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Implementation of the Acclimatization Process for Ginger Plantlets (*Zingiber officinale* Roscoe)

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Abstract

Ginger (*Zingiber officinale* Roscoe) is an important culinary and medicinal spice but is rarely cultivated due to the unavailability of seeds. Given the difficulties in adapting to plantlets produced in natural environments, it is important to analyze the survival conditions of ginger plantlets. For this reason, we varied the incubation temperature and humidity as well as the substrate during the weaning phase. Then, we varied the nutrients contained in the watering solution during the hardening phase. The statistical analysis showed that physical factors and substrates significantly influenced ($p < 0.0001$) plantlet survival. Nutrient solutions significantly influenced the phylogenesis, rhizogenesis, and height growth of the plantlets. The suitable physical factors for good development of plantlets are a temperature of 26.54˚C and a humidity of 96.16%. The 1C2T2TC substrate (1 Compost + 2 Soil + 2 Coconut Peat) had a significant survival rate of approximately 92.5%. During hardening, the Plantzym solution promoted good growth in terms of plantlet height (0.6 cm) and good development of roots (30 roots) and leaves (03 leaves). This work will make it possible to develop a technical seed production sheet for better development of ginger cultivation in Benin.

Keywords

Acclimatization, *Zingiber officinale*, Weaning, Nutrient Solutions, Substrates

1. Introduction

Ginger (*Zingiber officinale* Roscoe) is a plant species of the "*Zingiberaceae*" family that includes more than a thousand (1000) different species [1]. They are all perennial herbaceous plants, with a branched underground rhizome at the

origin of roots often forming tubers and several aerial stems bearing couplet leaves [2]. A scale world constitutes an important raw material in pharmaceutical industries. It is increasingly used in cooking, medicine, and food, particularly in manufacturing various drinks, such as sodas, flavored waters, teas, or even beers, and many food supplements contain it [3]. It has long been used empirically to treat various disorders [4]. In Benin, the majority of ginger is used for cooking, for making juice, and in pharmacopeia. Although the sector is not very popular, it is itself an important source of income. However, micropropagation has become an important method of seed multiplication for many horticultural crops [5] [6], particularly those that propagate vegetatively or slowly. In this context, *in vitro* culture techniques have been developed to produce healthy ginger plantlets [7]. Acclimatization constitutes one of the key steps in micropropagation for rapid large-scale multiplication of elite varieties. The survival rate of tissue culture-generated plants transferred from the laboratory to soil is generally low, due mainly to differences in temperature, humidity conditions, biotic stresses, and soil used [8].

Nutrient watering solutions, relative humidity, and temperature are important factors that determine the success of the acclimatization process [9]. In addition, the effect of substrate is strongly affected by the success of acclimatization [10]. However, few studies have considered the acclimatization process and the use of local products in the composition of the substrate. Cacaï *et al.* [11] indeed reported a positive effect of preacclimation of cassava plants at 25˚C and 90% humidity on the survival rate. The present work aimed to explore the optimal conditions for better acclimatization of ginger plantlets subjected to environmental conditions.

2. Materials and Methods

2.1. Materials

The plant material included ginger rhizomes obtained from an agro-pastoral farm located at Athiémè township in the Republic of Benin. The experiment was carried out in the Central Laboratory of Plant Biotechnology and Plant Breeding of the Department of Genetics and Biotechnologies of the University of Abomey-Calavi (Republic of Benin) from October 2022 to May 2023.

2.2. Methods

2.2.1. Preparing Plantlets for Weaning

The plantlets are delicately extracted from the jars with forceps by peeling off the agar from the bottom of the jar. The plants were then carefully washed with tap water to remove residual agar from the roots to avoid contamination by microorganisms [12] and washed once more with distilled water, taking care not to break the roots. Subsequently, the height of the washed seedlings was calibrated, and the plants were transplanted in the shade into cells filled with moistened substrates.

