Journal of Plant Sciences
2023; 11(1): 1-8
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20231101.11
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Effect of Substrate Type on Survival and Growth of Sweet Potato Vitroplants Under Acclimatization

Katembo Vihabwa Abishay[1], Agbidinoukoun Arnaud[2, *], Badou Bienvenu Témidouan[2], Tohoun Todedji Jean-Marie[2], Zoumarou Wallis Nouhoun[3], Ahanhanzo Corneille[2]

[1]Faculty of Agricultural Sciences, Adventist University of Lukanga, Butembo, Democratic Republic of Congo

[2]Central Laboratory of Biotechnology and Plant Breeding, Faculty of Sciences and Techniques, University of Abomey-Calavi, Cotonou, Republic of Benin

[3]Faculty of Agronomy, University of Parakou, Parakou, Republic of Benin

Email address:

arnaudag2002@yahoo.fr (Agbidinoukoun Arnaud)

[*]Corresponding author

To cite this article:

Katembo Vihabwa Abishay, Agbidinoukoun Arnaud, Badou Bienvenu Témidouan, Tohoun Todedji Jean-Marie, Zoumarou Wallis Nouhoun, Ahanhanzo Corneille. Effect of Substrate Type on Survival and Growth of Sweet Potato Vitroplants Under Acclimatization. *Journal of Plant Sciences*. Vol. 11, No. 1, 2023, pp. 1-8. doi: 10.11648/j.jps.20231101.11

Received: November 22, 2022; **Accepted**: December 16, 2022; **Published**: January 6, 2023

Abstract: The lack of healthy planting materials is one of the major constraints of sweet potato cultivation in Benin. One of the solutions to this problem is the use of vegetal biotechnology through micropropagation. At the end of the production of vitroplants, a crucial phase consists in acclimatizing them before their transfer to the real environment. The type of substrate used plays an important role in the success of acclimatization. This study aims at evaluating the effect of the type of substrates on the survival and growth of sweet potato vitroplants in acclimatization of the two accessions (Mèché and Bombo vôvô). For this, three substrates were assessed. These are Potting soil alone, Potting soil + Sawdust, and potting soil + Charcoal powder in proportion 2:1. These substrates were first sterilized separately in an oven at 150°C for 2 hours. The experiment was conducted in the greenhouse of Central Laboratory of Vegetal Biotechnology and Plant Improvement of the Faculty of Sciences and Techniques of the University of Abomey-Calavi following a model of random complete blocks in split plot design. The results show that the type of substrate influences the success of acclimatization in terms of survival rate, the gain of size and the number of neoformed leaves ($p= 0.0033$; 0.0019 and 0.0001). The Potting soil + Charcoal powder substrate influenced these parameters better than the other two with 95%, 3.13 cm and 3.165 respectively for survival rate, size gain and the number of neoformed leaves. It was followed by the Potting soil + Sawdust substrate. Acclimatized plants have been successfully introduced into the field, proving that this technique is fruitful in the increase of the production of healthy cuttings and make them available to farmers. The protocol used in this study can be applied in the acclimatization of sweet potato vitroplants.

Keywords: Acclimatization, Substrates, Sweet Potato, Benin

1. Introduction

Sweet potato is the seventh most important crop in the world after wheat, rice, maize, potato, barley and cassava. For its food, economic and nutritional value [1, 2], it is cultivated on a worldwide scale. Thus, in 2020, its world production was estimated at 89,487,835 tons on an area of 7,400,472 ha and, in Africa, at 28,798,773 tons on an area of 4,213,802 ha [3]. Its tubers are used in many culinary and agri-food preparations [1, 4, 5]. They are rich in carbohydrates, vitamins C, A and minerals [2, 6]. In spite of these assets of the crop, it faces several constraints of which the lack of fit materials of plantation of the potent varieties [7, 8] which is the main constraint in Benin and in Africa in general [9].

Since the sweet potato seed system is informal in Benin, farmers themselves multiply and share planting material without any special organization [7]. This favors the spread of viral diseases leading to a decrease in yield. Micropropagation is the best alternative to solve this problem with its advantages such as high reproduction and obtaining replicas selected for their robustness and plant sanitation. At the end of the production of vitroplants, a crucial phase consists in acclimatizing them before their transfer to the real environment [10-12].

