysis of Substrate Mixture Used for Acclimatization Process of *in Vitro* Taro Plantlets

2.4.1. Sand and Soil Analysis

Soils were air-dried and ground to pass through a 2 mm sieve. For C and N analysis, soils were further ground to pass through a 0.5 mm sieve. Soil pH in water was determined in a 1:2.5 (w/v) soil: water suspension. Organic C was determined by chromic acid digestion and spectrophotometric analysis [23]. Total N was determined from a wet acid digest [24] and analyzed by colorimetric analysis [25]. Exchangeable cations (Ca, Mg, and K) and micro elements (Cu, Zn, Mn, and Fe) were extracted using the Mehlich-3 procedure and determined by flame atomic absorption spectrophotometry. Available P was extracted using the Mehlich-3 procedure [26], and the resulting extracts were analyzed using the molybdate blue procedure described by [27].

Exchangeable acidity was extracted with 1M KCl and quantified by titration. P, Cu, Zn, Mn, and Fe expressed in ppm or ug/g or mg/kg; Organic C and Total N expressed as %, Ca, Mg, K and exchangeable acidity expressed in cmol (+)/kg which is same as me/100g. As quality control measures, inclusion of 5 internal reference samples in every batch was analyzed. Inclusion of four external reference samples from international soil exchange program in every batch wasanalyzed

2.4.2. Analysis of Rice Husk

Basic cations Ca, Mg, K, Na and micronutrients, Zn, Cu, Mn, Fe were exacted by

ashing in a muffle furnace at 500˚C diluted using a dilute acid mix of HCL/HNO_3 and analyzed by atomic absorption spectroscopy using the atomic absorption-spectrophotometer [28]. P was extracted as above and analyzed by using Murphy and Riley reagent and results were generated from colorimetric readings. Total N was determined from a wet acid digest [24] by colorimetric analysis [25].

2.4.3. Preparation of Substrate

The substrates used for this experiment were soil, sand and rice husk. These substrates were steam sterilized separately at 90˚C for 16 hours to ensure complete treatment. They were allowed to cool to environmental temperatures. At the end of the rooting stage, all plantlets with some roots were removed from the rooting media with a sterile forceps in order to avoid root damage. The root areas of the plantlets were washed in 5% sodium hypochlorite to remove nutrients and sugar which could attract fungi and bacteria. These plantlets were rinsed in three successive changes of sterile distilled water. They were planted in four different substrates, the first substrate consisted of sterile three parts of soil and one part of river sand mixed together (3:1), the second substrate consisted of sterile two parts of soil and two parts of river sand mixed together (2:2), the third substrate consisted of sterile two parts of soil, one part of rice husk and one part of river sand mixed together (2:1:1) and forth substrate consisted of sterile one parts of soil and three part of river sand mixed together (1:3). The various substrates were filled in plastic pots, placed in a propagator constructed with wood covered with transparent plastic sheets and the four local landraces of taro from the tissue culture laboratory were planted in a complete randomized design with three replicates of 10 plants of each cultivar per replicate. Taro plants were watered twice per week. The number of leaves, survived plants, senescence leaves, and diseased plant were counted. Petiole length and leaf diameter of taro plants were measured at weekly intervals for 6 weeks after establishment in the propagator (**Figure 1**). Acclimatized taro plants were removed from the propagator and placed under shade for one week following the adopted procedure of [22]. The process from shoot proliferation to acclimatization took 9 months to obtain plants that were transferred to the field.

Figure 1. Taro plants after six weeks of acclimatization in a propagator at IRAD Bambui.

2.4.4. Statistical Analysis

Analysis of variance (ANOVA) was performed using statistical software (JMP8, 2007) to evaluate growth and disease parameters of explants. Means were separated using student T-test (STT) at ($p = 0.05$).

3. Results

3.1. Nutrient Composition of Substrates

Analysis of the various substrates showed that the amount of potassium was the same for the four substrates: sand + soil (1:3), sand + soil (2:2), soil + sand (1:3) and rice husk + soil + sand (1:2:1). All the substrates contained all soil nutrients except Cu and Zn. Substrates: sand + soil (1:3) had the highest amount of nutrients compared to the other three substrates: sand + soil (2:2), soil + sand (1:3) and ice husk + soil + sand (1:2:1) (Table 1).