2.2.2. Treatment Substrates

Substrates were sterilized in an oven (BINDER) at 200˚C for 2 hours. This thermic sterilization is reinforced by chemical disinfection with fungicide (carbofuran) at 4% (g: g) [13]. The composition of the substrate is selected by mixing a compost, soil, sawdust, coconut peat, and charcoal in different quantity (Table 1). S1 is composed of one quantity of compost, and two quantity of soil whereas S2 is composed of one quantity of compost, two quantity of soil and two quantity of sawdust. S3 is composed of one quantity of compost, two quantity of soil, and two quantity of coconut peat while S4 is composed of one quantity of compost, two quantity of soil, and two quantity of charcoal (Table 1).

Table 1. Composition of the different substrates used.

Substrates name and Code	Composition
S1: 1C2T	1 Compost + 2 Soil (1C2T)
S2: 1C2T2SB	1 Compost + 2 Soil + 2 Sawdust
S3: 1C2T2TC	1 Compost + 2 Soil + 2 Coconut Peat
S4: 1C2T2CB	1 Compost + 2 Soil + 2 Charcoal

2.2.3. Transfer and Conditioning of Seedlings at Weaning

The repotted seedlings were subdivided into two batches for weaning. The first batch was placed in the greenhouse under a plastic tunnel covered with transparent white oilcloth for four weeks (Figure 1), and then a second batch was placed under growth room conditions in a transparent acclimatization tank. The acclimation tanks were hermetically closed for four (04) weeks to confine the atmosphere and reduce dehydration. The substrates were sufficiently humidified, and there was no addition of nutrient solution. Human interventions were limited weekly when necessary, as were phytosanitary treatments (fungal treatment). The hygrometer was placed inside the plantlets in the greenhouse and in the growth room from the start of conditioning to the end to record variations in temperature and relative humidity during the cultivation period. The relative humidity and temperature were measured weekly inside the acclimatization tank by spraying with water or manual misting four times a day for 1 min each.

Figure 1. Weaned plantlets were hermetically closed and sent to the growth room.

2.2.4. Transfer and Conditioning of Seedlings for Hardening

Seedling hardening was carried out under a shade house for 4 weeks by gradually reducing the ambient humidity to avoid direct contact of the plantlets with the sun's rays. The substrate (1C2T or manuring) was packed into previously perforated plastic pots, watered abundantly, treated with fungicides and left to settle for at least 24 hours before transplanting. The seedlings were transplanted individually into pots. During this phase, the seedlings received alternating running water and a nutrient solution. The water supply is supplied by sprinkling at a rate of one watering per day if necessary.

2.2.5. Preparing Watering Solutions

Four nutrient solutions were prepared and tested. The Plantzyme solution [14] was obtained by diluting 5 mL of crude solution in 1 L of distilled water. The NPK solution [15] was obtained by dissolving 6 g of 15 N-5P-30K-2MgO in 1 L of distilled water. The Plantzyme + 15 N-5P-30K-2MgO mixture was prepared by diluting 5 mL of pure Plantzyme and 6 g of NPK in 1 L of water [14].

2.2.6. Experimental Device and Statistical Analysis of the Results

The experimental design was a completely random block consisting of twenty plantlets per substrate with two repetitions for the weaning phase and ten plantlets per nutrient solution with two repetitions for the hardening phase. In order to see the effect of the temperature, relative humidity, and substrates on the survival rate, the generalized linear model with the binomial family was performed. Additionally, a generalized linear model was performed to explain the effect of nutrient solutions on the numbers of leaves and roots per plantlet. Analysis of variance (ANOVA) with Newman and Keuls test at the 5% threshold was performed by comparing the survival rate as well as the growth parameters (the height of plantlets and the length of roots) and establishing correlations between the number of leaves, number of roots, and height of the plantlets using simple Pearson linear regression. All analysis was done using XLSTAT software version 2014.

3. Results

3.1. Temperature and Relative Humidity Influence on Ginger during Acclimatization

Statistical analyses showed that the cultivation duration had a highly significant influence ($p = 0.003$) on plantlet survival. Similarly, the mean temperature (TM) and relative humidity (RH) significantly influenced ($p = 0.025$) the plantlet survival rate. On the other hand, the interaction between physical factors and culture duration did not significantly influence plantlet survival after 4 weeks of weaning ($p > 0.05$) (**Table 2**). Indeed, the survival rate, which was 100% during the first week of culture under both physical conditions, differed from that in the second week. Under weaning conditions (TM/26.54˚C/RH 96.16%), the survival rate increased from 100% to 90% in the 2nd week to 70% in the 3rd week and

4th week, while in the plantlets subjected to the greenhouse (TM29.94˚C/RH 67.38%), the survival rate increased from 100% to 70% in the 2nd week to 40% in the 3rd week and 4th week. In general, the highest survival rate (82.5%) was obtained under weaning conditions (26.54˚C/96.16%), while the lowest survival rate (62.5%) was obtained in the greenhouse (29.94˚C/67.38%).