Therefore, methods favoring the optimization of vitroplants survival during acclimatization should be considered. Its success depends on a number of factors among which we mention: conditioning at transplanting, choice of substrate, prevention of diseases and control of physical parameters. For the choice of the substrate, it must be of good quality with a good permeability, well aerated with a pH not too high [13].

For this purpose, several substrates have already been evaluated such as sand, sawdust, rice field waste as well as their mixture, compost+ sawdust+ sand, red soil and compost [14-17]. In Benin, the only study that addressed the aspect of acclimatization of sweet potato vitroplants on six accessions with a single type of substrate made of compost, sand and sawdust resulted in a success rate that varied between 56.6% and 83.33% [17]. It is necessary to optimize the acclimatization of sweet potato vitroplants in Benin in order to move from the informal to the formal seed system. Furthermore, charcoal powder does not seem to be used in acclimatization substrates, though it is used in agriculture as an approach to correct soil problems. [18]. This study intends to evaluate the effect of three substrates on the survival and growth of vitroplants in order to optimize the success of the acclimatization of sweet potato vitroplants in Benin.

2. Materials and Methods

2.1. Plant Material

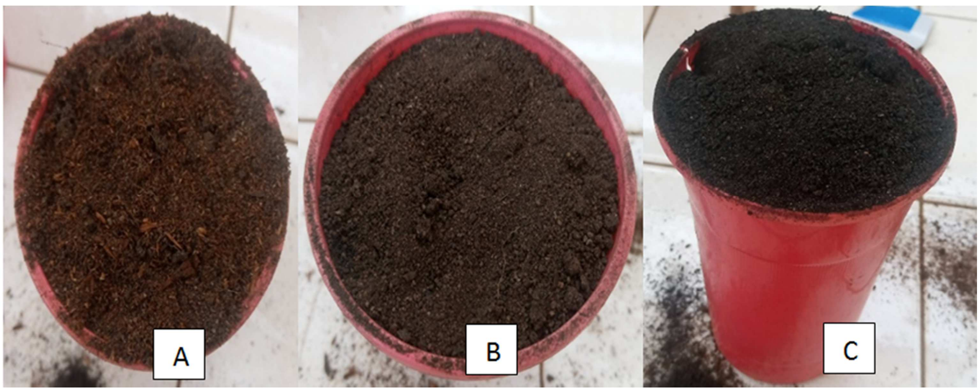
The plant material is made of seven-week-old vitroplants of two sweet potato accessions maintained in the glasshouse of the Central Laboratory of vegetal Biotechnology and Plant Improvement of the Faculty of Sciences and Techniques of the University of Abomey-Calavi (LCBVAP). These are Bombo vôvô and Mèché (Table 1). These accessions were chosen for their good yield and are among the most widely grown accessions in Benin [7].

Table 1. *Characterization of the studied accessions.*

Accessions	Tuber characteristics	Crop cycle (months)
Bombo vôvô	Purple skin with yellow flesh spotted with purple	3.5- 4
Mèché	Skin and flesh cream	2.5- 3

2.2. Composition and Sterilization of the Acclimatization Substrates

Three substrates previously sterilized in an oven at 150 °C for 2 h were used. The first one is made of potting soil alone and the other two are respectively made of Potting soil and sawdust on one hand, Potting soil and Charcoal powder on the other hand, in the proportion 2:1 (Figure 1). The Charcoal powder was chosen for its use in agriculture as an amendment [18]. It was crushed in a mortar and then sifted with a sieve of about 2 mm mesh to obtain its powder. As for the sawdust, it was taken as a positive control and the Potting soil alone as a negative control to assess the effect of the incorporation of the Charcoal powder in the substrate.



A= Potting soil + Sawdust, B= Potting soil alone, C= Potting soil + Charcoal powder

Figure 1. *Substrates under study.*

2.3. Transplanting of Vitroplants and Acclimatization Conditions

The vigorous vitroplants of about 8- 10 cm in size, 6-8 leaves with well-developed roots (2-4) were prepared for acclimatization by opening the cultivation-vases and clearing the cultivation media on the roots by mild rinsing with tap water without breaking the roots. Subsequently, the sterile substrates were moistened with Shive and Robbins' solution [19]. Transplanting of the vitroplants into plastic pots of 11.5

cm height and 5 cm diameter was done directly under greenhouse. After transplanting, the vitroplants were covered with transparent rubber in the plastic trays in order to increase the related humidity (Figure 2). After one week, these rubbers were removed to expose the plants to direct light under greenhouse for three weeks. The vitroplants were watered with Shive and Robbins solution at a frequency of once every 2 days. The acclimatization period was four weeks from 24th March to 20th April 2022.

