Research Article ISSN 2771-9014

Journal of Biotechnology and its Applications

Miniature Tubers in Immature Tissue Culture Jamaica Yams (Dioscorea sp.) and Biotechnology Applications for all Year Production

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Received: 02 Aug 2025; **Accepted:** 07 Sep 2025; **Published:** 17 Sep 2025

Citation: Collin SCANTLEBURY, Ryan FRANCIS. Miniature Tubers in Immature Tissue Culture Jamaica Yams (Dioscorea sp.) and Biotechnology Applications for all Year Production. J Biotechnology App. 2025; 4(2); 1-6.

ABSTRACT

Three-month old weaned tissue cultured yams (Dioscorea cayenenis var. yellow yam) were maintained in pots in shade-house conditions. Analysis of triplicate random samples, comprising 36 plants each, showed that a two month period of sharp decline in day-length from 13.2 hours to 12.5 hours was associated with a 50 – 70 % incidence of senescence and a 40 – 70 % incidence of tuberization. A novel observation was that tuberization was associated with an increase in daily minimum temperature. There was no significant difference in senescence or tuberization between samples and no correlation between senescence and tuberization frequency. The mean number of mini-tubers per sample of 12 plants ranged from 5.7 ± 0.6 to 8 ± 1.0 with statistically significant differences between samples. However, there was no difference in mean tuber mass between samples with overall mean mass of 2.0 ± 1.0 g per tissue culture (TC) plant. The mini-tuber diameter ranged from 13.0 to 14.0 mm but even in this narrow range, there was significant differences between samples. Despite apparent small ranges, ANOVA indicated that non-senescent vines showed significant differences between samples in number of shoots (1.1 ± 0.1 to 1.3 ± 0.6), shoot length (20.34 ± 2.89 to 34.47 ± 9.35 mm) and number of nodes per vine (7.2 ± 1.4 to 10.4 ± 3.0). Recommendations were made on how Biotechnology could be used to better facilitate all year production of yam.

Keywords

Tissue culture yams, Tuberization, Miniature tubers, All year production, Day length, Novel applications in yam production.

Introduction

Mozilla is a local yam (*Dioscorea sp.*) variety steeped in Jamaican folklore. The tale is that a Mozilla tuber may weigh hundreds of pounds. This is however the result of a few years' growth of the tuber underground. Yam is an annual crop limited by a 2 - 4 month period of dormancy in the tubers [1]. It is also a deciduous perennial crop cognizant of the underground tuber phase [2-4]. Traditionally a 9–12 month seasonal crop, it is harvested between year- end and January. There are generally two planting seasons. May is the start of the first rainy season and an anagram of yam and October is the start of the second rainy season. It is traditionally a rain fed crop, but irrigation has transformed Agriculture worldwide allowing crop establishment at any time of year. One strategy to tackle problems of food security is increased food production. For crops like yam this may not only involve increased acreages but additional planting times and harvesting at all times during the year is currently a physiological impossibility.

There are no novel biotechnologies that have not been applied in Jamaica Agriculture and Horticulture to solve yam propagation problems. Earliest publications on the use of rooted vine cuttings for yam propagation date back to just after the middle of the 20th century [5]. Rooting vine cuttings was revisited in the 70s [6], but in more recent times rooting of yam vines is a routine practice both without hormones [7] and with synthetic hormones [8,9].

Application of *in vitro* plant tissue culture specifically to propagate Jamaica yam has also been well documented [10-13]. Since these

reports, there have been several improvements and adaptations in major tissue culture activities. The semi autotrophic hydroponics (SAH) has evolved to address inefficiencies and facilitate the transition of *in vitro* plants to *ex vitro* shade-house environment [14-16].

The major constraint to all year round production of yam is that it receives various biochemical or phenology signals from light but there is variability of photoperiod throughout the course of the year. The vegetative phase may be maintained by extending the day length, while shorter day lengths induce senescence and tuberization in both yams and potatoes [17-19]. A number of varieties have been found in sweet potato that are day length neutral while others are stimulated to tuberize either by long days or short days [20]. To date reports of day length neutral yams are obscure. The search is on for yam analogues of the Jamaican Sorrel *Hibiscus sabdariffa* var. Bashment which flowers all year round.

