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Research Article

Emergence and Development Vigour of Yam Seedlings from Tissue Culture Generated Single Node Cuttings

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Abstract

Background and Objective: Traditionally, yams (*Dioscorea* spp.) are propagated through tubers and other technologies that use the edible portion as planting material. However, the propagation ratio of these techniques is limited in producing planting material for seed yam production within a short time compared to other grains and legumes. This research sought to establish an efficient source of planting material and technique to generate yam seedlings with the aim of mass propagating yam plantlets.

Materials and Methods: The study investigated the sprouting of nodal cuttings of yam plants of three locally popular white yam genotypes; Dente, CRI-Mankrong Pona and Pona. The nodes were harvested from vigorous growing tissue culture-generated plants in pots containing topsoil and vermiculite, as well as on an aeroponics system. The cuttings were nursed in seedling trays and monitored daily for 6 weeks for emergence-related parameters. Data collected were subjected to statistical analysis using ANOVA where significant differences were determined at a 5% significance level ($p < 0.05$). **Results:** Plants in the aeroponics system had a significant ($p < 0.05$) influence on emergence percentage, emergence energy, mean emergence time, the uncertainty of emergence and synchrony of emergence. Aeroponics also recorded the highest height of seedlings and number of leaves than other sources of systems used. The response of seedlings was significantly affected by the source of cuttings. The varietal effect on seedling emergence was observed to be significant ($p < 0.05$). **Conclusion:** Using single-node cuttings from plants growing on aeroponics or vermiculite was successful in producing seedlings in a relatively shorter time. The seedlings had high survival rates and could potentially serve as an alternative to traditional methods of producing planting material for yam propagation.

Key words: Synchrony of emergence, nodal cutting, emergence energy, seed yam, substrate influence, clonal material propagation

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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 (*Dioscorea* spp.) is, a highly important, vegetatively propagated staple food crop, with rising popularity worldwide that strengthens food security systems and directly serves as a source of nutrition and income generation. An estimated 94%, amounting to 50,974,634.23 MT of worldwide production in 2021 of yam was from Africa, with Nigeria and Ghana contributing 66 and 11%, respectively to the total worldwide production in 2021¹.

The cultivation of yam is traditionally done using small seed yam tubers or pieces of cut tubers known as setts. The planting material constitutes about 30% of the overall cost of ware yam production². The potential of yam production in Ghana is limited by the high cost of planting material, climate change, diseases and pests. The unavailability of adequate, clean planting material for yam production and continuous use of saved seed also renders the crop susceptible to the buildup of disease-causing pathogens and severe yield losses of up to about 80%³. Profitable yam cultivation is further constrained by lower multiplication ratios (<1:10) as compared to other root and tuber crops such as sweet potato and cassava, which use non-edible parts for propagation.

There is evidence to suggest that yam vine cuttings can provide a potentially viable solution to the problem of scarcity in the supply of planting material. Propagation through vine cuttings has reportedly been noted to have higher multiplication rates and produce viable seed tubers. It reduces the number of saved tubers that are used as planting material. Aighewi *et al.*⁴ suggested that cuttings from yam vines are an alternative remedy to the problem of inadequate seed yam planting material as the use of tubers as the seed is eliminated. *Dioscorea rotundata* plants produce vines for cutting and mini tubers within 90-120 days of planting, which could be used as genetic material for seed yam production and the conservation of germplasm⁵. The production of seed yam tubers through vine cuttings rapidly increases the propagation of clones, yielding seed yams devoid of heavy infestation from nematodes and other soil-borne pathogens. The technique is useful for the rapid multiplication of clonal materials which are highly sought after⁶.

Due to the high level of technical expertise needed for establishment, the development of seed yam seedlings for seed yam production using vine cuttings is not yet a commercial practice; nevertheless, seed yam seedling production using vine cuttings is feasible. To produce seed

yam seedlings, the study assesses the efficiency of vine cuttings of the popular white yam genotypes; Pona, Dente and CRI-Mankrong Pona harvested from mother plants growing on different substrates and sources.

MATERIALS AND METHODS

Location and climate: The study was conducted from October to November, 2020 and repeated from January to February, 2021 at the Biotechnology Section of the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) at Fumesua, Kumasi (6°43'01.1"N, 1°31'51.1"W). The Institute is located in the forest agroecological zone of Ghana with a mean annual precipitation of 50-86 inches with 2 rainy seasons; the major season from March to July and the minor from September to November.

Seedling establishment: Tissue culture plantlets were acclimatized and allowed to grow in the screenhouse and single node vine cuttings (SNVC) were harvested from the main stems of 4-month-old plants vigorously growing in pots containing vermiculite and topsoil (Fig. 1a) and on aeroponics (Fig. 1b) at 16 weeks after planting (WAP) using a clean and sharp pair of scissors (Fig. 2a). The single node cuttings (Fig. 2b) were carefully planted with the leaves remaining above in seed trays filled with sterilized topsoil (Fig. 3a) in a screenhouse and allowed to grow for 6 weeks (Fig. 3b). Node cutting spacing on the seed trays were 7×7 cm apart. Caution was exercised to prevent the single node cuttings from sustaining damage. Planted single nodes were watered manually by use of a watering can with a rose head.

