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Storage and Germination of "Akam" Aerial Tubers in Jamaica *Dioscorea alata* var. Renta yam

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ABSTRACT

Akam or aerial bulbils of Renta yam remained viable seven months after harvest without treatment. Half of the sample had individual tuber mass less than 5.5 g; approximately 20 % had mass 5.5 -10.5 g, 20% between 10.5 – 20.5 g and 10% between 20.5 – 40 g. Principal component analysis (PCA) showed that four main factors accounted for differences in viability after storage. These included ability to form roots, mass or size, ability to sprout and the how linear the shape of tubers was. Mean germination of tubers ranged from 84.75 ± 5.5 to 100 % among the four weight classes. Tubers heavier than 20.5 g had significantly higher germination (P=0.05). There was no difference in the number of emerging shoots between mass classes (1.0 ± 0.1 to 1.9 ± 1.0). There was also no significant differences for vine length (24.08 ± 12.16 to 96.10 ± 35.08 cm), shoot fresh weight (SFW) (4.35 ± 1.75 to 14.35 ± 4.65 g) and number of mini-tubers (0.85 ± 0.1 to 1.9 ± 1.0). However, the linear relationship between these parameters and tuber mass were all highly significant at P = 0.05. PCA identified three components that accounted for 90.2% of the variance in the shade-house grown tubers. The most important component PC1 described foliage parameters. PC2 described de novo regeneration of shoots and roots. PC3 related to determinants of tuber yield.

Keywords

Jamaica yam, Aerial tubers, Miniature tubers, Long-term storage, Germination.

Introduction

Aerial tubers or bulbils are miniature carbohydrate storage organs formed in leaf axils or at nodes of yam vines of certain varieties. They are produced in response to various cues of environmental stress [1]. Reportedly rare in nature [2], bulbils may not be so common in the wider Caribbean yam germplasm. They are however, well known among Jamaican farmers growing *Dioscorea alata* var. Renta yam. In the local patois, these miniature tubers are called "akam". Apart from insufficient size, whether they are edible is debatable. Their greater significance is their use in vegetative reproduction and an alternative means of dispersal. Analogous to seeds they show dormancy but germinate when conditions are suitable the subsequent growing season [2,3]. Bulbils represent a strategy of asexual reproduction that allows more of the harvestable yield to be marketed for consumption and not diverted for planting material.

Culturally and traditionally Jamaicans are partial to hard yam varieties like yellow yam (*Dioscorea cayenensis*) in preference to the soft yam varieties. Renta is a soft yam and like most originate from Asia [4]. It is an old popular cultivar and has shown resilience by its continual presence today, evolved through virus and anthracnose outbreaks. Archived material from the Caribbean Food Crop Society Conferences in early 70's cited Renta yam as unique to Jamaica and among top ten yam varieties in 60s and 70s, accounting for 15% of total production [5,6]. Renta is commercially significant in Jamaica and it is also grown in St. Lucia where is is called Wild Yam. There used to be a Renta yam

in Tobago 1960-70s but it was thought to be different from the Jamaica variety [5].

Recent local research on Renta yam has been limited mainly to the chemical investigations. These included starch characterization [7]. There was also interest in composition and anti-nutritional factors [8]. The research in phytochemical content and potential in the treatment of diabetes supported medicinal and possible pharmaceutical exploitation [9,10]. Apart from that, recent reports on Renta yam in Agricultural research are quite obscure.

Jamaica national yam germplasm held in international genebanks (CIPS, IIITAs, CIAT etc.). It is however felt that there must be viable local repositories to access germplasm. *In vitro* initiation and gene banking of Renta yam is part of germplasm collection and conservation at the Jamaica Scientific Research Council (SRC) as material becomes available. List of chemicals to enhance bulbil sprouting and breaking dormancy are in publication [11,12]. The research herein does not use plant growth regulators for germination. The purpose of the research was to gather benchmark or baseline data to develop competencies in long-term storage and germination of yam micro and mini tubers.

Methodology

Bulbils (82) were harvested from Renta yam vines growing at Brown's Hall, St. Catherine, an area of relatively higher elevation (Google map coordinates 19.057375, -77.126622), 7 January 2024. They were stored for 6 months, 25 ± 2 °C in a non-woven recyclable bag at ambient room conditions at the Biotechnology Department, Scientific Research Council, Kingston, Jamaica (Google map coordinates 18.0189,-76.7497). Data was collected for each of 82 pre-sprouted bulbils for 20 data fields (Table 1). The Microsoft® XL spreadsheet data was used in IBM SPSS version 30.0.0.0 (172) for Principal Component Analysis (PCA) to determine the main factors that account for differences between bulbils at pre-sprouting.

A frequency distribution graph was prepared to show the diversity in bulbil masses post storage among eight arbitrary mass classes with a class interval of 5 g. This was used to generate four suitable bulbil mass classes to be used in a randomized block design ANOVA pot trial with four different weight classes. Data was collected after 12 weeks. The ANOVA tested the hypotheses of no difference in the means between the four weight classes. The following parameters were investigated: - germination % (using arc sin transformed data); number of shoots to emerge from each aerial tuber (log x+1 transformation); total vine length (log x transformation); shoot fresh weight (SFW) (non-transformed data); and number of mini-tubers at 12 weeks (log transformed data). Data for each parameter was regressed on the mass of each weight class and the significance of the regression coefficient determined using the *t*-test. The data fields (15) of records collected at 12-week harvest were used in PCA to determine the main differences between aerial tubers post germination (Table 2).

Table 1: Data recorded from bulbils at pre-sprouting.