It has been observed for ages that yam tuberizes under short day length regardless of when planted [21]. Biotechnology has potential to manage tuberization since we have already been manipulating photoperiod with artificial light for flowering induction [22-24]. It is readily conceivable how yam may be grown under artificial lights with regulated photoperiod to regulate the vegetative and tuber phases. Use of plant growth regulators is also an option.

The purpose of the investigation is characterization of the growth, senescence and tuberization of yam seedlings of an obscure putative *D. cayenensis* cultivar in pots in shade-house conditions under reducing photoperiod for eight weeks. The analysis of the data would inform Biotechnology strategies that would allow yam to be planted and harvested all year round. The objective is sensitization on available biotechnologies to allow all year round crop production in yam.

Methodology

One hundred and twenty (120) tissue culture (TC) yellow yam plants (*D. cayenensis*), weaned March 2024 were moved from shade-house to a comparable semi-indoor environment 3 July 2024, following Hurricane Beryl. Plants were maintained in 4"x 6" potting bags at a SRC facility located at Google map reference 18.01888, -76.74954 for eight weeks. Minimal watering was applied (1.0 L m^{-2} wk^{-1}). On-line weather sources were consulted for environmental data for the period. Regression analysis was used to evaluate the changes in temperature and day-length data over the period. The significance of the regression coefficient *b* was analyzed using the standard error (S.E.) of the regression, $S.E._b$ and the *t*-test, where $t = b/S.E._b$ [25].

Data was collected from three replicate samples taken at random. Each sample comprised 3 trays of 12 potting bags. Data was recorded on the frequency of senescent or dying back plants and the frequency of tuberization in each tray. The data recorded from non-senescent vines included the average number of shoots per potting bag, the mean number of nodes per vine, the mean number of mini-tubers, average length of vines, average diameter of mini-tubers and mass of mini-tubers.

Simple one-way analysis of variance (ANOVA) using an arsine transformation was used to test the null hypotheses of no difference in the frequency of senescence, and the frequency of mini-tubers between samples. In the ANOVA, total sum of squares (SS) were partitioned as follows:

$SS_T = SS_{between} + SS_{within}$

In non-senescent vines simple one-way analysis of variance (ANOVA) with a logarithmic transformation was used to test the null hypotheses of no difference in number of shoots, length of vines, number of nodes and mean mini-tuber diameter between samples. A two-way ANOVA was computed for mini-tuber mass without transformation. Tukey's test (honest significant difference) was used to identify sample means that were significantly different.

Results and Observation

Over the research period, the mean temperatures ranged from the maximum 33.6 ± 1.1 °C to a minimum of 25.9 ± 2.0 °C. The mean temperature range was 8.0 ± 2.3 °C. The regression analysis of the maximum daily temperatures on time gave $t = -|0.369|$ which did not exceed the tabled value 2.660 at $df = 60$, P = 0.01. The linear relationship between maximum temperature and time was not statistically significant (Figure 1).

The minimum temperature increased steadily with the duration of the investigation. The calculated $t = 5.543$ exceeded the tabled value. The relationship between minimum temperature and time was statistically significant at *df* 60 and P = 0.01. The difference between the maximum daily temperature and daily minimum decreased continuously as the experiment progressed. The linear relationship between the temperature difference and time was statistically significant with $t = -|4.393|$ exceeding the tabled value at P = 0.01.

The average day length for the period was 12.9 ± 0.2 hours but the hours of daily sun light decreased linearly from 13.2 hours at the start of the investigation to 12.5 hours by the end of the period (Figure 2). The significance test of the regression line showed *t*

= -|58.00|which exceeded the tabled value at 60 *df* of 2.660, P = 0.01. The linear relationship between the day length and time was statistically significant.