Climatic data in terms of related humidity and temperature were taken by the related humidity sensor every five days per week during four weeks of acclimatization. They varied from 75% to 80.4% and from 30.3°C to 31.5°C for respectively related humidity and temperature (Figure 3).

Figure 2. *Vitroplants in acclimatization in the first week.*

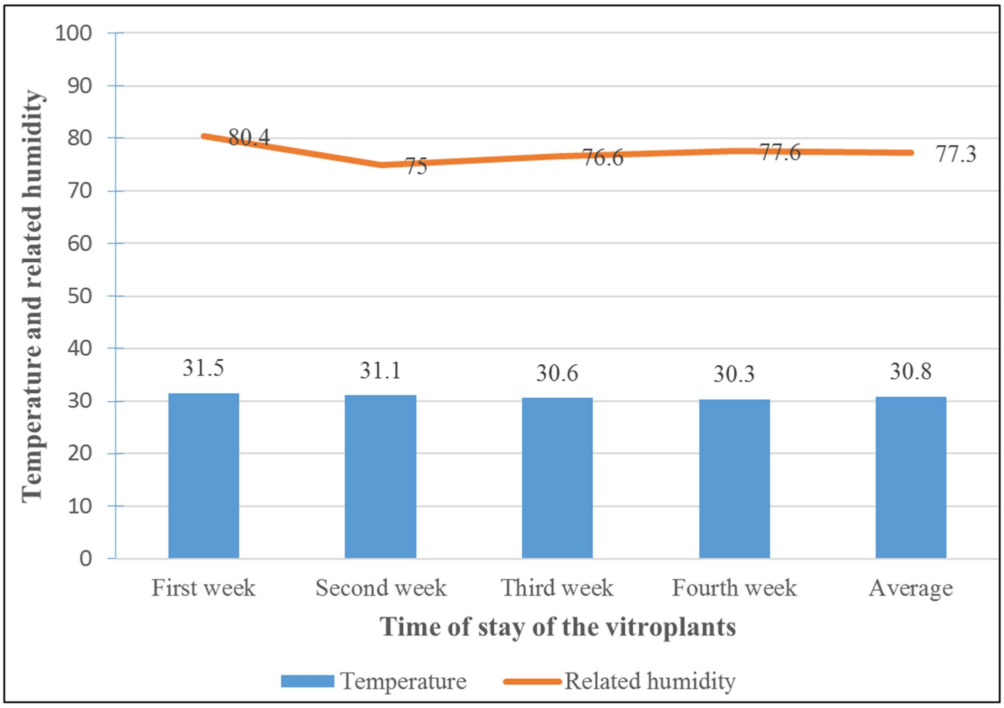


Figure 3. *Climatic data in acclimatization greenhouse.*

2.4. Experimental Device

The experiment was conducted in the greenhouse following a model of random complete blocks in split plot design [20]. Two factors were assessed: the substrate with three modalities (Potting soil alone, Potting soil + Sawdust and Potting soil + Charcoal powder) and the accession with two modalities (Mèché and Bombo vôvô). A total of six treatments were assessed. Ten vitroplants were tested per treatment with two repetitions.

2.5. Data Collection

After four weeks of acclimatization, data were gathered. These were the number of surviving vitroplants, the number of neoformed leaves and the gain of size. The surviving vitroplants and neoformed leaves were counted. The size of the vitroplants after four weeks of acclimatization was measured using a measuring stick from the crown to the apex.

2.6. Statistical Data Analysis

XLSTAT 2014 software was used for descriptive and inferential statistics. Analysis of variance under the generalized linear model was applied for survival rate, gain of size and number of neoformed leaves.

