

Asian Journal of
Agricultural
Research



Research Article

Influence of Aeroponics, Vermiculite and Top Soil on Growth of Tissue Culture Plantlets for Seed Yam Seedling Production

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Abstract

Background and Objective: Yam (*Dioscorea* spp) is an important staple food crop that serves as an income for local and export markets. Production potential and demand for the crop have not been fully met because of the unavailability of quality planting materials. This study was conducted to investigate the influence of vermiculite, topsoil and aeroponics systems on three yam varieties for seedling production. **Materials and Methods:** The experiment was carried out in 2019 and 2020 in a screen house for 4 months. Virus-free tissue cultures of yam plantlets were sourced and established on an aeroponics system and in buckets containing vermiculite and topsoil. Measurement of plant height, number of leaves, number of branches, internode length and relative chlorophyll were measured. **Results:** Analysis of Variance indicated that varietal response on aeroponics was significantly higher ($p < 0.05$) than topsoil and vermiculite. However, no significant differences ($p > 0.05$) were recorded in the varietal effect. Varietal response and performance of Dente, Mankrong Pona and Pona to media were significantly affected by the use of aeroponics. Effects of vermiculite and topsoil were comparable for some parameters measured. **Conclusion:** All three varieties exhibited great potential for the improvement of plants for the production of seed yam seedlings. Alternatively, planting materials could be grown on vermiculite and topsoil for similarly positive results.

Key words: *Dioscorea* spp., varietal response, aeroponics, vermiculite, seed yam, vine, tissue culture

Citation: Arthur, M., M.D. Quain, D. Pukinka, C. Afriyie-Debrah, E.A. Adjei and E.A. Gaveh, 2023. Influence of aeroponics, vermiculite and top soil on growth of tissue culture plantlets for seed yam seedling production. Asian J. Agric. Res., 17: 16-24.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Yam is a tuber crop that belongs to the Dioscoreaceae family. Yam species of economic value comprise of *Dioscorea rotundata*, *D. dumetorum*, *D. cayenensis*, *D. alata*, *D. esculenta* and *D. bulbifera*. *Dioscorea rotundata*, also known as white yam or white guinea yam in West Africa, is most extensively cultivated¹. It is reported that Africa contributed 97.1% of the world's production of yams in 2018. This sums up to over 8.7 million hectares at a yield rate of 83515 hg ha⁻², ².

As a source of food, yams provide cash and dietary carbohydrate to millions of people, which plays a very important role. Yams have longer dormancy periods where physiological activity is reduced. As a result, they store better and longer than other tropical root and tuber crops. As a food security crop and source of income, yams supplement the requirements for food consumption in the typical African household and serve as a source of feed for livestock. The cultivation of yam crops is a good source of employment opportunities in agriculture for persons who grow, harvest, process, transport and market yams. The production of yams is therefore of critical importance to meeting the food security, nutritional and income generation needs of people in rural areas of Ghana and other yam producing countries. Yam cultivation has changed from being a crop for food security to a cash crop that helps smallholder farmers make money. It is the primary source of food and income for at least 60 million smallholder farmers and the households they support. Along the value chain, the marketing, processing and value addition of yam tubers (ware yam) generates significant income for rural households. Additionally, selling extra yam seed generates additional revenue. The high cultural, religious and social qualities of yam are particularly significant in the customary ceremonies and cultural rituals of the civilizations that cultivate it in addition to its dietary and nutritional benefits³.

Traditionally, yam is propagated through the use of edible tubers. There is a persistent undersupply of seed tubers at planting time because, in traditional systems, the tubers reproduce slowly with a multiplication ratio that ranges between 1:4 and 1:8⁴. The challenge of a low multiplication rate is heightened by the long tuber dormancy period and long growth cycle of yam. The use of edible tubers for propagation coupled with a low rate of multiplication renders quality seed yams very expensive and scarce.

Through research, high propagation ratio techniques have been developed for yams. Technologies, which include the Adaptive Yam Miniset Technique (AYMT), *in vitro* and Temporary Immersion Bioreactor System (TIBS) tissue culture techniques and yam vine propagation techniques all, have ratios of 1:30, 1:200 and 1:240, respectively, which have not been fully exploited because of low rate of adoption⁵. One tenable solution to the problem of low multiplication ratio is to adopt the vine technology in aeroponics or other growing media for the seed yam production system. Tissue culture techniques can be used to generate clean planting materials and aeroponics is a system that is used for post-flask mass propagation of clean seed yam planting material, which ultimately produces higher yields and reduces disease occurrences on the field. Thus, a screen house experiment was conducted to investigate responses to tissue culture-generated seedlings of three yam varieties as grown on vermiculite, topsoil and aeroponics systems.