3.2. Acclimatization of Taro Plantlets

3.2.1. Effect of Substrate Mixtures on Acclimatization of *in Vitro* Taro Plantlets

The acclimatization process lasted for 6 weeks; it was observed that all four landraces leaves and petioles grew well in the different substrates during the establishment process. The best growth rate was recorded on substrate sand + soil (1:3) on the four different landraces (Table 2). The other substrates showed moderate growth of landraces. No diseased plant was observed in all the substrates. Table 2 shows the effect of substrate mixtures on the mean number of-petiole length at 1 to 6 weeks of adaptation of the four landraces. There was an increase in mean petiole length on all the plants on different substrates from 1 to 6 weeks with longest petiole length observed in sand + soil (1:3) substrate on all landraces. Sand + soil (1:3) substrate recorded the longest mean petiole length of 11 ± 91 cm at 5 and 6 weeks of establishment on landrace L3. The shortest petiole length of 1.5 ± 0.17 cm was recorded on landrace L2 at 1 and 2 weeks of establishment on sand + soil (3:1) substrate. Significant variation was observed on mean petiole length of landraces from 1 to 6 weeks on the different substrates.

Table 3 shows a significant difference in leaf diameter at 1 and 6weeks of acclimatization of the different plantlets in the different substrates. Landraces planted in sand + soil (1:3) substrate showed large size of leaf diameter compared to other substrates. The largest mean leaf diameter of 6.63 ± 0.14 cm was recorded on landrace L3 on sand + soil (1:3) substrate at 6 weeks and the smallest mean leaf diameter of 0.71 ± 0.06 cm was recorded on landrace L2 on rice husk + soil + sand (1:2:1) substrate at 1 week of acclimatization (Table 3).

From the results obtained at 1 to 6 weeks of acclimatization (Table 4), there was a significant difference in the mean number of leaves ($p = 0.05$) amongst the different plants in the different substrates. The highest mean number of leaves of 3.33 ± 0.22 was recorded on landraces L1 and L4 on substrates sand + soil (1:3) and sand + soil (2:2) respectively at 6 weeks of acclimatization. The lowest mean

number of leaves of 1.00 ± 0.04 was recorded on landrace L4on sand + soil (2:2) substrate at the first week of acclimatization.

Table 1. Chemical composition of substrate mixture used for acclimatization process of *in vitro* taro plantlets.

Total	Soil + Sand (3:1) (g)	Soil + Sand (2:2) (g)	Soil + Sand (1:3) (g)	Ricehusk + Soil + Sand (1:2:1) (g)
N	56.3	39.1	21.8	40.2
Ca	4.7	4.3	3.9	4.1
Mg	1.0	0.9	0.7	1.1
K	1.4	1.1	0.8	3.0
Na	0.1	0.1	0.1	0.7
P	0.1	0.1	0.1	0.1
Zn	0.0	0.0	0.0	0.0
Cu	0.0	0.0	0.0	0.0
Mn	1.0	0.8	0.7	0.9
Fe	1.5	2.0	2.4	1.6

Table 2. Effect of substrate mixtures on petiole length for an interval of 1and 6 weeks of establishment.

PETIOLE LENGTH (cm)					
LANDRACES	WEEK	SA1S03	SA2SO2	SA3SO1	R1SO2SA1
L1	1	7.99 ± 1.99a	7.25 ± 1.46ab	5.58 ± 1.96abcde	3.96 ± 0.82defg
	6	11.51 ± 1.00ab	9.18 ± 1.21cde	7.83 ± 0.58defg	6.71 ± 0.17fghi
L2	1	3.34 ± 0.54efg	2.33 ± 0.47fg	1.5 ± 0.17g	1.67 ± 0.23g
	6	7.35 ± 0.91efgh	3.66 ± 1.58k	6.15 ± 0.16fghij	4.00 ± 0.24jk
L3	1	6.22 ± 1.82absd	4.83 ± 1.09bcdef	7.00 ± 0.16ab	6.83 ± 0.94abc
	6	11.91 ± 0.87a	4.90 ± 0.48ijk	9.63 ± 0.70bcd	8.25 ± 0.41cdef
L4	1	7.21 ± 0.67ab	4.08 ± 0.39cdefg	1.75 ± 0.23g	3.00 ± 0.83efg
	6	10.13 ± 0.81abc	6.06 ± 0.60fghij	5.16 ± 0.36hijk	5.65 ± 0.73ghijk