3. Results

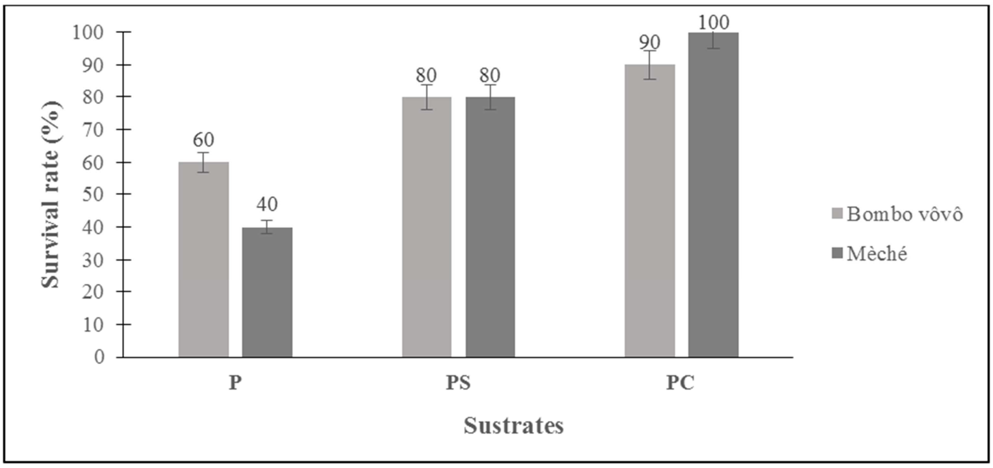
3.1. Effect of Substrate and Genotype on Vitroplants Survival

The analysis of the results indicates that only the type of substrate significantly influenced the survival rate of vitroplants in acclimatization ($p < 0.05$) (Figure 4). The highest survival rate was obtained with the Potting soil + Charcoal powder substrate while the lowest was recorded with the Potting soil alone substrate (Figure 4).

Table 2. *Analysis of the survival rate of vitroplants as a function of substrate and genotype.*

Source	DF	Sum of squares	Mean squares	F	Pr > F
Genotype	1	0.0167	0.0167	0.1011	0.7517ns
Substrate	2	2.1000	1.0500	6.3708	0.0033**
Genotype*Substrate	2	0.2333	0.1167	0.7079	0.4972ns
Error	54	8,9	0.1648		

ns= not significant; **= highly significant.



P= Potting soil alone, PS= Potting soil + Sawdust and PC= Potting soil + Charcoal powder.

Figure 4. *Survival rates as a function of substrate and genotype.*

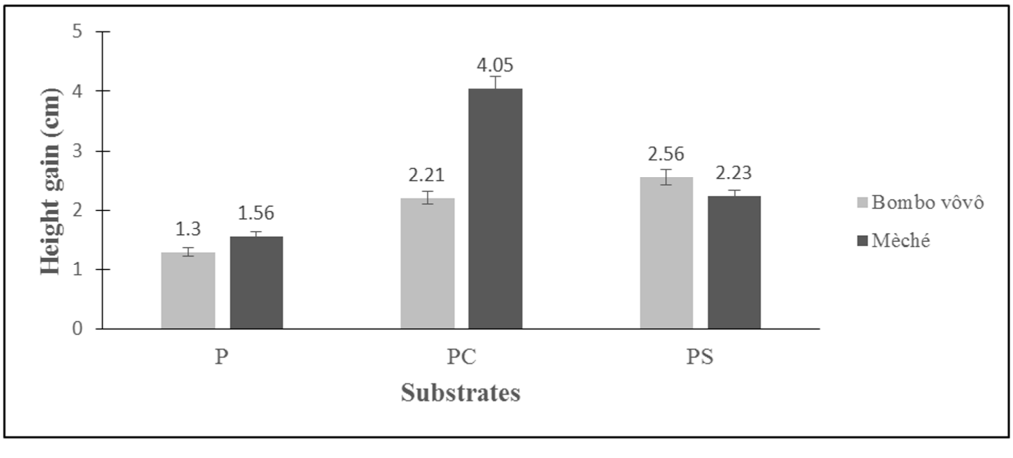
3.2. Size Gain (cm) of Vitroplants According to Substrate and Genotype

The results of the analysis of variance indicate that the size gain was significantly influenced by substrate type. Neither genotype nor genotype-substrate interaction influenced this parameter (p> 0.05) (Table 3). The lowest size gains were noted with the Potting soil substrate alone while the highest were obtained on the Potting soil with Charcoal powder (Figure 5).