Experimental design: The set-up constituted the nursing and establishment of single-node seedlings in seed in a Randomised Complete Block Design (RCBD). The factors consisted of nodes harvested from the different sources and the genotypes as another factor. Three sources of vines were cut (aeroponics, vermiculite and topsoil) and three genotypes were used (Dente, CRI-Mankrong Pona and Pona) with 3 replications.

Data collection and statistical analysis: A node was considered to have emerged when its radicle came out of the substrate and was visible. Days to the emergence of node cuttings, daily emergence count, node shoot height and number of leaves from shoots were measured at 3, 4, 5 and 6 WAP for the experiment. All data were statistically analyzed

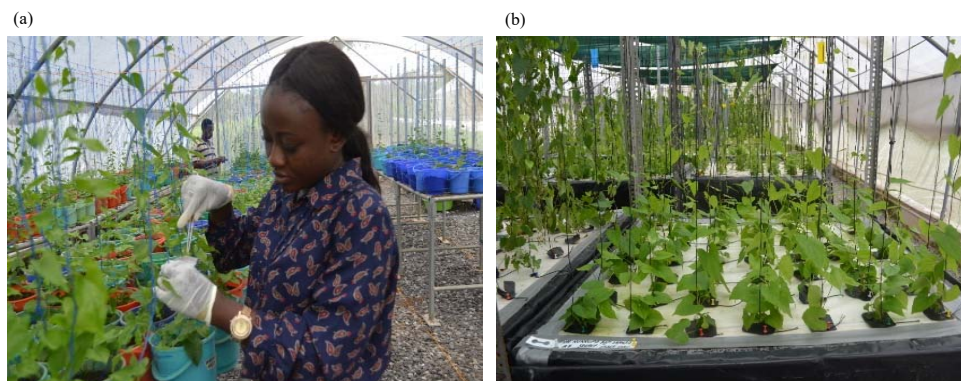


Fig. 1(a-b): Yam plants growing in (a) Vermiculite, topsoil and (b) Aeroponics

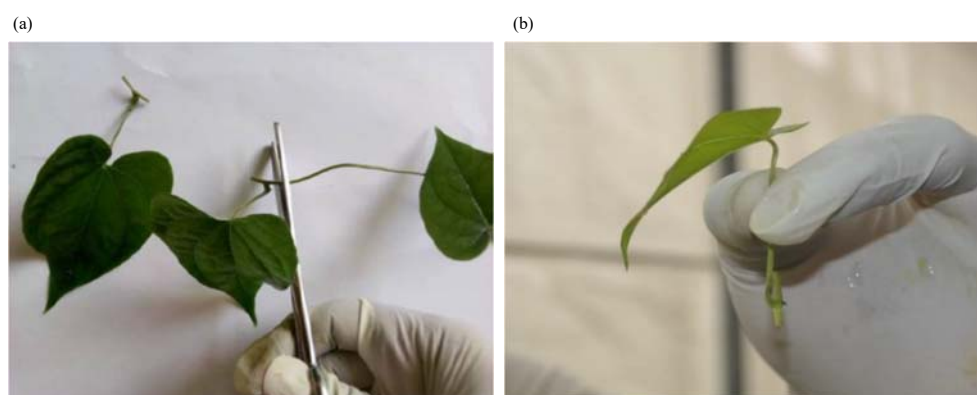


Fig. 2(a-b): Yam vine (a) Scissor cut and (b) Single node



Fig. 3(a-b): Single node cuttings in seed trays at (a) Planting and (b) Emergence at 6 weeks after planting

using GenStat version 15 ANOVA, with the F test being significant at 5%. Means were separated using LSD at $p < 0.05$.

Percentage of emergence (EP): The total number of seedlings within a setup that emerged and the potential to complete emergence, expressed as a percentage on the final day of the experiment. It was defined by Ranal and de Santana⁷ as n

is the total number of seedlings that emerged and TnP as the total number of nodes planted or nursed with a minimum value of 0% and maximum value of 100%, fitted into the formula:

$$EP = \frac{\sum n}{TnP} \times 100$$

Emergence energy (EE): Refers to the element of the seed (planting material) vigor that corresponds to uniformity and speed of seedling emergence. Higher values typically are indicators of vigorous seeds. Emergence energy was calculated as used by Domin *et al.*⁸, where n is the number of seeds that emerged at time t and nT is total number of seeds planted:

$$EE = \frac{n}{nT} \times 100$$

Mean emergence time (MET): Defined as a measure of the speed of emergence, where the number of cuttings germinated on day D, is represented by n and the number of days from the start of planting is represented by Dn. It is given as⁹:

$$MET = \frac{\sum Dn}{\sum n}$$

Uncertainty of emergence (U): Depicts how the emergence of nodes or planting material is consistent and predictable with limited variability. Low uncertainty means that emergence is predictable and has a narrow confidence interval whereas high uncertainty makes for increased variability and a wider confidence interval. Uncertainty of emergence is defined as recommended by Shannon¹⁰ with the formula:

$$U = - \sum_{i=1}^k ni \log_2 ni, \text{ being } ni = \frac{ni}{\sum_{i=1}^k ni}$$

where, ni is the number of seeds that emerged on the ith time and k is the total number of time intervals.