Means followed by the same letters in the same row are not significantly different at p = 0.05 (STT). Values are means number of taro plant petiole length followed by standard error. SA1S03 = Sand + soil (1:3), SA2SO2 = Sand + soil (2:2), SA3SO1 = Sand + soil (3:1), R1SO2SA1 =Rice husk + soil + sand (1:2:1).

Table 3. Effect of substrates mixture on leaf diameter at 1 to 6 weeks interval of establishment.

LEAF DIAMETER (cm)					
LANDRACES	WEEK	SA1SO3	SA2SO2	SA3SO1	RISO2SA1
L1	1	1.95 ± 0.24bc	1.72 ± 0.13bcd	2.00 ± 0.35bc	1.77 ± 0.25bc
	6	5.90 ± 0.38ab	5.33 ± 0.92abc	5.23 ± 0.54bc	3.90 ± 0.08def
L2	1	1.46 ± 0.15cde	1.00 ± 0.16def	1.30 ± 0.03cdf	0.71 ± 0.06f
	6	4.65 ± 0.53bcde	2.21 ± 0.98g	2.60 ± 0.08fg	3.53 ± 0.29efg
L3	1	3.00 ± 0.24a	1.66 ± 0.19cd	1.52 ± 0.17cde	1.49 ± 0.10cde
	6	6.63 ± 0.14a	3.65 ± 0.13ef	5.03 ± 0.43bcd	6.55 ± 0.11a
L4	1	1.69 ± 0.25bcd	2.41 ± 0.66ab	1.30 ± 0.03cdef	0.89 ± 0.20ef
	6	4.85 ± 0.31bcde	4.20 ± 0.09cde	3.76 ± 0.14def	5.23 ± 0.48bc

Means followed by the same letters in the same row are not significantly different at p = 0.05 (STT). Values are means number oftaro plant leaf diameter followed by the standard error. SA1S03 = Sand + soil (1:3), SA2SO2 = Sand + soil (2:2), SA3SO1 = Sand + soil (3:1), R1SO2SA1 = Rice husk + soil + sand (1:2:1).

Table 4. Effect of substrates mixture on number of leaves at 1 to 6 weeks' interval of establishment.

		NUMBER OF LEAVES			
LANDRACES	WEEKS	SA1SO3	SA2SO2	SA3SO1	RISO2SA1
L1	1	2.33 ± 0.21a	1.50 ± 0.21cdef	2.16 ± 0.17ab	1.83 ± 0.26abcd
	6	3.33 ± 0.22ab	3.16 ± 0.50a	2.66 ± 0.20a	3.00 ± 0.34a
L2	1	2.16 ± 0.15a	1.66 ± 0.18bcde	2.00 ± 0.04abc	2.00 ± 0.04abc
	6	2.66 ± 0.35abcd	1.66 ± 0.74b	3.00 ± 0.02a	3.00 ± 0.42a
L3	1	1.83 ± 0.15ab	1.50 ± 0.19cdef	2.00 ± 0.04abc	1.33 ± 0.22def
	6	3.16 ± 0.18abc	3.00 ± 0.02a	3.00 ± 0.022a	2.50 ± 0.23ab
L4	1	2.00 ± 0.27abc	1.00 ± 0.04f	2.00 ± 0.35abc	1.16 ± 0.17ef
	6	2.50 ± 0.35ab	3.33 ± 0.21a	2.66 ± 0.19a	2.50 ± 0.32ab

Means followed by the same letters in the same row are not significantly different at p = 0.05 (STT). Values are means of taro plant number of leaves followed by standard error. SA1S03 = Sand + soil (1:3), SA2SO2 = Sand + soil (2:2), SA3SO1 = Sand + soil (3:1), R1SO2SA1 = Rice husk + soil + sand (1:2:1).