MATERIALS AND METHODS

Location and climate: The experiment was carried out at the Biotechnology Section of the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) in Fumesua, Kumasi (6°43'01.1"N and 1°31'51.1"W) from January to April, 2019 and repeated within the same period in 2020. The annual mean rainfall is experienced twice at the station, from April to July and from September to November. The normal prevailing temperature is a monthly average of about 25.5°C.

Experimental design: The setup involved growing yam plantlets in 3 liter (L) buckets filled with 2 kg of sterilized topsoil, vermiculite and an aeroponics system. The experimental design used was a split plot in a Randomized Complete Block Design (RCBD) with the aeroponics system, vermiculite and topsoil as the main plots and the varieties as the sub-plot and replicated three times. There were 3 levels for the main plot (aeroponics, vermiculite and topsoil) and 3 levels for the subplot (Dente, Mankrong Pona and Pona).

Plant establishment: Tissue culture plantlets were obtained and hardened in a screen house in a humidity chamber made from clear plastic bags to prevent water loss. After 6 weeks the plants were transplanted to an aeroponics system (Fig. 1a) and 3 L plastic buckets filled with 2 kg of sterilized topsoil and

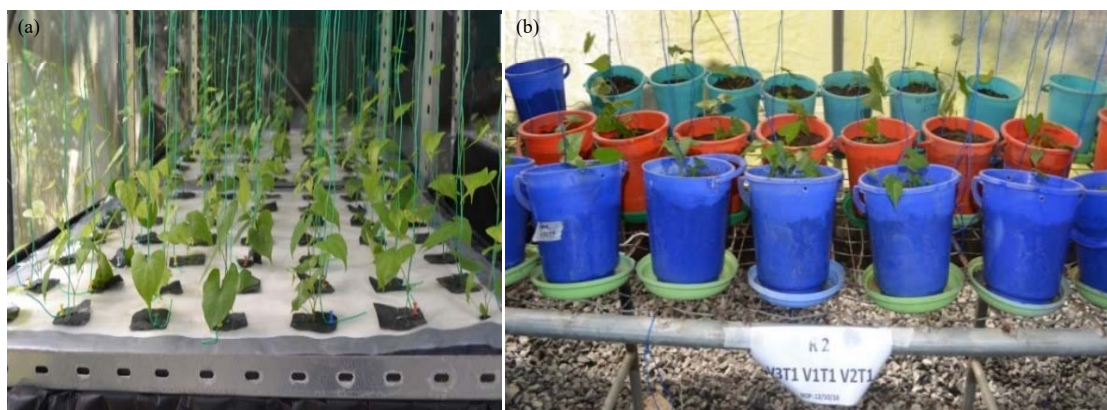


Fig. 1(a-b): Yam seedlings growing in (a) An aeroponics system and (b) Vermiculite and topsoil

vermiculite (Fig. 1b). The topsoil was sterilized at 121 °C for 3 hrs to eliminate pathogens and weeds. Irrigation was done every three days with the application of 300 mL of nutrient solution to the plants in the buckets filled with topsoil and vermiculite. Each plant was trellised at 4 weeks after planting.

Data collection and statistical analysis: The pH readings of the aeroponics nutrient solution were taken on alternate days using a digital pH meter (JENCO® VisionPlus pH630). Data on the following agronomic parameters were taken: Plant height in cm from the base to the tip, the number of leaves per plant, number of branches per plant, mean internode length per plant and mean relative chlorophyll at 0 weeks after planting (WAP), 4, 8, 12 and 16 WAP. All data were compiled, tabulated and analyzed statistically by subjecting to ANOVA using GenStat version 15 where the F test was significant at 5%. Mean separation was done using LSD.

RESULTS

Characterization of soil, vermiculite and aeroponics nutrient solution: Initial laboratory analysis of soil, vermiculite and aeroponics nutrient solution conducted yielded results presented in Table 1. The organic carbon content of the soil and vermiculite was 0.8 and 3.99%, respectively, whilst total N was 0.112 and 0.025%, respectively. Generally, the fertility of the media used was low.