Table 2. Influence of temperature and relative humidity on the survival rate of plantlets after four (04) weeks of incubation.

Source	DDL	Khi² (Wald)	Pr > Wald	Khi² (LR)	Pr > LR
Physical factor	1	4.675	0.031	4.988	0.025*
Time of Culture	3	2.292	0.514	13.787	0.0003**
Physical factor * Time of culture	3	0.005	0.999	0.005	0.999ns

ns: not significant; *: significant at 0.05; **: significant at 0.001; DDL = degree of freedom; Pr = probability.

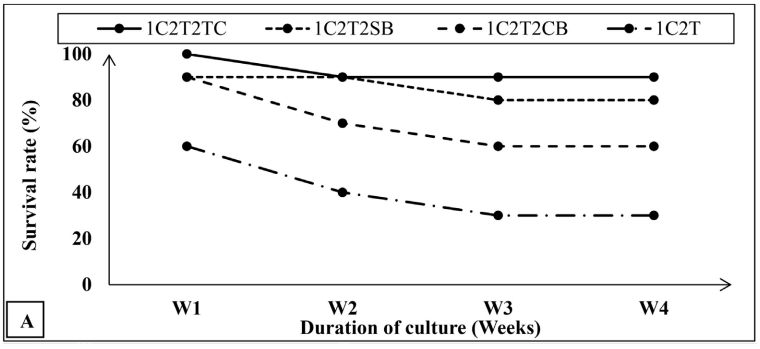
3.2. Substrates Influence on Ginger Plantlets during Acclimatization

Substrates tested, culture duration, and their interaction strongly influenced ($p < 0.0001$) the plantlet survival rate (**Table 3**). Indeed, during the first week, the survival rate was 100% on the 1C2T2TC substrate (1 Compost + 2 Soil + 2 Coconut Peat), 90% on the 1C2T1CB (1 Compost + 2 Soil + 2 Charcoal) and 1C2T2SB (1 Compost + substrates 2 Soil + 2 Sawdust) and 60% on the 1C2T substrate (1 Compost + 2 Soil). At the end of the fourth week of incubation, these percentages increased to 90%, 80%, 60% and 30%, respectively (**Figure 2(A)**). For all substrates combined, the survival rate was generally 85% in the first week, 72.5% in the second week and 65% in the third and fourth weeks of culture

Table 3. Influence of substrate on the survival rate of plantlets after four (04) weeks of incubation *in vitro* culture.

Source	DDL	Khi² (Wald)	Pr > Wald	Khi² (LR)	Pr > LR
Substrates	3	3.302	0.347	623.173	<0.0001***
Time of culture	3	2.397	0.494	3094.409	<0.0001***
Substrates * Time of culture	9	0.645	0.999	3094.409	<0.0001***