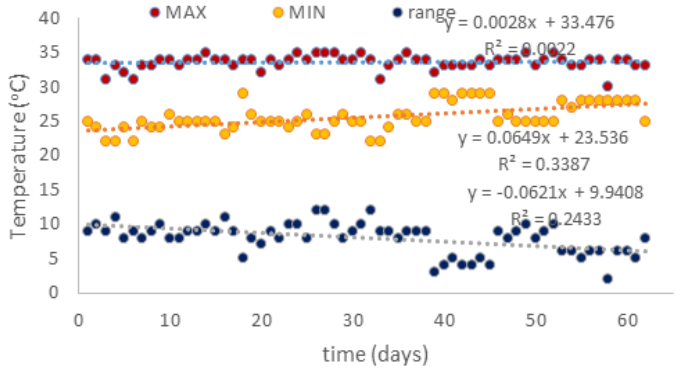

Figure 1: Temperature in Hope Gardens for the Research Period (Data sourced from Accuweather[1,2])

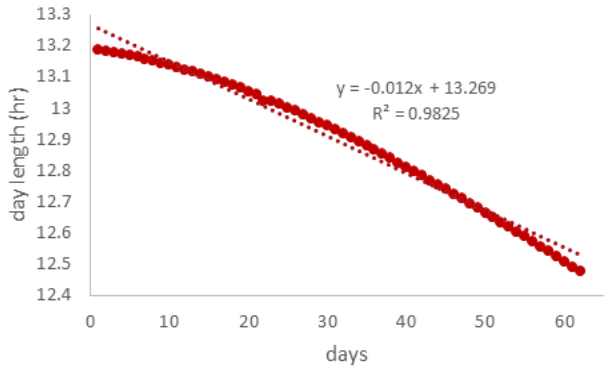

Figure 2: Day length data in Hope Gardens for the Research Period. (Data sourced from www.timeand date.com)

Table 1: Senescence and tuber formation frequencies (%).

Sample #	Replication	Senescence	Tuberization
1	1	50.0	66.67
	2	63.6	63.64
	3	54.5	45.45
2	4	66.7	66.67
	5	58.3	63.64
	6	50.0	66.67
3	7	66.7	41.67
	8	58.3	50.00
	9	50.0	50.00
	Mean	56.6	57.16
	St. dev	6.1	10.22

In the ANOVA of the frequency data for senescence and tuberization, the within samples variance (s^2_{within}) was larger than the between sample variance ($s^2_{between}$) so no need to compute F. These frequencies were therefore not significantly different between samples so the data was pooled (Table 1). Senescence was observed in all replications (between 50 to 70 %) as well as mini-tubers (between 40 to 70 %), but there was a very weak correlation between the two activities (r = -0.123). The Table 1 means for frequency of senescence and tuberization were similar but Figure 3 illustrates that while both were observed, the frequencies were not always equal and there was no identifiable pattern between senescence and tuberization.

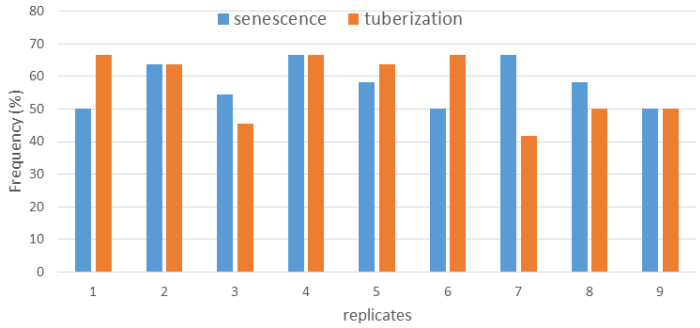

Figure 3: Frequency of senescence and mini-tuber formation.

The mean number of mini-tubers per TC plant ranged from 5.7 ± 0.6 to 8.0 ± 1.0. In the ANOVA the calculate F 1182.99 exceeded the tabled value 5.14 for $df_{2,6}$ at P < 0.05. The T statistic 0.10 indicated that sample 1 ≠ sample 3 and sample 2 ≠ sample 3 (Figure 4).