Effect of substrates mixture on the number of senescence leaves at 1 to 6 weeks' interval of establishment showed that there was no senescence leaves on landraces L1, L2 and L4 on the various substrates; L1 on substrate rice husk + soil + sand (1:2:1), L2 on substrates rice husk + soil + sand (1:2:1), sand + soil (2:2) and sand + soil (3:1) and L4 on substrate sand + soil (3:1) at 1 to 6 weeks of acclimatization. Maximum mean senescence leaves of 1 was recorded on L1 on sand + soil (2:2) substrate at 1week of acclimatization. Minimum mean senescence leaves of 0.25 were recorded on landrace L1 on substrate sand + soil (1:3) at 4 to 6 weeks, substrate sand + soil (2:2) at 2 to 3 weeks, landrace L2 on sand + soil (1:3) at 2 to 3 weeks, landrace L3 on substrate sand + soil (3:1) at 3 and 5 weeks, sand + soil (3:1) at 2, 4 and 5 weeks, landrace L4 on substrate rice husk + soil + sand (1:2:1) at 1 week and on substrate sand + soil at 5 weeks (**Figure 2**).

Bars represent mean number of senescence leaves with standard errors.

Mean (SEN 1 WKS) = Mean senescence leaves at 1 week; Mean (SEN2 WKS) = Mean senescence leaves at 2 weeks; Mean (SEN 3 WKS) = Mean senescence leaves at 3 weeks; Mean (SEN4 WKS) = Mean senescence leaves at 4 weeks; Mean (SEN 5 WKS) = Mean senescence leaves at 5 weeks; Mean (SEN 6 WKS) = Mean senescence leaves at 6 weeks

3.2.2. Substrate Mixtures on Different Landraces

SA1SO3 = sand + soil (1:3); SA2SO2 = sand + soil (2:2); SA3SO1 = sand + soil (3:1); R1SO2SA1 = rice husk + soil + sand (1:2:1). All landraces survived on all the substrates except landrace L2 on substrate sand + soil (2:2) at 3 to 6 weeks of acclimatization (**Figure 3**).

Bars represent mean number of survived plantlets with standard errors.

Mean (SUR 1 WKS) = Mean number of survived plants at 1 week; Mean (SUR 2 WKS) = Mean number of survived plantlets at 2 weeks; Mean (SUR 3 WKS) = Mean number of survived plant at 3 weeks Mean (SUR 4 WKS) = Mean number