Effect of substrate and variety on the length of vine: The length of vines at all data collection days was presented in Fig. 2(a-c). Varietal differences in vine length were

significant ($p < 0.05$) at 4, 8, 12 and 16 WAP. The effect of the different media on varieties was statistically significant on all measurement days. Differences in vine length among the varieties were significant ($p < 0.05$) at 4, 8, 12 and 16 WAP under all three growing systems. On the days of taking measurement, Mankrong Pona had the longest vines (90.80 cm) and Pona produced the shortest vines (62.00 cm). At 4 WAP, the setups using topsoil (Fig. 2c) and vermiculite (Fig. 2b) had an identical effect on the vine length of Mankrong Pona, Dente and Pona, but were significantly shorter than their counterparts growing on aeroponics (Fig. 2a). The length of vines of the varieties was significantly affected by all three growing systems at 8, 12 and 16 WAP. There was, however, no significance in the interaction of variety and substrate at any of the data sampling times. Observed growth rates in Mankrong Pona at the end of 16 WAP, were 20.28, 17.96 and 16.12 in aeroponics, vermiculite and top soil, respectively. Similar trends were observed in Dente and Pona. Pona reported the lowest rates in all cases. The lowest growth rate in the study was 13.46 when Pona was grown on topsoil.

Number of leaves developing on various media: The results for the number of leaves at 0, 4, 8, 12 and 16 WAP were presented in Fig. 3. Number of leaves produced was similar across all varieties at 0 WAP. The response of plants on aeroponics, topsoil and vermiculite as a measure of the number of leaves at 4, 8, 12 and 16 WAP was statistically significant in all varieties. The number of leaves of Pona ranged from 5 to 36 and were statistically similar across all media at 8, 12 and 16 WAP ($p < 0.05$). Differences in the number of leaves of Mankrong Pona (6-34) and Pona on aeroponics were similar but significantly higher than Dente.

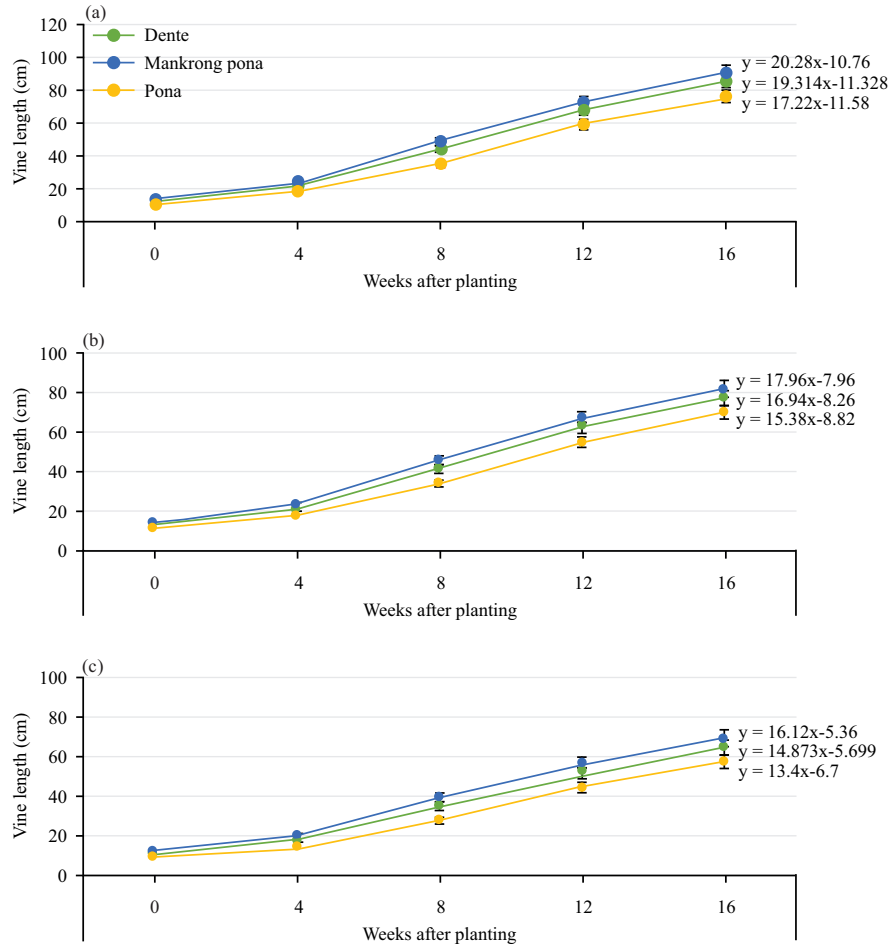


Fig.2(a-c): Vine length at planting and subsequent weeks after planting on top soil (a) Aeroponics, (b) Vermiculite and (c) Top soil