Synchrony of emergence (Z): Indicates the uniformity of emergence of all nodes within the population (with a minimum value of 0 and a maximum value of 1:

$$Z = \frac{\sum_{i=1}^k C_{ni,2}}{C \sum_{ni,2}}, \text{ being } C_{ni,2} = \frac{ni(ni-1)}{2}$$

In this formula, $C_{ni,2}$ is the combination of nodes that emerged at the ith time, 2 by 2 and ni is the number of seeds that emerged on the last day¹¹.

RESULTS

The results from the study indicated that single-node vines successfully emerged into seedlings with 100% survival for all nodes for all sampling periods with varying effects on parameters related to seedling emergence and growth. In the study, some significant differences between the source of cuttings ($p < 0.05$) were found.

Percentage of emergence: The percentage of seedling emergence from nodal cuttings harvested from aeroponics, topsoil and vermiculite recorded no significant difference across Dente, CRI-Mankrong Pona and Pona varieties as shown in Fig. 4. Significant differences ($p < 0.05$) were, however, recorded for cuttings of the varieties harvested from the different sources. It was observed that the emergence percentage of Dente cuttings was 100.00, 87.00 and 95.00% for aeroponics, topsoil and vermiculite, respectively. Emergence of cuttings for CRI-Mankrong Pona was 100.00% for aeroponics, 86.50% for topsoil and 94.50% for vermiculite. Pona recorded 100.00, 85.00 and 91.50% for aeroponics, topsoil and vermiculite, respectively.