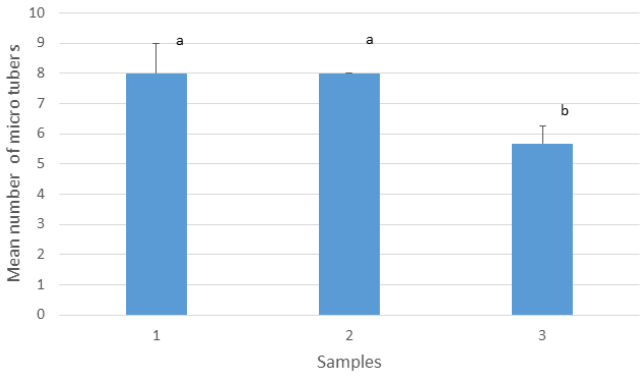

Figure 4: Number of Micro tubers per plant (mean ± St. Dev).

The mean masses of mini-tubers per tray of 12 plants ranged from 1.47 ± 1.03 to 2.52 ± 1.07 g (Table 2). The mean masses between samples and replications are illustrated (Figure 5) but there was no significant difference in mean masses of mini-tubers between samples (Table 3). The calculated F values did not exceed the tabled critical values for $df_{2,38}$ (trays and samples) and $df_{4,38}$ (interaction) of approximately 5.2 and 3.8 respectively, at P = 0.01. There was also no difference in masses between trays (replications). There was also no interaction. The pooled average of mini-tubers per TC plant was therefore 2.0 ± 1.5 g.

Table 2: Masses (g) of mini-tubers (mean ± St. Dev).

Sample	tray 1	tray 2	tray 3
1	2.33 ± 0.95	2.48 ± 2.08	2.52 ± 1.07
2	1.52 ± 1.51	1.96 ± 1.81	2.45 ± 2.18
3	1.48 ± 0.61	1.50 ± 1.60	1.47 ± 1.03

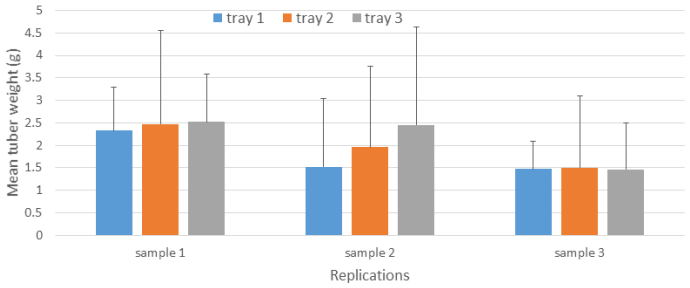

Figure 5: Masses (g) of mini-tubers in samples (mean ± St. dev).

Table 3: ANOVA Summary table for mass of mini-tubers.

Source of variation	Sum of squares	*df*	Variance	F
Between samples	9.433	8		
Trays	0.706	2	0.353	0.150
Samples	7.166	2	3.581	1.523
Interaction	1.566	4	0.391	0.167
Within samples	89.320	38	2.350	

The means of mini-tuber diameter were similar between samples, all between 13.0 - 14.0 mm (Table 4). However, ANOVA showed that the difference in means of the three samples, where n = 3 in each case, was statistically significant ($F_{2,6}$ = 351.892, P < 0.05). The calculated Tukey test statistic T = 0.189 for pairwise comparison of means indicated that sample 1 ≠ 2, 1 = 3; 2≠ 3 (Figure 4).

Table 4: Average mini-tuber diameter (mm) per sample.

Sample	Mean	St. Dev
1	13.35	2.93
2	13.81	2.27
3	13.38	1.52

The yams that survived (non-senescent plants) comprised mostly single shoots (vines). There was very little difference observed between samples (Table 5). The ANOVA with calculated F = 509.37 exceeded the tabled value 5.14 for $df_{2,6}$ at P < 0.05. As such, the number of shoots was significantly different between samples. The calculated Tukey statistic was T = 0.157. The pairwise mean comparisons that exceed T and were therefore significantly different were: 1 = 3; 1 ≠ 2; 2 ≠ 3 (Figure 6).

Table 5: Mean number of shoots or vines per pot.

Sample	Mean	St. Dev
1	1.1	0.1
2	1.3	0.6
3	1.1	0.1

The non-senescent vines ranged from 20.34 ± 2.59 to 34.47 ± 9.35 cm (Table 6). The calculated ANOVA value of F = 181.767 where n = 3 in each case, at $df_{2,6}$ exceeded the tabled value 5.14, P < 0.05. As such the mean shoot length was significantly different between samples. The Tukey value T = 0.263 indicated that all sample were significantly different (Figure 7).