Table 1: Initial physicochemical properties of soil, vermiculite and aeroponics nutrient solution

Parameter	Soil	Vermiculite	Nutrient solution
EC ($\mu\text{S cm}^{-1}$)	110	1210	1150
pH	8.14	6.59	6.50
Available P (mg kg^{-1})	3.492	18.99	15.60
Total N (%)	0.112	0.025	1.77
K	0.228	0.428	1.10
Ca	6.8	20.80	3.80
Mg	5.4	25.00	16.40
Na	0.0019	0.0782	7.00
Al	-	-	-
H	-	-	-
Organic C (%)	0.8	3.99	-
Organic matter (%)	1.38	6.88	-

EC: Electrical conductivity, P: Phosphorus, N: Nitrogen, K: Potassium, Ca: Calcium, Mg: Magnesium, Na: Sodium, Al: Aluminium, H: Hydrogen and C: Carbon

Ranking of the highest number of leaves produced was Pona, Mankrong Pona and Dente on aeroponics, vermiculite and soil. The effects of aeroponics (Fig. 3a) and vermiculite (Fig. 3b) were not significantly different from one another, but either effect was significantly higher than topsoil (Fig. 3c) at 4, 8, 12 and 16 WAP. Variety and media

interactions were significant at 8, 12 and 16 WAP. Considering the rate of leaf growth, Pona had the highest value of 8 under aeroponics and the lowest value of 6.6 when grown on topsoil. Dente recorded the lowest leaf growth rates of 6.9, 5.9 and 5.2 on aeroponics, vermiculite and topsoil, respectively.

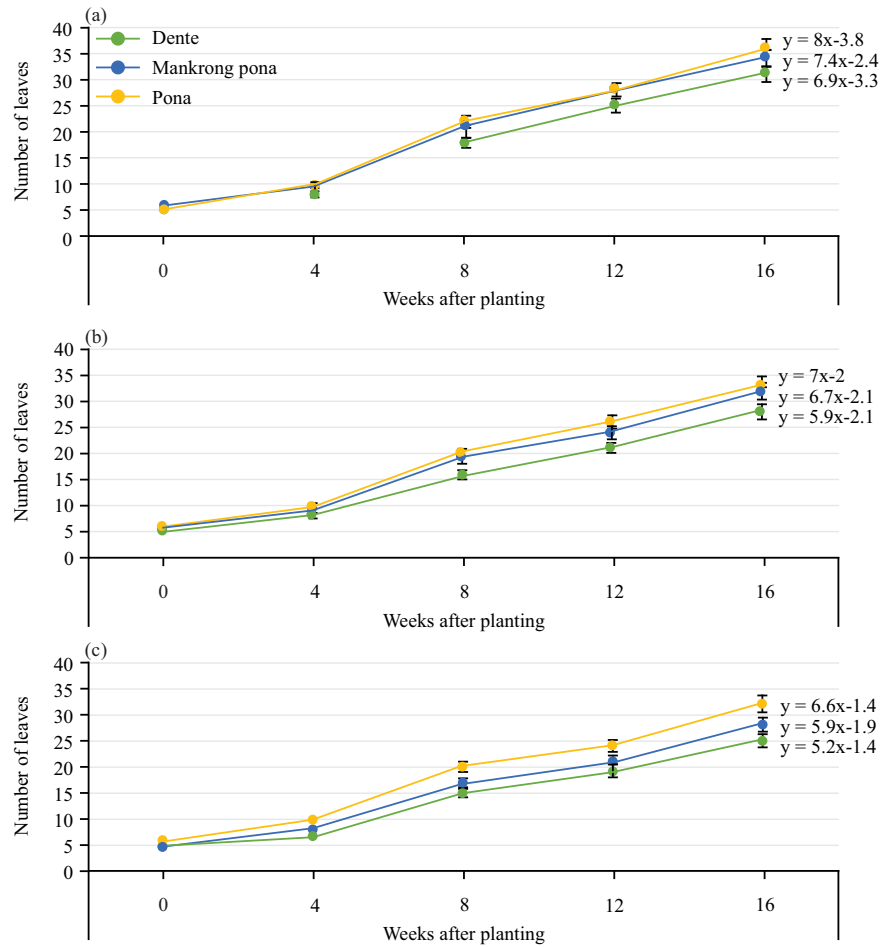


Fig.3(a-c): Number of leaves at planting and subsequent weeks after planting, (a) Aeroponics, (b) Vermiculite and (c) Top soil