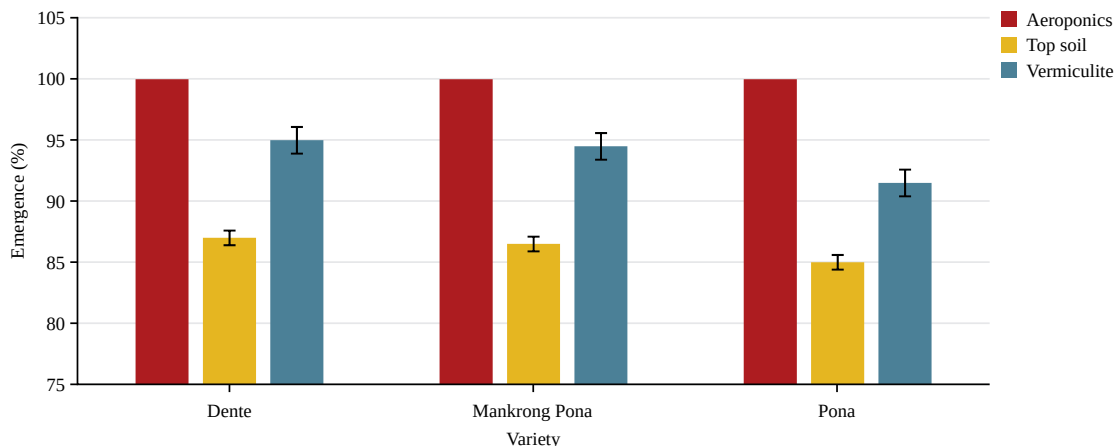


Fig. 4: Effect of source of cuttings on percentage emergence of seedlings

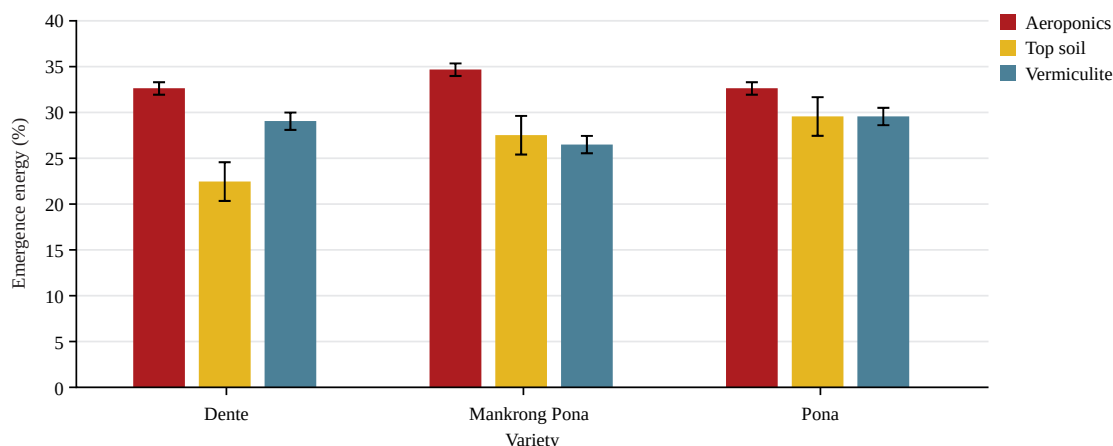


Fig. 5: Effect of source of cuttings on emergence energy of seedlings

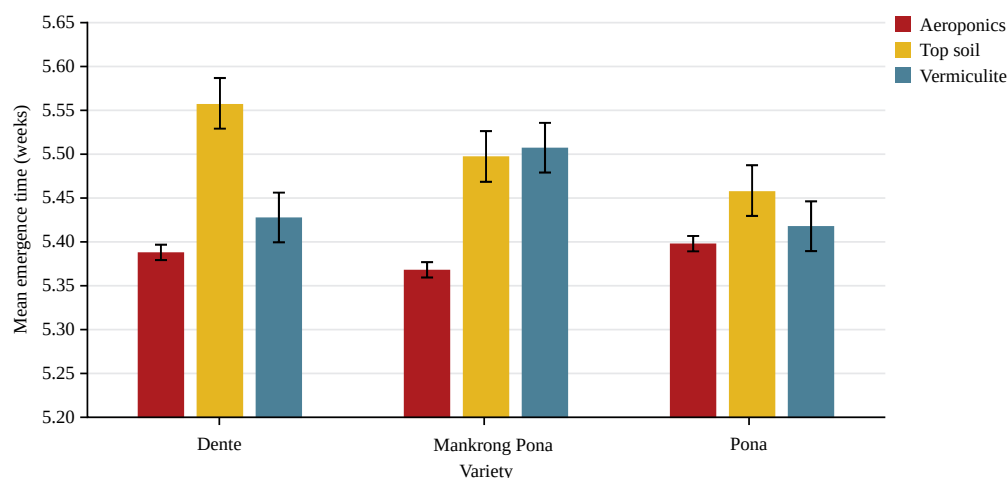


Fig. 6: Effect of source of cuttings on mean emergence time of seedlings

Emergence energy: The results in Fig. 5 demonstrated that emergence energy for Dente was 32.00, 22.05 and 28.50% for aeroponics, topsoil and vermiculite, respectively. With respect to CRI-Mankrong Pona cuttings, emergence energy was 34.00% in aeroponics, 27.00% in topsoil and 26.00% in vermiculite. Cuttings of Pona recorded emergence energy of 32.00% in aeroponics and 29.00% each in both topsoil and vermiculite. Although similarities were seen in topsoil and vermiculite, both of them were significantly ($p < 0.05$) different from aeroponics across all varieties. Emergence energy was relatively lower in seeds (single nodes) from topsoil and vermiculite compared to aeroponics.

Mean emergence time: The mean emergence time was the shortest for all genotypes in aeroponics; 5.39 weeks for Dente, 5.37 weeks for CRI-Mankrong Pona and 5.40 weeks for Pona.

Topsoil and vermiculite did not vary significantly ($p < 0.05$) for this parameter in CRI-Mankrong Pona (5.50 and 5.51 weeks) but did vary from aeroponics. For Pona, vermiculite (5.42 weeks) did not differ significantly from aeroponics (5.40 weeks) and topsoil (5.46 weeks), however, aeroponics and topsoil differed significantly. The mean emergence time for Dente also showed a different pattern. Aeroponics, vermiculite and topsoil varied significantly, with topsoil taking the longest time to emerge at 5.56 weeks (Fig. 6).

Uncertainty of emergence: The uncertainty of emergence was recorded at 1.48, 1.53 and 1.61, respectively for aeroponics, topsoil and vermiculite in Dente (Fig. 7). In CRI-Mankrong Pona cuttings, aeroponics was 1.48, topsoil recorded 1.56 and vermiculite observed 1.50. Cuttings of Pona had values of 1.50 for aeroponics, 1.59 for topsoil and