Table 6: Length (cm) of surviving shoots (mean ± St. Dev).

Sample	Mean	St. Dev
1	34.47	9.35
2	29.62	8.21
3	20.34	2.59

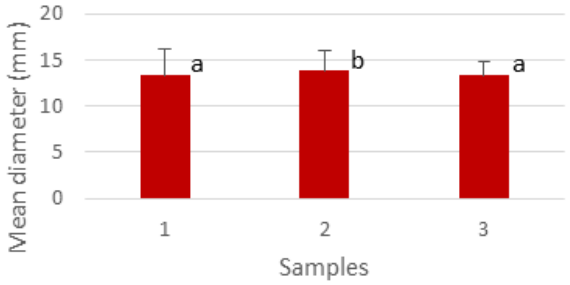

Figure 6: Mini-tuber diameter per sample (mean ± St. Dev).

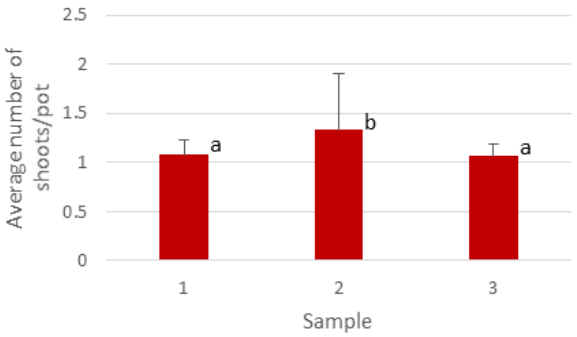

Figure 7: Number of vine or shoots per pot (mean ± St. dev).

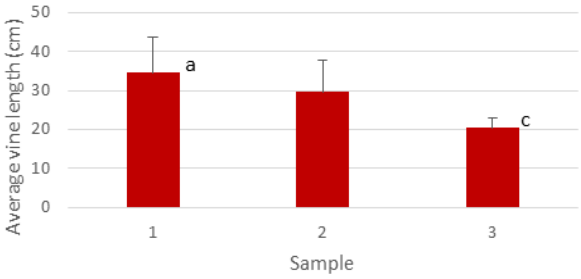

Figure 8: Length (cm) of non-senescent vines (mean ± St. Dev).

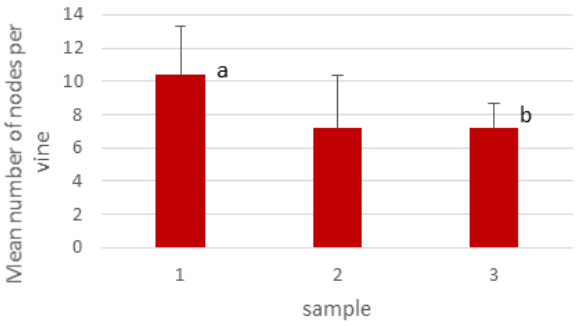

Figure 9: Number of nodes per vine (mean ± St. Dev).

The average number of nodes on non-senescent vines was variable between samples. The sample means were 10.4 ± 3.0, 7.2 ± 3.1 and 7.2 ± 1.4 nodes respectively. The calculated F, 102.41 where n =3 in each case, at $d_{2,6}$ exceeded the tabled value 5.14, $P < 0.05$. As such, the mean number of nodes was significantly different between samples. The Tukey value T = 0.350 indicated that sample $1 \neq 2$, $1 \neq 3$, $2 = 3$.

Discussion

It is interesting that 4"x6" potting bags may be used to produce yam tubers. While not large enough for consumption, they are useful and viable propagules. It shows the potential for increased size. Pot culture of yams is a novel concept to apply to Jamaica yams especially the *D. cayenensis* var. Yellow yam or similar varieties with very large tubers. Yam is traditionally grown in yam mounds or hills started to digging out deep pits in anticipation of a huge tuber and fixing a pole for vines to run [26,27]. Pot culture is herein proposed as one possible alternative to regulate tuber size (mass). This is currently achieved by manipulating the spacing between plants or varying the volume of the growing vessel. Conventionally yams are grown in raised mounds, and raised beds or ridged in open field but production under protected cover and commercial shade-houses has not been explored.