Length of internodes developing on various media:

Results of aeroponics, vermiculite, and topsoil effects on the internode length of Mankrong Pona, Pona, and Dente at 0, 4, 8, 12 and 16 WAP were shown in Fig. 4(a-c). Internode lengths did not differ significantly ($P > 0.05$) for Dente on vermiculite (Fig. 4b) and topsoil (Fig. 4c) at 12 and 16 WAP, but was significantly different from aeroponics (Fig. 4a). Mankrong Pona showed significantly shorter internode lengths in topsoil at 12 and 16 WAP, whereas, significantly shorter lengths were observed at 8, 12 and 16 WAP in topsoil. Internode growth rate was very low for Pona as it ranged from 0.8 to 0.77, however, Mankrong Pona and Dente had higher growth rates ranging from 1.11 to 0.8.

Effect of media and variety on the number of branching vines

The number of branching vines emanating from the base of the three yam varieties at 0, 4, 8, 12 and 16 WAP under

the various media were presented in Fig. 5. The highest number of branching vines was observed in Pona on aeroponics (2, 3, 3) (Fig. 5a), vermiculite (2, 3, 3) (Fig. 5b) and topsoil (2, 3, 3) (Fig. 5c) at 8, 12 and 16 WAP, respectively. Dente and Mankrong Pona recorded a similar number of vines across all the media types but had a significantly lower number of vines than Pona. The rate of branching was highest in Pona, at a value of 0.6 under all conditions. Dente and Mankrong Pona recorded a value of 0.3 in all cases.

Leaf chlorophyll content of different varieties on various media:

The relative chlorophyll from leaves of the three yam varieties were shown in Table 2. Dente, Mankrong Pona and Pona showed significant differences although the difference between aeroponics, vermiculite and topsoil was not significant ($p < 0.05$) at all measuring times. At 4 WAP, varieties