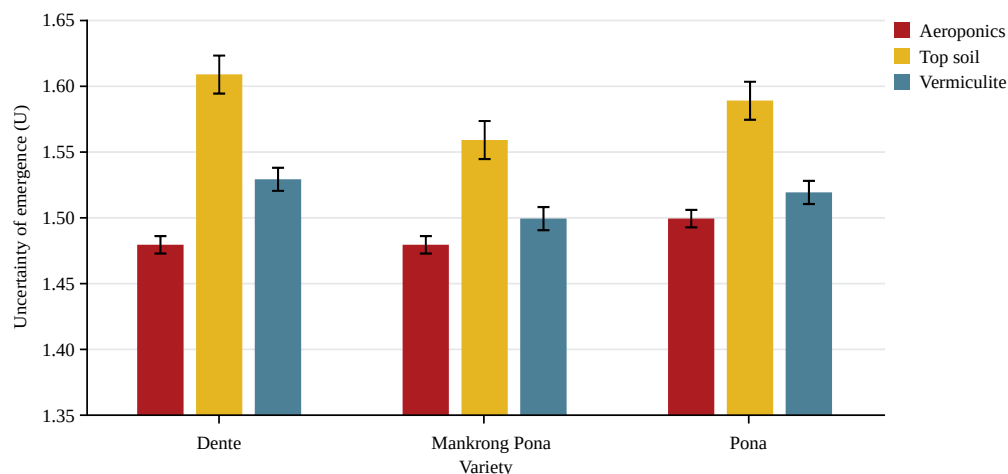


Fig. 7: Effect of source of cuttings on uncertainty of seedling emergence

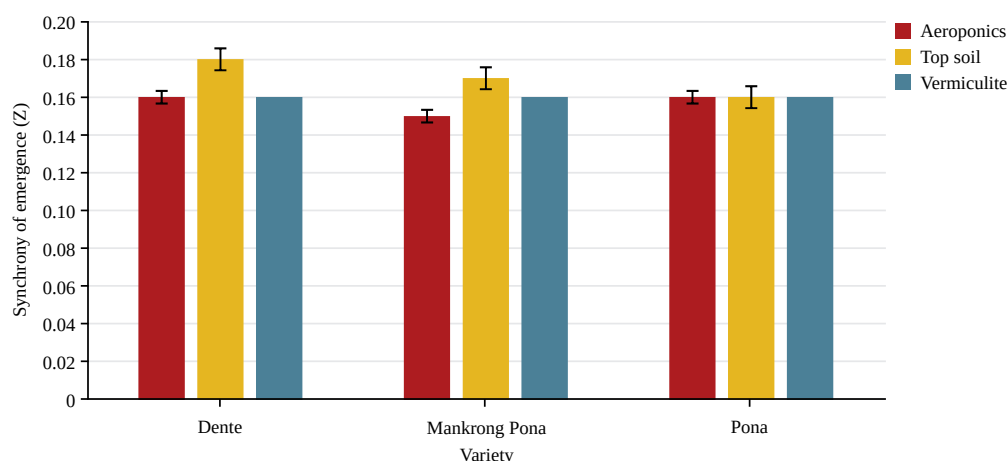


Fig. 8: Effect of source of cuttings on seedling emergence synchrony

1.52 for vermiculite. With the exception of CRI-Mankrong Pona seedlings from topsoil and vermiculite, a significant ($p < 0.05$) difference was observed for all other uncertainty of emergence values.

Synchrony of emergence: The synchrony of emergence was 0.16 in aeroponics, 0.18 in topsoil and 0.16 in vermiculite for Dente. The CRI-Mankrong Pona saw emergence synchrony values ranging from 0.15, 0.17 and 0.16 in aeroponics, topsoil and vermiculite, respectively. In Pona, the synchrony of emergence recorded was 0.16 across all sources showing that all the seedlings synchronized in emergence around the same time (Fig. 8).

Height of shoots at emergence: For Dente, no difference was observed in seedling height for aeroponics, topsoil and vermiculite at 3 and 4 WAP, respectively (Fig. 9a). However,

significant ($p < 0.05$) differences were observed in aeroponics (2.37 cm), topsoil (1.60 cm) and vermiculite (2.07 cm) at 5 and 6 WAP (aeroponics; 4.73 cm, topsoil; 3.27 cm and vermiculite; 4.13 cm).

In CRI-Mankrong Pona, a similar trend was observed. The seedling height of cuttings from aeroponics (0.27 cm), topsoil (0.20 cm) and vermiculite (0.20 cm) at 3 and at 4 WAP, did not record any significant ($p < 0.05$) differences. At 5 and 6 WAP, topsoil (1.60 and 3.27 cm), vermiculite (2.07 and 4.13 cm) and aeroponics at 5 WAP (2.34 cm) and 6 WAP (4.73 cm), respectively all showed statistically significant differences in height (Fig. 9b).

Pona seedling height recorded from aeroponics, topsoil and vermiculite-derived cuttings was 2.27, 0.20 and 0.20 cm at 3 WAP; 0.70, 0.53 and 0.60 cm at 4 WAP; 2.40, 2.00 and 2.23 cm at 5 WAP, respectively. At 6 WAP, the height of seedlings were 4.80, 4.03 and 4.50 cm, respectively for aeroponics, topsoil and