***: significance at the threshold of 0.0001; DDL = degree of freedom; Pr = probability.



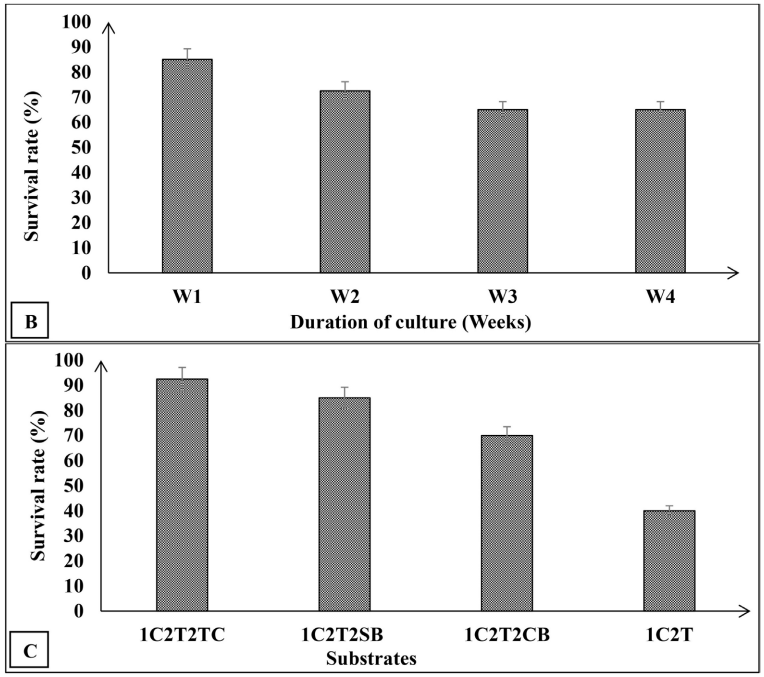


Figure 2. Survival rate of plantlets on substrates as a function of culture time (A), (B) and substrate (1C2T2TC, 1C2T2SB, charcoal) (C).

(**Figure 2(B)**). For the substrates tested, the survival rate was 92.5% for the 1C2T2TC substrate, 85% for the 1C2T2SB substrate, 70% for the 1C2T2CB substrate and 40% for the 1C2T substrate (**Figure 2(C)**).

3.3. Effect of Watering Nutrient Solutions on the Growth and Development of Ginger Plantlets

3.3.1. Number of Newly Formed Leaves

Nutrient solution, culture duration, and their interaction had highly significant influences ($p < 0.0001$) on the number of leaves formed (**Table 4**). Indeed, with the watering solution consisting of 15 N-5P-30K-2MgO, no leaves formed during the first and second weeks. With the Shives and Robbins nutrient solution, no leaves formed during the first week. At the end of the third week, an average of two leaves had formed on the plants. The acclimated plantlets that were watered with Plantzymes formed 1.3 ± 0.12 leaves during the first week, 1.5 ± 0.12 leaves during the second week, and 3.1 and 3.5 leaves during the third and fourth weeks of culture, respectively. With respect to the watering solution containing 15 N-5P-30K-2MgO + Plantzyme, leaves formed after the second week, with an average of 1.2 leaves. Thus, the average numbers of newly formed leaves were 1.2, 1.3, 2.2, and 3.5 for the 15 N-5P-30K-2MgO, 15 N-5P-30K-2MgO + Plantzyme, Shives and Robbins, and Plantzyme watering solutions, respectively. In general, the greatest percentage (3.5 leaves) of newly formed leaves was obtained with the Plantzyme watering solution, and the lowest percentage (1.2 leaves) was obtained with the 15 N-5P-30K-2MgO nutrient solution (**Figure 3** & **Figure 4**).

Table 4. Effects of watering solutions on the number of newly formed leaves per plantlet after four (04) weeks of incubation in a greenhouse.

Source	DDL	Khi² (LR)	Pr > LR
Nutrient solutions	3	45.304	<0.0001***
Time of culture	3	61.403	<0.0001***
Nutrient Solutions * Time of culture	9	14.450	<0.0001***