Table 3. *Analysis of in vitro plant size gain as a function of substrate and genotype.*

Source	DF	Sum of squares	Mean squares	F	Pr > F
Genotype	1	5.6019	5.6019	2.6964	0.1064ns
Substrate	2	29.2203	14.6102	7.0325	0.0019**
Genotype*Substrate	2	11.9323	5.9661	2.8718	0.0653ns
Error	54	109.8418	2.0341		

ns= not significant; **= highly significant.



P= Potting soil alone, PS= Potting soil + Sawdust and PC= Potting soil + Charcoal powder.

Figure 5. *Vitroplant size gain as a function of substrates and genotypes.*

3.3. Number of Neoformed Leaves of Vitroplants According to Substrate and Genotype

The results of the analysis of variance revealed that only the substrate still influenced very significantly the number of neoformed leaves (p < 0.0001) (Table 4). The highest numbers of neoformed leaves were observed with the Potting soil and Charcoal powder substrate while the lowest were recorded with the Potting soil substrate alone (Figures 6, 7).

Table 4. *Analysis of the number of neoformed leaves of vitroplants as a function of substrate and genotype.*

Source	DF	Sum of squares	Mean squares	F	Pr > F
Genotype	1	0.0065	0.0065	0.0084	0.9275ns
Substrate	2	21.1441	10.5721	13.6951	0.0001***
Genotype*Substrate	2	1.7414	0.8707	1.1279	0.3312ns
Error	54	41.6857	0.7720		

ns= not significant; ***= very highly significant.

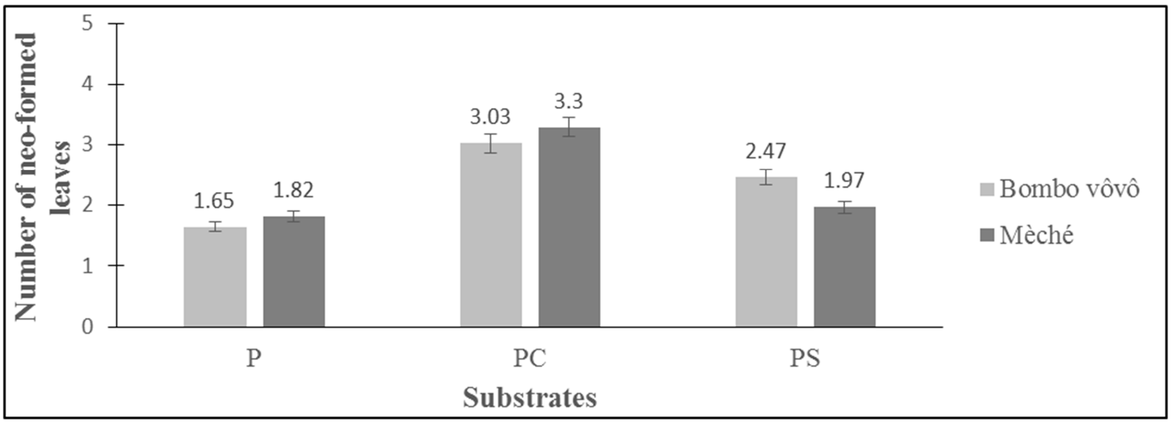


Figure 6. *Number of neo-formed leaves of vitroplants as a function of substrate and genotype. P= Potting soil alone, PS= Potting soil + Sawdust and PC= Potting soil + Charcoal powder.*

Figure 7. *Vitroplants after four weeks of acclimatization. A-C: Bombo vôvô accession on Potting soil + Sawdust, Potting soil alone and Potting soil + Charcoal powder substrates respectively. D-F: Mèché accession on Potting soil + Sawdust, Potting soil alone and Potting soil + Charcoal powder substrates respectively.*

After the acclimatization phase (April 20, 2022), the vitroplants were sent to a real environment for a period of four months.

Figure 8. shows these vitroplants in the growth phase (A and B) and maturity (C and D) respectively for the accession Mèché and Bombo vôvô.