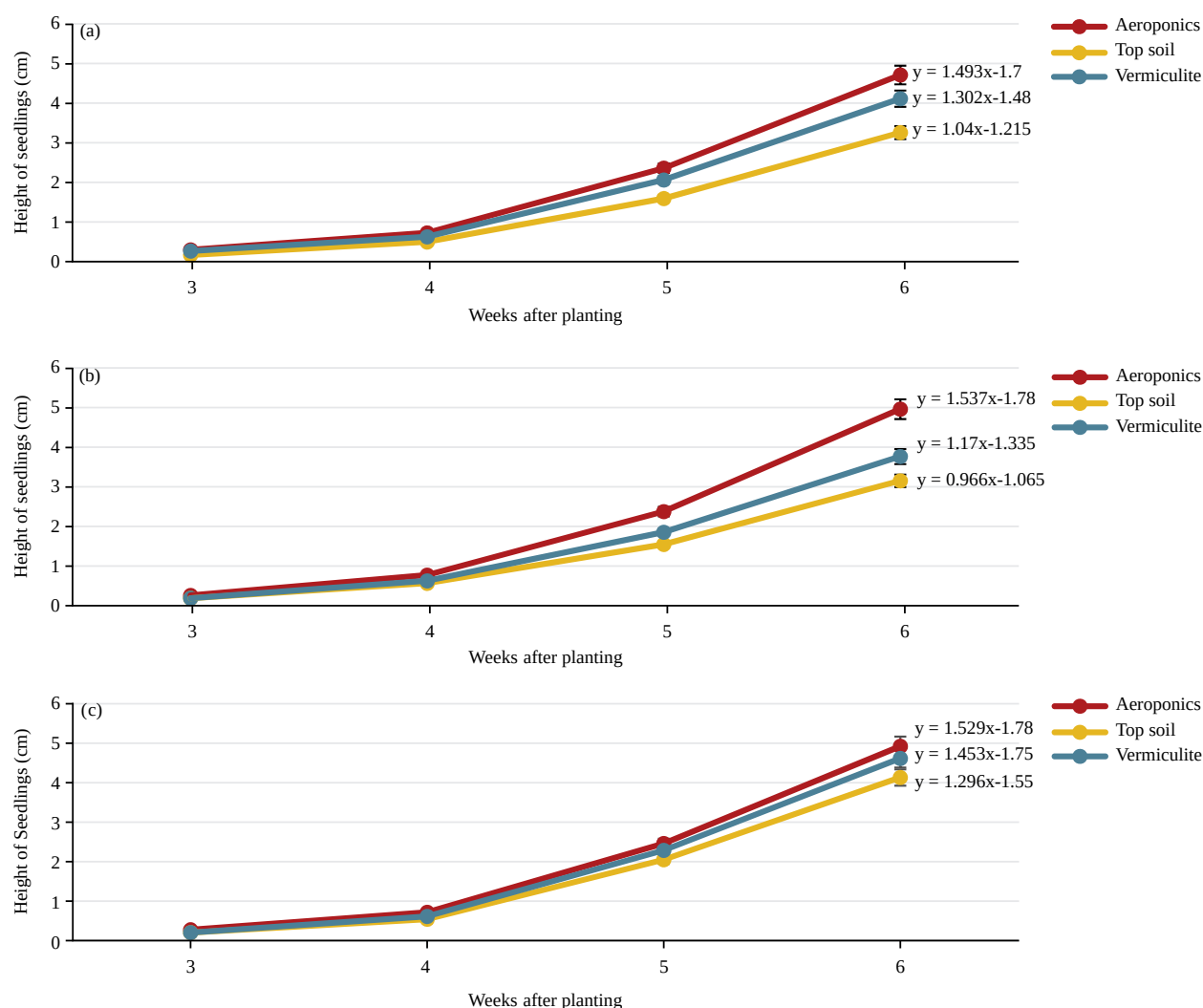


Fig. 9(a-c): Effect of source of cuttings on average height of seedlings (cm) at emergence and subsequent weeks after planting, (a) Dente, (b) CRI-Mankrong Pona and (c) Pona

vermiculite (Fig. 9c). No significant ($p < 0.05$) differences were seen in seedling height for aeroponics and vermiculite generated seedlings at 6 WAP but was different from topsoil-generated seedlings. In all cases, single node cuttings from aeroponics produced the tallest seedlings, followed by vermiculite and then top soil producing the shortest seedlings, as demonstrated in Fig. 9(a-c).

Number of leaves developed: After the nursed single node cuttings emerged into seedlings, the number of leaves on each plant was counted at 3, 4, 5 and 6 WAP. Number of leaves on Dente plants recorded for aeroponics, topsoil and vermiculite at the different sampling periods were 1.4, 0, 1 at 3 WAP; 2.4, 1, 2 at 4 WAP; 2.9, 1.3, 2.4 at 5 WAP and 3.5,

2, 3 at 6 WAP, respectively (Fig. 10a). Number of leaves on CRI-Mankrong Pona observed for aeroponics, topsoil and vermiculite were 1, 0, 0.5 at 3 WAP; 2.1, 1, 1.3 at 4 WAP; 2.5, 1.3, 1.5 at 5 WAP and 3, 2, 2.3 at 6 WAP, respectively (Fig. 10b).

Number of leaves on Pona plants recorded for aeroponics, topsoil and vermiculite at the different sampling periods were 1, 0, 0.4 at 3 WAP; 2, 1, 1.4 at 4 WAP; 2.4, 1.4, 1.8 at 5 WAP and 3.5, 2, 2.6 at 6 WAP, respectively (Fig. 10c). Across all genotypes, sampling periods, the source of cuttings significantly affected the number of leaves which developed ($p < 0.05$) for all seedlings. Most leaves were counted from aeroponics-generated seedlings and then vermiculite, with topsoil-derived seedlings recording the least number of leaves for all the genotypes used (Fig. 10a-c).