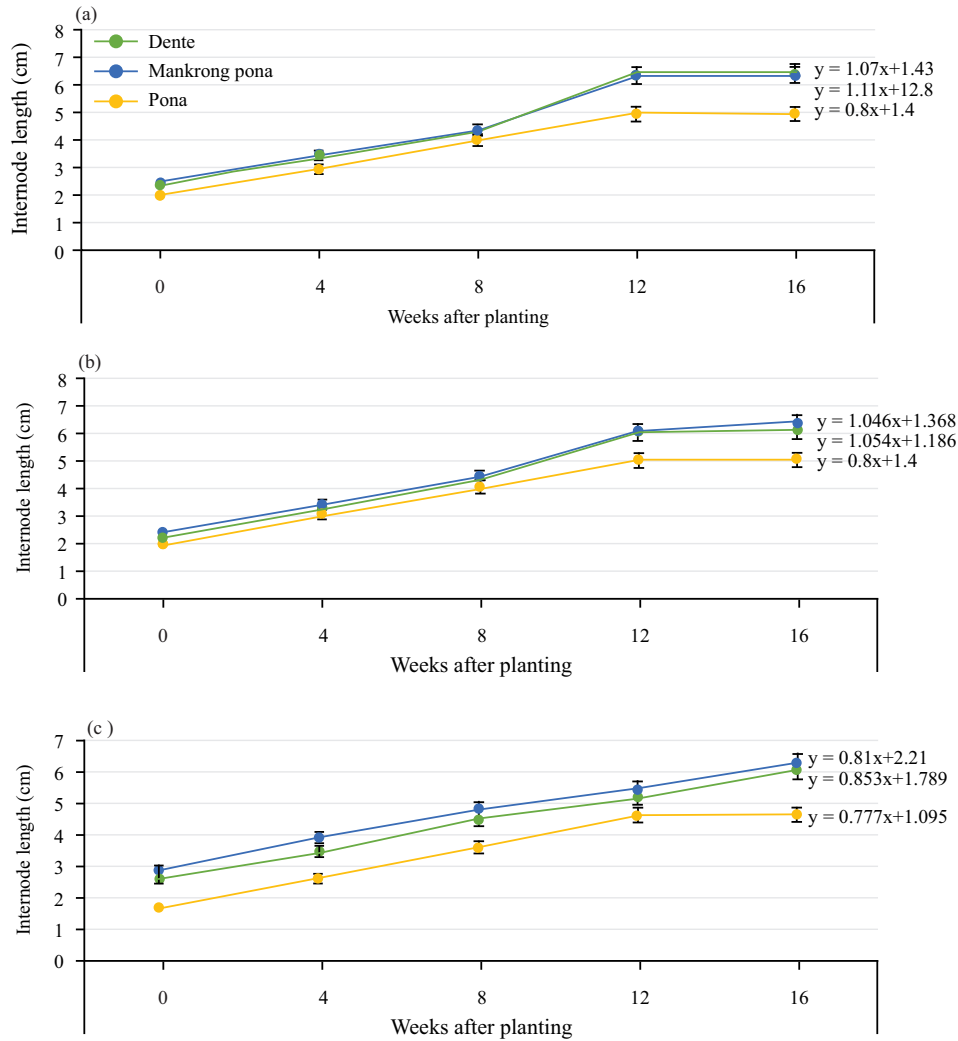


Fig. 4(a-c): Internode length at planting and subsequent weeks after planting on top soil (a) Aeroponics, (b) Vermiculite and (c) Top soil

Table 2: Relative chlorophyll content of leaves at planting and subsequent weeks after planting (WAP)

Media	0 WAP	4 WAP	8 WAP	12 WAP	16 WAP
Aeroponics	12.47 ^a	21.91 ^a	34.49 ^a	48.56 ^a	68.78 ^a
Vermiculite	12.60 ^a	20.20 ^b	30.11 ^b	41.38 ^b	55.04 ^b
Top Soil	12.36 ^a	19.96 ^b	29.22 ^c	39.16 ^c	50.76 ^c
LSD	0.56	0.50	0.62	0.60	0.68
CV (%)	6.16	5.80	4.70	3.40	2.80
Variety	0 WAP	4 WAP	8 WAP	12 WAP	16 WAP
Dente	12.02 ^a	20.33 ^a	30.58 ^a	42.80 ^a	57.51 ^a
Mankrong pona	12.67 ^a	20.84 ^a	31.42 ^b	42.87 ^a	58.09 ^b
Pona	12.73 ^b	20.89 ^a	31.82 ^b	43.42 ^a	58.98 ^b
LSD	0.56	0.50	0.62	0.60	0.68
CV (%)	6.16	5.80	4.70	3.40	2.80

Figures with the same letters within the same column are not significantly different at $p \leq 0.05$ and *: Not significant

on aeroponics recorded a significantly higher relative chlorophyll content than vermiculite and topsoil. Significant differences were observed at 8, 12 and 16 WAP with aeroponics recording the highest and topsoil recording the

least. At the end of all sampling periods, relative chlorophyll content was highest in Pona (58.98) whereas Dente and Mankrong Pona yielded 57.51 and 58.09 relative chlorophyll content, respectively.