It has been a novel observation that increasing minimum daily temperature is associated with mini-tuber induction. The regression analysis determined that the change in temperature as well as the expected change in day length was significant. It is well established that short day length is a trigger for tuberization. There was no significant change in mean daily temperature so this is unlikely to be a major factor. However, the effect of minimum or lowest daily temperature on tuberization has not been hitherto reported in literature. Research is now required to investigate the effect of temperature both at fixed short and fixed long day length. If biotechnology is to be optimally exploited to grow yams all year round then the effect of the lowest day time temperature may warrant closer investigation.

Ideally tissue culture yam plants are grown in protected under cover facilities to produce tubers which may be significantly reduced in size compared with yields from conventional tubers or mini-setts. It is these seed tubers or generation zero (G_0) material that is used for field planting to generate commercial yields. Unwittingly, TC yam plants are used for direct field planning and do not survive because they were not intended for direct field planting. The investigation has shown that even in immature plants, not ready for field planting, with insufficient biomass for rooting, tuberization may be initiated by shortened day length, consistent with literature [28].

One goal of artificial manipulation of tuberization will be uniformity of the process. Even within narrow margins, mean number of shoots (1.0 - 1.3), which was very negligible, statistically there were significant differences between groups. Also, unrealistically number of nodes ranging from 7 - 10 and vine length 20 – 30 cm were significantly different. These were due to the controlled environment of the investigations and the construct of the ANOVA. Otherwise, to the average onlooker these would be normal uniform observations.

The idea of all year round production is not novel. The constraints are well articulated in literature [29]. With the availability of more advanced biotechnology techniques, it is becoming more foreseeable. The following table (Table 7) summarizes four relevant components with potential for improvement from biotechnology interventions: – the conservation of all available germplasm, virus elimination where required, under cover protected *ex vitro* bulking of materials, and under cover protected growing of yam.

Conclusion

The results herein was a demonstration of a natural phenomenon of tuberization by both *D. alata* and *D. cayenensis* yams at SRC even in immature weaned tissue culture plants. There was no use of plant growth regulators (PGRs) and the frequency of tuberisation was high. The relevant biotechnologies exist to optimize frequency, size and mass of these miniature tubers as viable multiplication propagules. As SRC continues its yam *in vitro* germplasm conservation in the Caribbean and provision of yam propagation services via local government agencies RADA, it is also looking to the future. This is a vision to meet increased

Table 7: Potential for Biotechnology Interventions in Yam Production.

	Activity	Key Function	Techniques/methods
1	Collection of yam accessions (quarantined conservation)	Disease diagnostics	Morphological symptoms; ELISA; PRC kits for DNA/RNA isolation and comparison with known libraries
		DNA fingerprinting	DNA extraction, PCR amplification; random amplified polymorphic DNA analysis (RAPD), Amplified fragment length polymorphism (AFLPs). Microsatellites, SSR
		Transportation	Temperature and humidity controlled chambers; sanitization
		Physical isolation	Insect proof netting
2	TC for Virus elimination indexing (bulking and *in vitro* gene banking)	Virus elimination	*In vitro* meristem tip culture; heat treatments ; see disease diagnostics above
		Rapid multiplication	*In vitro* shoot –tip and nodal cultures
		Storage/conservation	*In vitro* maintenance on modified slow growth media; reduced temperature
3	Explant propagation in under cover protected environment	Weaning and hardening	Semi Auto Trophic Hydroponics, humidity/misting bins
		Ex vitro propagation	*Ex vitro* rooting of cuttings, with and without auxins
		Maximizing propagation from tubers	Mini setts for in vitro initiation or *ex vitro* bulking
4	Yam production in under cover protected environment	Foliage generation phase	Modified photoperiod – long days
		Tuber production phase	Modified photoperiod – short days

demand for by facilitating production all year round. In addition, SRC will provide the necessary advanced disease diagnostics and mitigation strategies.

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