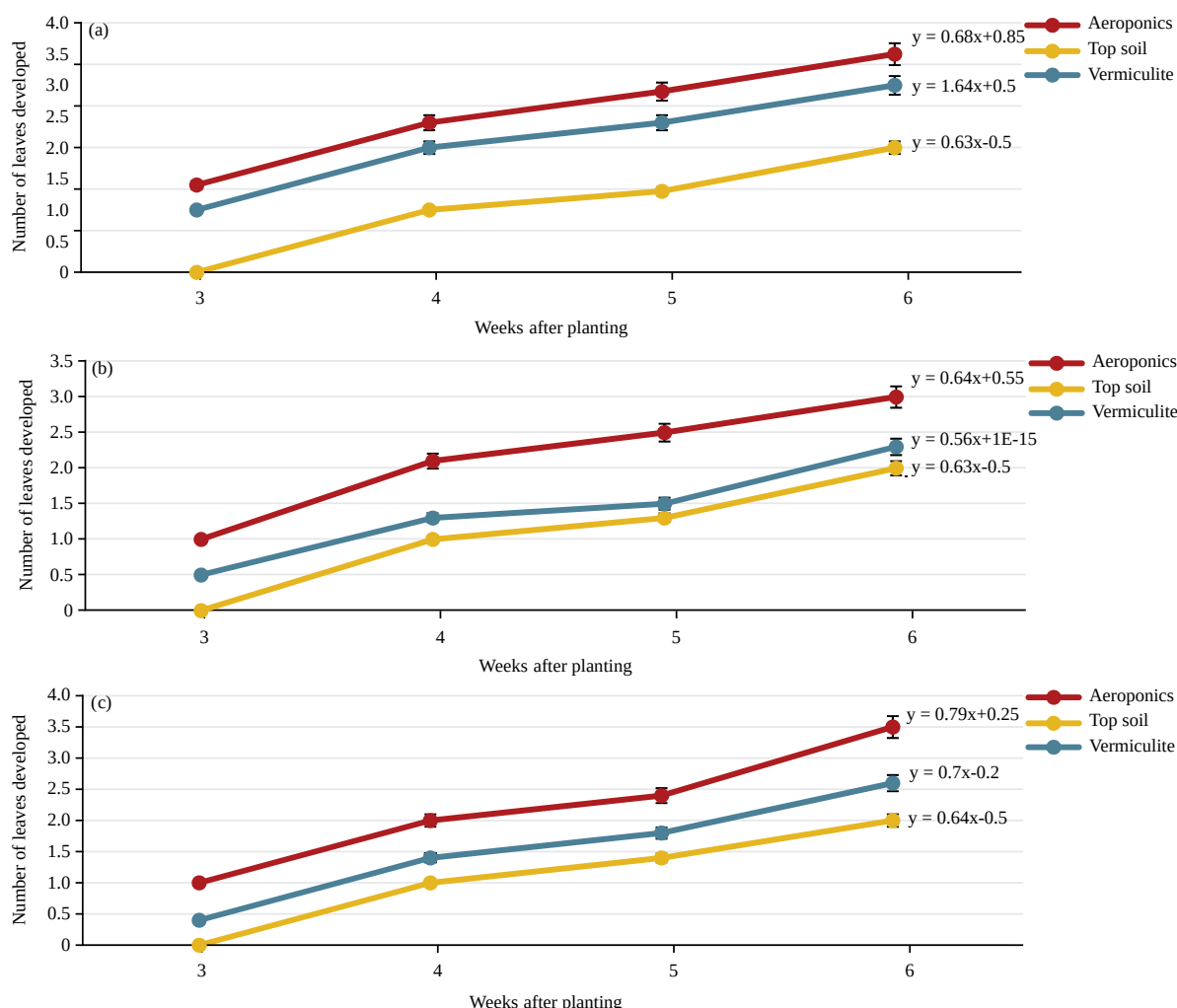


Fig. 10(a-c): Effect of source of cuttings average number of leaves at emergence and subsequent weeks after planting, (a) Dente, (b) CRI-Mankrong Pona and (c) Pona

DISCUSSION

The emergence of seedlings from nursed single nodes that were harvested was identical between varieties with no significant differences but was strongly influenced by the sources from which they were harvested. Aeroponics had the greatest effect on the emergence of single-node seedlings when compared to vermiculite and topsoil. Early emergence of the seedlings was apparent as early as 3 weeks in nodes harvested from aeroponics and continued till as late as 6 weeks after planting for plants harvested from topsoil. The cumulative number of shoots originating from aeroponics was significant and is predominantly associated with higher agronomic and growth parameters in the growth of yam vines as documented by Arthur *et al.*¹². In a study on the

propagation of wild yam using nodal cuttings by Zulu *et al.*¹³, cuttings under different treatments were rooted or sprouted within 30 days after planting. It was also reported that the treatments used provided similar emergence and survival rates when compared to yams cultivated from tubers. This observation can further be supported by a finding published by Maroya *et al.*¹⁴ which asserted that plants growing on aeroponics accumulate considerably more nutrients into their cells for subsequent planting material production. The nodal cuttings harvested then begin to express vigor in the development of shoots in the nursery and canopy formation in the nursery and on the field. Balogun¹⁵ opined that the ability of yams to sprout varied with the stage of physiological maturity and the extent of pathogen infections. Single node seedling establishment had 100% survival and shoot

formation for all emergence parameters because the initial source of plantlets, transplanted to the screenhouse and subsequently harvested were from clean and disease-free tissue culture material.