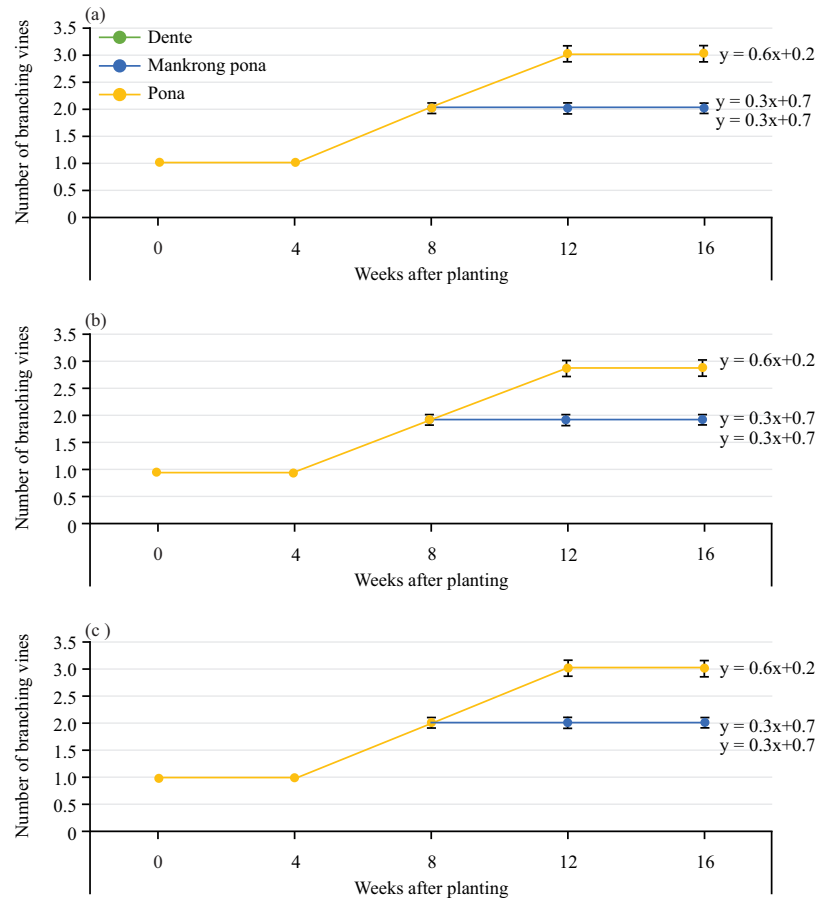


Fig.5(a-c): Number of branching vines at planting and subsequent weeks after planting on top soil (a) Aeroponics, (b) Vermiculite and (c) Top soil

DISCUSSION

Growth parameters are important indicators required to assess the performance of the crop. The following parameters evaluated in this study, the length of the vine, number of leaves, number of shoots and internode length were important to plant productivity. This facilitated the establishment of the effect of three different substrates (aeroponics, vermiculite and topsoil) on the growth of three yam varieties.

The superiority in the mode of growth was in the order of aeroponics, vermiculite and topsoil. Aeroponics and vermiculite may have contributed to the higher number of leaves by promoting less resistance to nutrient uptake and better physicochemical conditions. This implies that the lower density makes room for better absorption of available nutrients with less restriction and greater radicular development. The use of aeroponics and vermiculite enables further nutrients to be well managed and reduces the chances of hyperactive accumulation and its resulting side effect.

According to Maathuis⁶, nitrogen and other nutrients present in the media and their availability is an essential requirements of yam for optimal development and growth. For that reason, it was expected that the rate of growth per plant would be significant accordingly. The length of yam vines and the number of leaves of the three varieties were greatly influenced by the mode of growth and plant architecture.

The significant effects on the growth parameters of yam cultivars were attributed to the media and system on which the yam plants were grown. Water and nutrients required by the plants, when adequately provided to the root zone by the type of media and its distribution system, result in the desirable performance of any crop⁷. Due to the increased adoption of the single nodal cutting technique, the number of leaves and vine length have come to be of great importance in the propagation and multiplication of planting materials for seedling production. Diby *et al.*⁸ opined that with adequate fertilization, the yam plant tends to allocate a lower percentage of dry matter to the roots as the roots do not need to scavenge for nutrients. These are efficient for plants with a

higher demand for increased oxygen nutrient levels. Root and tuber crops such as yams have a high production potential and larger root surface area consisting of a biomass of tubers being formed, which has a great demand for nutrients and water as well as a high respiration rate⁹. The positive effects resulting from the use of vermiculite are consistent with a study conducted by Costa *et al.*¹⁰. It was observed that vermiculite favors physical and chemical conditions required to grow plants, as this substrate is characterized by the lower density of substrate and promotes greater cation exchange capacity. It has also been studied that in the production of seedlings, vermiculite is commonly used due to properties such as grain size, water holding capacity, low density, porosity and ability to stimulate a higher speed of emergence in seedlings¹¹.

The application of aeroponics in the production of potato seed Chiipanthenga *et al.*¹² in a study suggested that the system was efficient and yielded benefits such as nutrient availability for the plant, rapid production of planting material and improved growth and survival of potato plants.