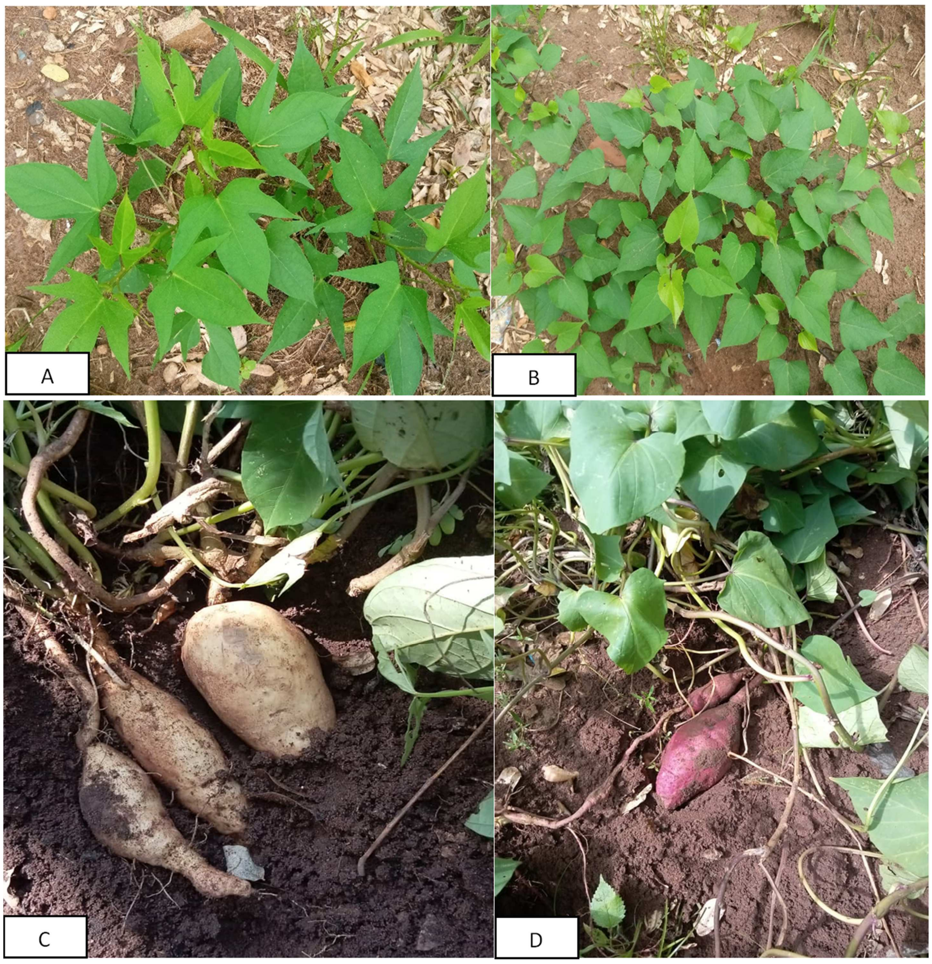


Figure 8. *Vitroplants in real environment. A= Mèché accession in growth phase, B= Bombo vôvô accession in growth phase, C= Mèché accession in maturity phase and D= Bombo vôvô accession in maturity phase.*

4. Discussion

Acclimatization is the final and very important phase of micropropagation because its success constitutes the achievement of micropropagation [10]. It is in this perspective that this study focuses on the evaluation of substrates. Thus, from the results obtained, it was found that the type of substrate influenced all the studied parameters, including the survival rate, the gain of size and the number of neoformed leaves ($p < 0.05$). For the survival rate, the mixture of Potting soil and Charcoal powder in the proportion 2:1 favored the survival of the vitroplants, followed by the substrate of the mixture of Potting soil + Sawdust. Also, the same mixture favored leaf emission than the others. This can be explained by the improved physical properties of this mixture. In all cases, the substrate made up of Potting soil alone showed the weak results. This could be explained by the lack of aeration of this substrate. The Charcoal powder and sawdust absorb a quantity of water of spraying by maintaining humidity necessary to the plants. Currently, charcoal powder is used in agriculture to correct the physical and chemical properties of the soil. [18, 21]. Accordingly, the sawdust is used as substrate in the technique of plant stemming from the fragmentation or macro propagation for its interest to maintain the humidity of the substrates without making it hydromorphic and its results have proved it in this technique [22, 23]. A study has shown that the survival rate of sweet potato vitroplants varies according to the substrate and that the mixture red soil - sand - compost at the proportion 1:2:1 has presented a high survival rate than the substrates taken alone, respectively for the varieties Kullufo and Tulla [24]. The effect of the type of substrate on the survival rate of vitroplants was also noted for banana-tree in acclimatization [25].