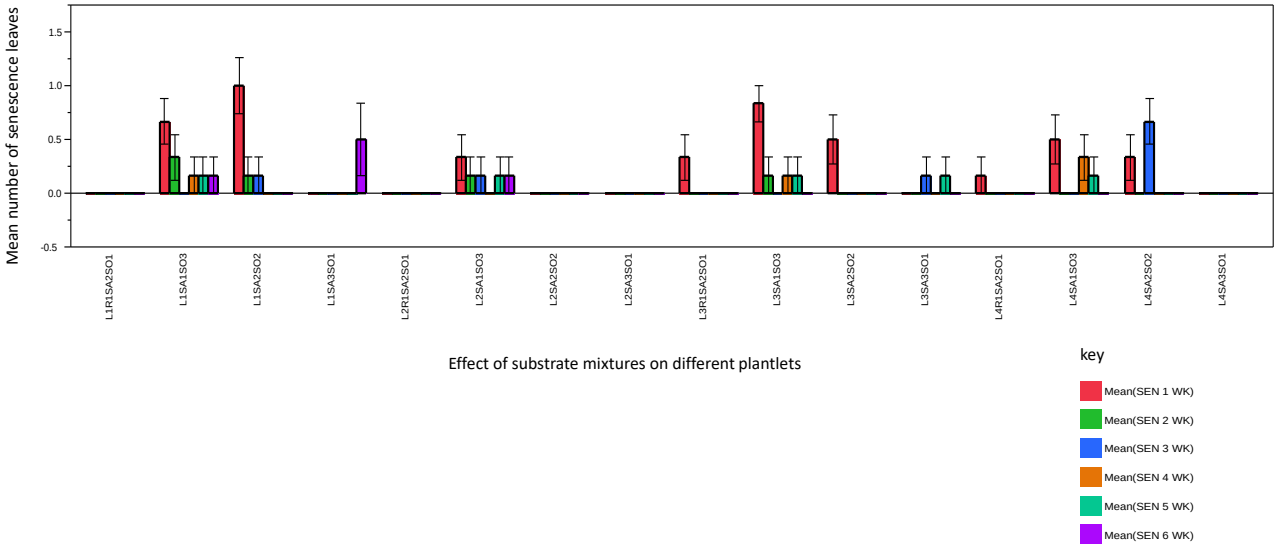

Figure 2. Effect of substrate mixtures on number of senescence leaves for an interval of 1 to 6 weeks of establishment.

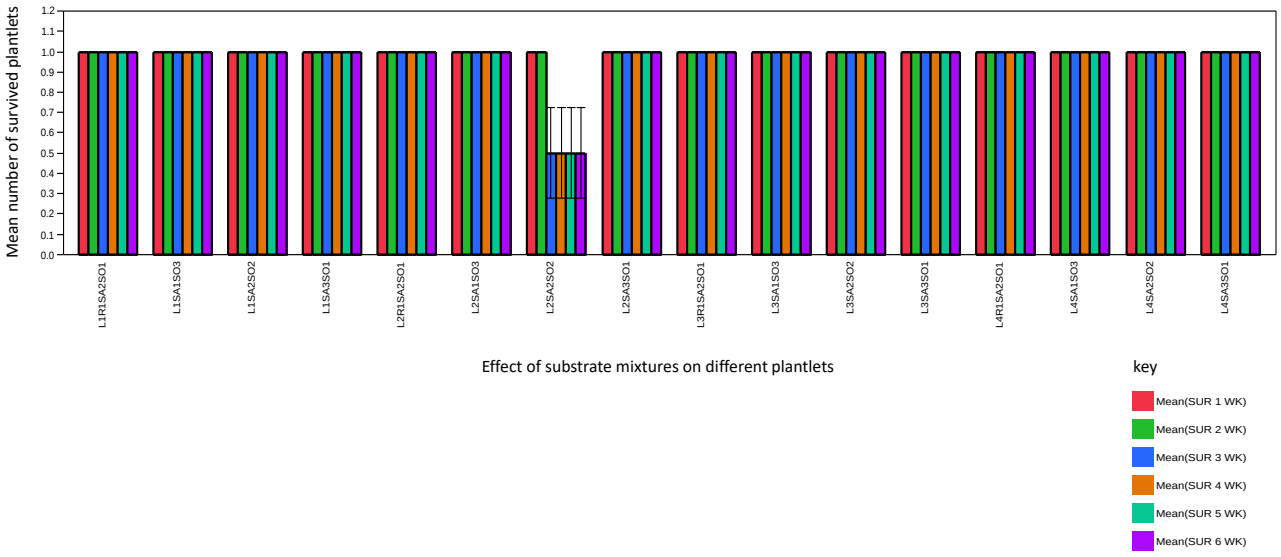

Figure 3. Effect of substrate mixture on acclimatization of taro plantlet on number of survived leaves at 1 to 6 weeks' interval of adaptation.

of survived plants at 4 weeks; Mean (SUR 5 WKS) = Mean number of survived plants at 5 weeks; Mean (SUR 6 WKS) = Mean number of survived plant at 6 weeks

3.2.3. Substrate Mixtures on Different Landraces

SA1SO3 = sand + soil (1:3); SA2SO2 = sand + soil (2:2); SA3SO1 = sand + soil (3:1); R1SO2SA1 = rice husk + sand + soil (1:2:1).

4. Discusion

Results from the 6 weeks acclimatization process showed that the best growth rate was recorded on substrate sand + soil (1:3) on the four different landraces.