The relative chlorophyll content of leaves was slightly affected by substrates and the variety. Relatively shorter internode length influenced the stem length of Pona. Pona, which had more and broader leaves, recorded a greater relative chlorophyll than Mankrong Pona and Dente. This is indicative that the photosynthetic apparatus of Pona was more functional than the other varieties, which would thus influence other growth factors. The driving force for increased growth and yield of yam in any media is the stimulation of photosynthesis. Over an extended period of exposure to different substrates with varying delivery systems, the effect of relative chlorophyll becomes more consequential and in this case, increases. The entire length of the internodes of Pona was the shortest for all three systems whereas Mankrong Pona and Dente had similar internode lengths on the various systems. These results indicated that internode lengths had an identical rate of growth within each media for Mankrong Pona and Dente between 0 WAP and 12 WAP, except for Pona growing on topsoil. Results of this study prove that different varieties may have the same length of vine, but the variety with the shorter internode length would have the greater number of nodes and consequently the greater number of leaves. It can also be deduced that the internode length is dependent on the variety and its architecture.

The results showed that there was no further increase in the internode length after 12 WAP for Pona. Mankrong Pona and Dente, however, showed slight additions to their internode length at 12 and 16 WAP. These differences may be

attributed to environmental conditions and the genetic makeup of the varieties. The high percentage of leaves and short internode length of Pona suggests its preferred attribute for adoption in yam single-node seedling production. A study by Diby *et al.*⁸ established that plant growth rate is a function of the leaf area available for photosynthesis and the number of leaves present on a plant determines this. Thus, the presence of many leaves is essential to the plant having a positive growth rate. The growth rate of the three varieties was highest in Pona for all substrates. This could also be considered a characteristic of Pona when compared to that of Mankrong Pona and Dente. For all varieties, the growth rate was best promoted by the use of aeroponics. Several leaves, length of vine, length of internode and relative chlorophyll content. Aeroponics and vermiculite may have contributed to the higher number of leaves by promoting less resistance to nutrient uptake and better physicochemical conditions. For that reason, it was expected that the rate of growth per plant would be enhanced accordingly. The length of yam vines and the number of leaves of the three varieties were greatly enhanced by the substrate which provided the least resistance and restriction to the absorption of nutrients, water and oxygen by the plants. The results also suggest that the superior performance of aeroponics was because nutrients and water were available and accessible at all times. In contrast, the growth rate was lower for all varieties growing on topsoil for all parameters measured. A major contributing factor to the relatively poor growth rate of varieties on topsoil could be as a result of low fertility as shown in Table 1. This suggested that with adequate fertilization, the plant growth rate could increase on topsoil.

Evaluation of different substrates which included vermiculite for the production of cobrina seedlings¹⁰, *Physalis peruviana*¹³ and papaya¹⁴ showed that vermiculite, when compared to fine sand, provides sufficient conditions for plant growth and promotes adequate expression of growth rate, vigor and plant development. Traditionally, seed yam has been propagated using milked tubers from farmer fields. As an alternative to this, single node cuttings harvested from yam vines are rooted, sprouted and cultivated on suitable media for about six to eight months. Seed yam mini tubers ranging from 50-600 g have been generated using this method and are a system that rapidly multiplies cultivars for breeding programs and farming activities¹⁵. The results of this study show that the greatest number of nodes, which are successfully produced, can serve as a guide to the seed yam system for commercial seed yam production using single nodes.

This work is focused on using 3 yam varieties and 3 growing systems. However, further studies can be carried out by exploring other economically important yam varieties in addition to using other substrates and high propagation ratio technologies, which could provide superior results to the highest performing system used, which is the aeroponics system. The limitations of the research included the advanced level of technical expertise required to operate and maintain the aeroponics system as well as the high dependence on electrical power. Interruptions to the power supply made the system vulnerable to failure and could have caused irreversible damage and losses to the plants.

CONCLUSION

Varietal response and performance of Dente, Mankrong Pona and Pona to media were significantly affected by the use of aeroponics. All three varieties exhibited great potential for the improvement of plants for the production of seed yam seedlings. The results of the study show the most significant growth of vine length, number of leaves, internode length and the number of stems for the varieties.

SIGNIFICANCE STATEMENT

The findings of this study provide detailed information on the effects of aeroponics, topsoil and vermiculite on agronomic performance characteristics and growth rates of various significant yam varieties. The outcomes are significant because they offer information that will help in the selection of promising varieties with strong vegetative capacity. The seed yam growers would be informed of the optimal substrate or growing system to use in the production of vines for single-node seedling production. Further study into yam varieties with a high potential for single-node production could build on this study. In conclusion, the results of this experiment can be used to improve the formal seed yam system.

ACKNOWLEDGMENT

The authors wish to acknowledge the contribution of the management and staff of the CSIR-Crops Research Institute- Biotechnology Laboratory for generating planting materials that were used for this study.

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