These good physical conditions of mixing substrates would favor the rooting of the vitroplants and consequently the absorption of minerals from the solution of Shive and Robbins ensuring the growth of the vitroplants, which would also explain the gain of size and the number of neoformed leaves. Ashiono and co-workers on Eucalyptus in the nursery found similar results on seedling growth. Greater height was observed with the sawdust and cow manure mixture substrate than with the substrates taken alone and the other mixtures [26]. Likewise, Suárez and his collaborators found a greater size with the mixture substrate (peat + sand) followed by peat + rice bran than peat alone in *Gynerium sagitatum* Aubl vitroplants during acclimatization [27].

5. Conclusion

The results obtained in this study allowed to identify two substrates favorable to the acclimatization of sweet potato vitroplants. These are the Potting soil mixed with Charcoal powder and the Potting soil mixed with Sawdust, each in the proportion 2:1. The protocol used in this study can be applied in the acclimatization of sweet potato vitroplants and Charcoal powder can be used in the composition of substrates for seed production and in large quantities from sweet potato vitroplants.

Acknowledgements

The authors thank the Central Laboratory of Vegetal Biotechnology and Plant Breeding of the Faculty of Sciences and Techniques of the University of Abomey-Calavi for the technical support in this study.

References

[1] Kpomasse, C. F., Padonou, S. W., Ahounou, J. L., Houssou, P. (2014). Towards the development of sweet potato-based couscous for human consumption in Benin, African Journal of Biotechnology, 13 (43), 4165-4168.

[2] Sanoussi, F., Adjatin, A., Dansi, A., Adebowale, A.-R., Sanni, L., et Sanni, A. (2016). Mineral Composition of Ten Elites Sweet Potato (*Ipomoea Batatas* [L] Lam) Landraces of Benin, International Journal of Current Microbiology and Applied Sciences, 5, 103-115.

[3] FAOSTAT (2020). Crops and livestock products, Food and Agriculture Organization of the United Nations, Statistical Databases. http://faostat3.fao.org/browse/Q/QC/E.

[4] Adabe Kokou, A., Kokou, E., Abdou, M., Jeoffray, D. (2019). Production et transformation de la patate douce, ISF, Cameroun.

[5] Ndangui, C. B. (2015). Production et caractérisation de farine de patate douce (*Ipomoea batatas*. Lam) : optimisation de la technologie de panification, Thèse de doctorat, Université de Lorraine, France.

[6] Djinet, A. I., Nana, R., Tamini, Z., Badiel, B. (2014). Mise en évidence des valeurs nutritionnelles de dix (10) variétés de patate douce [*Ipomea batatas* (L.) Lam.] du Burkina Faso, International Journal of Biological and Chemical Sciences, 8, (5).

[7] Doussoh, A. M., Dangou, J. S., Houedjissin, S. S., Assogba, A. K., Ahanhanzo, C. (2016). Analyse des connaissances endogènes et des déterminants de la production de la patate douce [*Ipomoea batatas* (L.)], une culture à haute valeur socioculturelle et économique au Bénin, International Journal of Biological and Chemical Sciences, 10 (6), 2596-2616.

[8] Gbenou, P. (2020). Atouts et contraintes lies à la production de la Patate Douce *(Ipomoea batatas*) dans la Commune de Sô-Ava au Benin, International Journal of Progressive Sciences and Technologies, 23 (2).

[9] Stathers, T., McEwan, M., Gibson, R., Mwanga, R., Carey, E., Namanda, S., Abidin, E., Low, J., Malinga, J., Agili, S., Andrade, M., Mkumbira, J. (2013). Tout ce que vous avez toujours voulu savoir à propos de la patate douce: Atteindre les agents du changement, manuel de formation des formateur (FdF) 3: Les systèmes semenciers de la patate douce, Centre International de la Pomme de Terre, Kenya.

[10] Hazarika, B. N. (2003). Acclimatization of tissue-cultured plants, Current science, 1704-1712.

[11] Acquaah, G. (2009). Principles of plant genetics and breeding, 2e éd, Oxford, Wiley- Blackwel.

[12] Loberant, B., Altman, A. (2010). Micropropagation of plants, Encyclopedia of industrial biotechnology: bioprocess, bioseparation, and cell technology, Wiley, New York, p. 1-17.