***: significance at the threshold of 0.0001; ns: not significant; DDF = degree of freedom; Pr = probability.

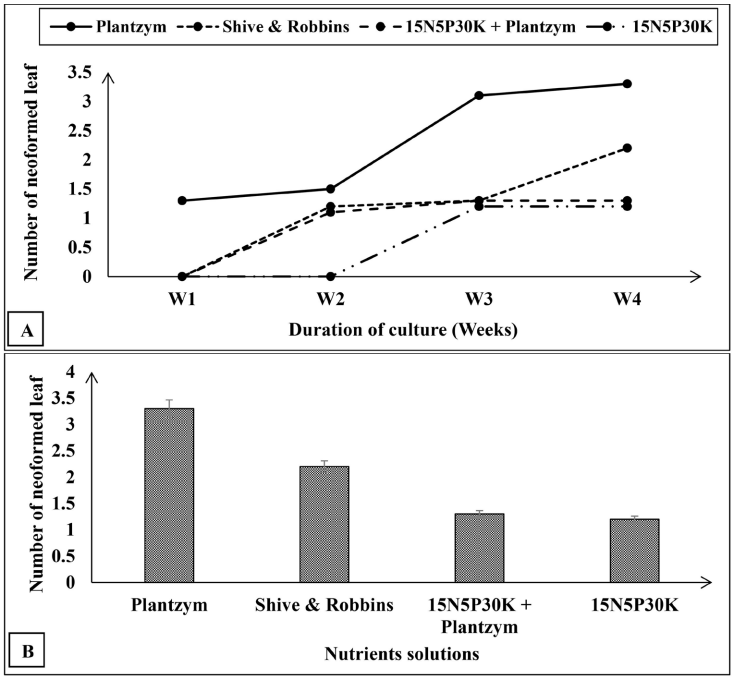


Figure 3. Average number of newly formed leaves; (A) during time of culture and (B) type of nutrient solutions.

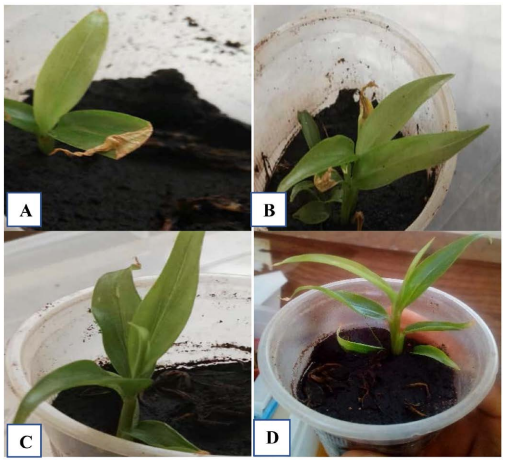


Figure 4. Effect of nutrient solutions on plantlets growth; (A) plantlets watered with the 15 N-5P-30K-2MgO solution, (B) plantlets watered with the solution of 15 N-5P-30K-2MgO + Plantzyme, (C) plantlets watered with Shives and Robbins solution, (D) plantlets watered with Plantzyme solution.

3.3.2. Gain in Height of Plantlets

The nutrient solution, duration of culture and their interaction strongly influenced ($p < 0.0001$) the height of the plantlets (Table 5). Indeed, with the watering solution consisting of 15 N-5P-30K-2MgO, there was no height gain during the first two weeks. The height increased to 0.1 cm during the third and fourth weeks of culture.

Table 5. Effects of watering solutions on the height growth of the plantlets during 4 weeks of culture in a greenhouse.

Source	DDL	SC	MC	F	Pr > F
Nutrient solutions	3	2.630	0.876	266.911	<0.0001***
Time of culture	3	1.376	0.458	139.655	<0.0001***
Nutrient solutions * Time of culture	9	0.540	0.060	18.268	<0.0001***

SC: Sum of squares; MC: Mean squares; Pr = Probability ***: significance at the threshold of 0.0001.