The lower performance of cuttings from topsoil could be attributed to the relatively high pH of 8.14. Vermiculite and aeroponics pH recorded were 6.59 and 6.50. Substrate pH has a noticeable influence on the growth of plants. A pH of 5.5-6.5 has proven to provide the most favorable conditions for the uptake of nutrients, growth and biomass allocation in yams¹⁶. Microorganisms that transform and release organic matter and some fertilizers function best in this pH range. The pH of top soil in this study was 8.14 which is somewhat above the optimum pH required for yam plants. This implies that although nutrients may be available in the medium, the nutrients dissolve less easily, as well as reduce the absorption of phosphate, manganese and iron in particular but also zinc, copper and boron leading to deficiency of nutrients in the plant. Omokhua¹⁷ in an experiment using different substrates to grow *Tectona grandis* reported that plant growth is affected by the availability or lack thereof, of nutrients during plant propagation. A good substrate should contain proportionate amounts of micro and macro nutrients necessary for appreciable growth and development of seedlings. The study suggested that the use of topsoil alone with less-than-optimal physiochemical characteristics with lesser pore spaces facilitated the unavailability of air and water within the soil, organic carbon, nitrogen and phosphorus which are major nutrients essential for cell formation and the optimum growth of plants.

Single node cuttings harvested from vines growing on vermiculite gave appreciable emergence and vegetative growth levels. This is due to its good water-holding capacity, ability to allow excess water to drain and good aeration capacity, providing a conducive environment for the growth of the mother plant. A study by Wisdom *et al.*¹⁸ using substrates to root *Raphanus sativus*, a combination of vermiculite and pine bark was reported to have good properties that support plant anchorage, porosity and oxygen circulation in the rhizosphere. It was noted, however that, frequency of irrigation would have to be limited.

Height of shoots from nursed single-node vines ranged from 0.17 and 0.30 cm at 0 WAP to 3.10 and 4.87 cm at 6 WAP. Shoot height was not significant for variety; however, the source of cuttings had a significant effect. The height of shoots emerging from single-node seedlings of aeroponics showed superior growth compared to the other sources. The increase in plant height of seedlings was influenced by the quality of a single node that was nursed. This high performance was

probably due to the young age of the vine cuttings and its succulent leaves which is a characteristic of a perfect vine for growth. Kabeya *et al.*¹⁹ reported that there is increased activity of the meristem of the nodal cutting thus the growth of the shoot is promoted and accelerated in younger and greener plants compared to the older and mature parts of the plant. Mounirou *et al.*²⁰ also suggested that germination and emergence start after 3 WAP and continue for 5 additional weeks.

Regarding the number of leaves on the shoots, results from the different sources were found to be different which implied significance. The effect of source and variety on the low number of leaves observed here could be connected to findings by Sanchez *et al.*²¹ that the development of leaves occurs at a slower rate as compared to shoot development and elongation. This is due to the slow development and differentiation of cells in the apical meristem for the different genotypes, even in improved soils. Active cell division in the region of the meristem has been discovered to mark the start of root formation and then shoot and finally leave. Paul *et al.*²² suggested that the variation of yam genotypes in sprouting and the number of leaves produced for yam planting material could be a result of genotypic differences and to a lesser extent the characteristics of the substrates in serving as a conducive environment for the growth of plant parts.

The results obtained in this study can be used to optimize mass production of yam seedlings, especially *D. rotundata* genotypes. They generally showed the possibility of planting material production using single node cuttings from different sources and their effect on emergence of sprouts.

CONCLUSION

Viable seed yam seedlings were produced through the use of single node vine cuttings from 'Dente', 'CRI-Mankrong Pona' and 'Pona'. The concept of using single-node yam vine cuttings as an initial source of planting material for seed yam production is a technology that has a high potential for replacing traditional means of seed production for yam propagation. These seedlings have high establishment rates and are a source of nematode and disease-free quality planting material for farmers.

SIGNIFICANCE STATEMENT

The research conducted details the best three sources of yam vines for single node cuttings towards seedling production. The results of the study confirmed the successful production of seed yam seedlings in a screenhouse as a source

of planting material. Additionally, the growth, establishment, development and emergence parameters of the yam vines from single node cuttings into seed yam seedlings were documented. The study can form the basis for mass propagation of planting material for seed yam production.

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