[13] Ourèye, S. M. (2013). Les vitrométhodes dans les stratégies de reboisement pour la Grande Muraille Verte: 129–150. *In*: Dia, A., Duponnois, R, (Eds.), Le Projet Majeur Africain de La Grande Muraille Verte : Concepts et Mise En Œuvre, Synthèses. IRD Éditions, Marseille.

[14] Wondimu, T., Feyissa, T., Bedadav, G. (2012). Meristem culture of selected sweet potato (*Ipomoea batatas* L. Lam.) cultivars to produce virus-free planting material, The Journal of Horticultural Science and Biotechnology, 87, (3), 255-260.

[15] Ubalua, A. O., Okoroafor, U. E. (2013). Micropropagation and postflask management of sweet potato using locally available materials as substrates for hardening, Plant Knowledge Journal, 2, (2), 56-61.

[16] Alula, K., Zeleke, H., Manikandan, M. (2018). *In vitro* propagation of sweet potato (*Ipomoea batatas* (L.) Lam.) through apical meristem culture », Journal of Pharmacognosy and Phytochemistry, 7 (1), 2386-2392.

[17] Doussoh, A. M., Dangou, J. S., Cacaï, G. H. T., Houedjissin, S. S., Ahanhanzo, C. (2018). Effect of cytokinins and auxin on bud burst and direct organogenesis *in vitro* of some sweet potato landraces (*Ipomoea batatas* L.) grown in Benin, Journal of Applied Biosciences, 131, 13347-13358.

[18] Lele Nyami, B., Kachaka Sudi, C., Lejoly, C. (2016). Effet du biochar et des feuilles de *Tithonia diversifolia* combiné à l'engrais minéral sur la culture du maïs (*Zea mays* L.) et les propriétés d'un sol ferralitique à Kinshasa (RDC), Biotechnol. Agron. Soc. Environ, 20, (1), 57-67.

[19] Shive, J. W., Robbins, W. R. (1942). Sand and water culture method used in the study of plant nutrition. *In* Hewitt, E. J., (Ed.s), Technical communication NO. 22 of the commonwealth bureau of horticulture and plant crops, 22 (86).

[20] Dagnelie, P. (2003). Principes d'expérimentation: planification des expériences et analyse de leurs résultats, Belgique, Presses agronomiques de Gembloux.

[21] Houben, D., Hardy, B. Faucon, M. P., Cornélis, J. T. (2017). Effet du biochar sur la biodisponibilité du phosphore dans un sol limoneux acide, *Biotechnol.* Agron. Soc. Environ, 21, 1-9.

[22] Chom Thungon, S., Hazarika, D. N. Langthasa, S., Goswami, R. K., Kalita, M. K. (2017). Standardization of Growing Media for Macropropagation of Malbhog (AAB) Banana, International Journal of Agriculture Innovations and Research, 5 (4), 597-599.

[23] Oselebe, H. O., Nwosimiri, K., Okporie, E. O., Ekwu, L. G. (2008). Macropropagation of Musa genotypes on soilless media, *J*ournal of agriculture, Biotechnology and ecology, 1 (1), 105-115.

[24] Mengs, B., Chimdessa, M., Abraha, E. (2018). *In vitro* propagation of sweet potato (*Ipomoea batatas* (L.) Lam.) trough lateral bud culture, International Journal of Innovative Pharmaceutical Sciences and Research, 6 (7), 2347-2354.

[25] Mekonen, G., Chimdessa, M. E., Muthsuwamy, M. (2021). *In vitro* Propagation of Banana (*Musa paradisiaca* L.) Plant Using Shoot Tip Explant, Turkish Journal of Agriculture - Food Science and Technology, 9 (12), 2339-2346.

[26] Ashiono, F. A., Wangechi, H. K., Kinyanjui, M. J. (2021). Effects of Sawdust, Forest Soil and Cow Dung Mixtures on Growth Characteristics of Blue Gum (*Eucalyptus saligna)* Seedlings in South Kinangop Forest, Nyandarua, Kenya », Open Journal of Forestry, 7 (4).

[27] Suárez, I. E., Yépez, J. E., López, C. M. (2020). Effect of different substrates on acclimatization and costs of arrow cane (*Gynerium sagitatum* Aubl.) micropropagated plants », Temas Agrarios, 25 (1), 77-84.