The other substrates showed moderate growth of plants. This could be due to the chemical characteristics of the growth medium which exert an effect on the growth of plants. According to [29], both physical and chemical characteristics of the growth medium exert a substantial effect on the growth of plants. Among the physical properties or characteristics, a ratio and water holding capacity are probably the most important factors while, among the chemical characteristics we have nutritional status and salinity level which have a crucial role on plant development [30]. Substrate sand + soil (1:3) contained high level of macronutrients (N, Ca and Mg) which favour the growth of leaves, petioles, and the survival of the plants. The other substrates contained low levels of macronutrients which reduced the growth of the plant landraces in these substrates. These macronutrients played different important roles in plant growth and development. Nitrogen is responsible for the synthesis of proteins, nucleic acids and other organic compounds such as coenzymes and chlorophyll [31]. According to [32] optimum rate of Nitrogen increases photosynthetic processes, leaf area production, leaf area duration as well as net assimilation rate. All plants including cereals, oil seeds, fibre, and sugar-producing and horticultural plants require a balanced amount of nitrogen for vigorous growth and development proper growth and development of plants require an optimum supply of nitrogen. Little application of nitrogen on crops directly reduces growth, and crop yield while excess of N also causes negative effects on plants [33] [34]. Calcium formed the lamella (calcium pectate) between plant cell walls and normal cell wall development. Reports from [35], state that plants growing with adequate Ca in their natural habitat have shoot Ca concentration between 0.1% and 5%. Calcium is an essential plant nutrient, as divalent cation (Ca^{2+}) is required for structural role in the cell wall and membranes, as counter cations for inorganic and organic anions in the vacuole, and as an intracellular in the cytosol. Magnesium is part of the structure of chlorophyll which absorbs sun light energy during photosynthesis thereby increasing the growth of the plant and productivity. Iron is an essential micronutrient for almost all living organisms because it plays a critical role in metabolic processes such as DNA synthesis, respiration and photosynthesis. Further, many metabolic pathways are activated by iron and it is a prosthetic-group constituent of many enzymes [36].

No landraces showing disease symptom was observed in all the substrates. Shoot tip meristem which was used for this experiment is the disease-free part of the plant. Meristem culture technique is used to produce plants free of viruses and fungi especially in vegetative propagated plants [17]. According to [18] [37], different explants can be used to produce disease free planting materials.

All plantlets survived on all the substrates except landrace L2 on substrate sand + soil (2:2) at 4 to 6 weeks of acclimatization. The plants that did not survive had shorter and smaller roots during the acclimatization processes; these roots were unable to absorb enough macronutrients from the first to the second week. As from the 3rd week nutrients were leached by water from watering and

also a reduction of nutrients in substrates as it was absorbed by the plant roots during the first two weeks thus plants died off. Amongst nitrogen limiting factors, leaching is one of the major problems. Application of N to crops, dissolve in irrigation water and leaches down from the top soil surface to the downward portion. This process causes N deficiency which reduces the growth and development of plants [34]. Nitrogen deficiency in plants can be prevented using mulch, organic and inorganic nitrogen fertilizer and N-fixing plants (legumes) [38]. Plantlets or shoots that have grown *in vitro* have been continuously exposed to a unique micro environment that has been selected and optimum conditions for plant multiplication, contribute a culture which induce phenotype that cannot survive the environmental conditions when directly placed in a propagator [39].

5. Conclusion

Based on the results of this study, Substrate sand + soil (1:3) were the best substrate for acclimatization of the four different types of plantlets. The other substrates showed moderate growth of plantlets. This is the first protocol for acclimatisation of four Cameroonian landraces of taro and this information will provide bases for future studies on acclimatization of tissue culture landraces of taro. This will also facilitate the rapid multiplication of disease-free plants for farmers to plant and improve the production of taro in Cameroon.

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Authors' Contributions

This work was carried out in collaboration among all authors. Author MEB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FVY and IB managed the study design, statistics and the literature searches. Author ART managed the analyses of the study. All authors read and approved the final manuscript.

Conflicts of Interest

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

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