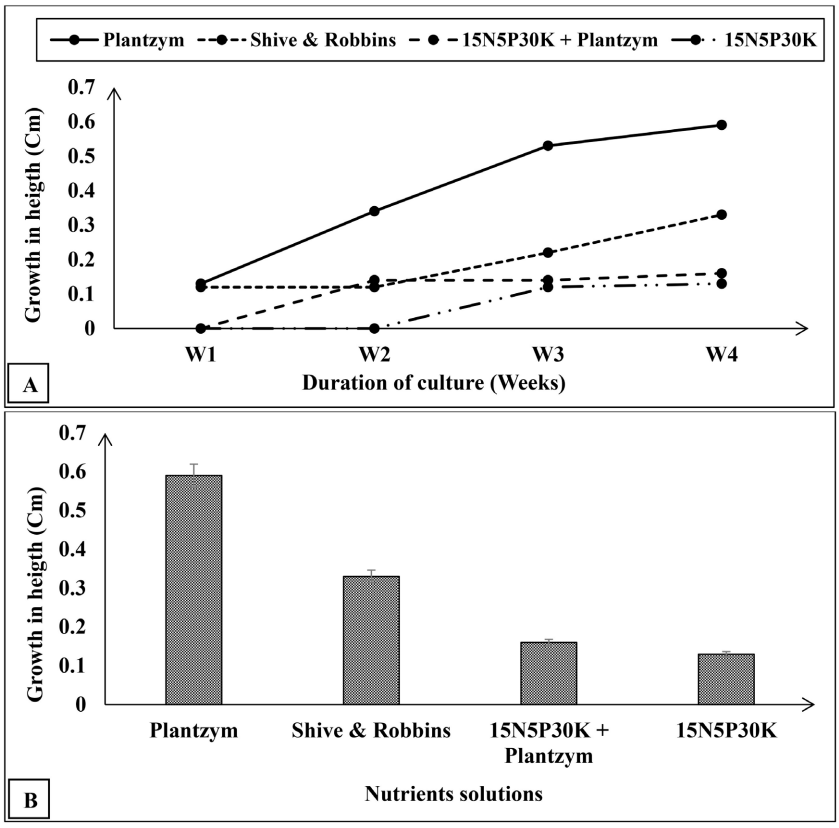


Figure 5. Plantlet growth height; (A) culture time and (B) nutrient solution type.

By using Shives and Robbins nutrient solution, the height gain was 0.13 cm in the first and second weeks and reached 0.23 cm and 0.35 cm, respectively, during the third and fourth weeks of culture. Furthermore, the height of the acclimatized plantlets watered with Plantzym increased by 0.12 cm, 0.33 cm, 0.53 cm and 0.6 cm during the first, second, third, and fourth weeks of culture, respec-

tively. With respect to the mixed solutions (15 N-5P-30K-2MgO + Plantzyme), there was no increase in height during the first week, but the height increased to 0.13 cm during the second, third, and fourth weeks. In general, the greatest height gain (0.60 cm) was obtained with the Plantzyme solution (Figure 5(A)), and the lowest height gain (1.1 cm) was obtained with the 15 N-5P-30K-2MgO solution (Figure 5(B)).

3.3.3. Number and Length of Roots

The results revealed that nutrient solution had a highly significant ($p < 0.001$) influence on both root length and number (Table 6). Likewise, the coefficients of determination (R^2) are highly significant for both variables. The number of roots formed was 30, 25, 14, and 12 for the Plantzym, Shives and Robbins, NPK + Plantzyme and NPK nutrient solutions, respectively (Figure 6). Root lengths of 10 cm, 8 cm, 5 cm, and 3 cm were noted for the Plantzym and Robbins solutions, NPK + Plantzyme, and NPK, respectively (Figure 6).

Table 6. Effect of watering solutions on the average number and length of roots.

Source	Root length	Root number
R^2 (%)	31.04	98.83
Pr	0.0036**	<0.0001***
Nutritient solutions	0.0036**	<0.0001***

: significance at the 0.001 level; *: significance at the threshold of 0.0001; R^2: coefficient of determination; Pr = probability.

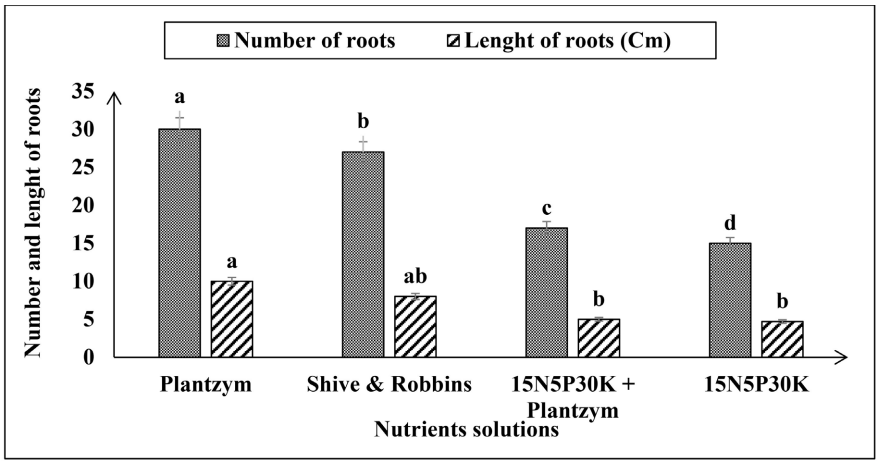


Figure 6. Average number and length of roots in different nutrients solutions.

3.3.4. Correlations between Growth Parameters

The results showed a highly significant and positive correlation between the studied growth parameters. The correlation between the average length of the roots and the average number of neoformed roots was 0.544. The number of roots, number of newly formed leaves and height increased strongly ($r > 0.8$). On the other hand, the correlations between average root length, the number of neoformed leaves (0.43) and the increase in height were weak (0.43) (Table 7).

Table 7. Pearson correlation test between growth parameters.

Variables	LMR	NRF	NFN	GT (cm)
LMR (cm)	1	0.544***	0.438**	0.432**
NRF	0.544***	1	0.825	0.832***
NFN	0.438**	0.825***	1	0.851***
GT (cm)	0.432**	0.832***	0.851***	1

MRL = average root length; NRF = number of neoformed roots; NFN = number of neoformed leaves; GT = gain in height.

4. Discussion

Plantlet seedling production requires acclimatization as a final step conditioned by several factors. This study showed that temperature and humidity significantly influenced the survival rate of ginger plantlets preacclimated for four (04) weeks. The highest survival rate (82.5%) was obtained under weaning conditions (TM/26.54˚C/RH 96.16%), in contrast to that under greenhouse conditions (TM29.94˚C/RH 67.38%). Physical factors such as temperature and relative humidity must be rigorously controlled during the acclimatization process [16]. The effects of these gradual conditions on plantlet cassava acclimatization were noted at 25˚C ± 1˚C and an average humidity of 90% for seven days of cultivation before passage through a tunnel [11]. Thus, for the weaning phase of ginger plantlets, an average temperature of 26.5˚C and a relative humidity of 96.16% are adequate for the survival of ginger plantlets in preacclimation.

There was a highly significant difference in plantlet survival among the various pineapple substrates. The mixture of 1 Compost + 2 Soil + 2 Coconut Peat substrate (1C2T2TC) generated the highest survival rate (92.5%), while this rate was 40% for the control substrate composed of 1 Compost + 2 Soil (1C2T). The positive effect of coconut fiber proportions on the survival and growth of acacia plants was reported by Bongoua-Devisme *et al.* [17]. Compared with charcoal, sawdust improved the survival rate because it not only increased the water retention capacity of the acclimation substrate but also promoted the root development of the seedlings [18]. The successful acclimatization of plantlets depends on the nature and composition of the substrate [16]. These results are consistent with those of Bongoua-Devisme [17], who noted that coconut dust absorbs and retains water from the soil longer and provides good physical support for seedlings *in vitro*. A mixture of compost and potting soil is generally considered to improve plant growth [19]. This study proved that these two components are not sufficient. Other components, such as charcoal, sawdust and coconut dust, are indispensable for water retention.

Their results showed that the nutrient solution and cultivation duration had a highly significant influence on the number of newly formed leaves, height, and average number of roots emitted. Compared with the other watering solutions, the Plantzyme solution generated more newly formed leaves and more roots and better elongated roots. The ability of this liquid organic fertilizer to facilitate the

penetration of water into the soil and reduce water loss due to evaporation and dispersion is known [14]. Despite its lower N, P, and K contents than the NPK nutrient solution used, the Plantzyme solution therefore played a key role in ginger plantlet's acclimatization to natural conditions. Plantlets treated with NPK nutrient watering solutions (20-10-10) had a better survival rate (48.57%), better phylogenesis (2.125) with the RB 89509 variety and greater growth in height (1.18 cm) with the BF92/0267 variety [11].

In this work, the NPK nutrient watering solution used alone was less inefficient and considerably reduced the Plantzyme effect. Compared to the Shives and Robbins solution. Plantzymes are obtained by enzymatic hydrolysis of proteins of vegetative origin and contain more nutrients. The production process guarantees the presence of essential amino acids.

5. Conclusion

This study deepened our knowledge of ginger (Zingiber officinale) plantlet production. A temperature of 26.54˚C and humidity of 96.16% appeared to be the optimal average temperature for good survival of plantlets during three weeks of culture. Notably, preacclimation requires weaning conditioning of the plantlets. In addition, the suitable substrate for preacclimation is the 1C2T2TC substrate (1 compost + 2 soil + 2 coconut peat), which is watered with Plantzyme solution, a very effective biostimulant for plantlet regeneration. The application of these different factors resulted in the optimal growth of ginger plantlets.

Conflicts of Interest

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

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