#	Parameter (trait)	Remarks
1	ID #	
2	Bulbil mass	
3	Bulbil shape	
4	Description of bulbil end	
5	Volume of bulbil	
6	Density of bulbil	Derived
7	Length of bulbil	
8	Width of bulbil	
9	Quotient of length/width	Derived
10	Pre-sprouting	Presence or absence
11	Number of sprouted shoots	
12	Sprouted shoot length 1	
13	Sprouted shoot length 2	
14	Total shoot length	Derived
15	Number of roots	
16	Length of longest root	
17	Length of second longest root	
18	Length of third longest root	
19	Total root length	Derived
20	Average root length	Derived

Table 2: Data recorded from bulbils 12 weeks after germination.

#	Parameter (trait)	Remarks
1	ID #	
2	Mass of bulbil	
3	Density of bulbil	
4	Shoot emergence	Presence or absence
5	Total shoot length	
6	Number of micro-tubers	
7	Total number of nodes	
8	Total number of nodes/shoot	Derived
9	Total number of leaves	
10	Total number of leaves/shoot	Derived
11	Total fresh weight	
12	Average shoot fresh weight	Derived
13	Original tuber mass	
14	Total new tuber mass	
15	Total tuber mass	

Results and Observations

Akam or aerial bulbils of Renta yam remained viable seven months after harvest without any special treatment at ambient temperature in a non-woven recyclable bag (Figure 1). Almost half the bulbils collected weighed less than 5 g; approximately 20 % had mass 5 -10 g, 20 % between 10 - 20 g and 10 % between 20 – 40 g (Figure 2). The frequency distribution was used to select tubers for sufficient material for the replicated ANOVA. The class intervals were adjusted for the ANOVA trial (Figure 3).

Figure 1: Renta yam bulbils prepared for pre-sprouting.

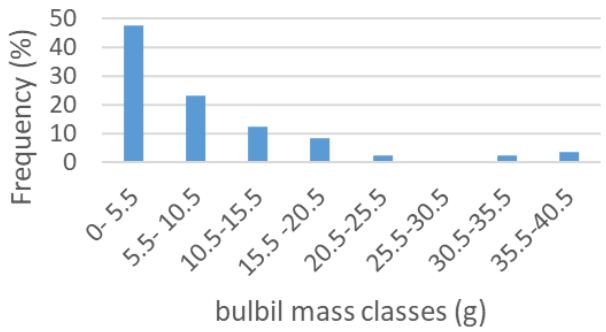

Figure 2: Frequency distribution of bulbil masses in original sample.

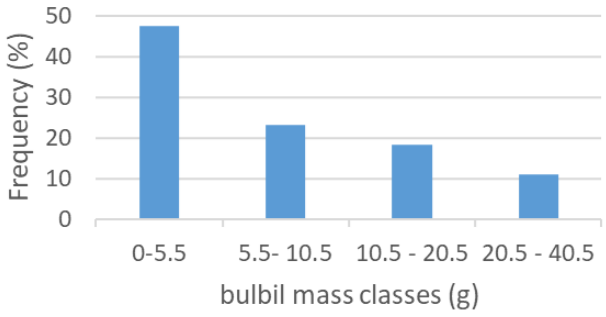

Figure 3: Adjusted class intervals and frequency.

In the PCA of the data on viability of aerial tubers after storage (Table 1), removal of fields for tuber shape, description of tubers ends, density and length of second longest shoot resulted in higher extraction values ≥ 0.7 for the remaining traits (Table 3). Four principal components explained the observed variance between aerial tubers at the sprouted stage. These components accounted for 89 % of the observed variance (Table 4). Each represented traits that are themselves correlated and inherted disticntly from those of other components.

Communalities

	Initial	Extraction
mass (g)	1.000	.963
volume (cm3)	1.000	.915
length (mm)	1.000	.956
width (mm)	1.000	.932
length/width	1.000	.923
pre-sprouted*1	1.000	.952
# sprouts	1.000	.957
shoot length 1 (mm)	1.000	.895
total shoot length (mm)	1.000	.886
# roots	1.000	.791
root length 1*2	1.000	.750
root length 2	1.000	.815
root length 3	1.000	.692
tot. root length (mm)	1.000	.953
av. Root length (mm)	1.000	.953

Extraction Method: Principal Component Analysis.

Table 3: Table of Communalities.

The first principal component PC1 correlated with traits exclusively related to root formation on sprouted tubers (Table 5). The second principal component PC2 correlated with the mass and three dimensional measurement of the aerial tubers. PC3 related to evidence of sprouting and PC4 related to shape of the tuber (ratio length to width). These factors were all independent of each other.

Sprouted tubers (Figure 4) germinated into vigorous vines in the shade-house (Figure 5) and produced mini tubers by 12 weeks. It was unclear if the original tuber had shrunk to form the second older tuber structure seen alongside a newly formed tuber initial (Figure 6), a vestigial tuber.

Tables 6 summarized the computations of F and showed that germination rates were significantly different between the four mini-tuber mass classes. The calculated value of F for the main effect (difference in weight) of 5.16 at 3, 9 *df* exceeded the tabled critical value of 3.86 at P = 0.05. As such, the difference in germination rates between weight classes was highly significant.

Table 4: Total variance explained.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	8.480	56.534	56.534	8.480	56.534	56.534	4.988	33.251	33.251
2	2.521	16.803	73.338	2.521	16.803	73.338	3.604	24.030	57.281
3	1.320	8.800	82.137	1.320	8.800	82.137	3.104	20.697	77.978
4	1.012	6.750	88.887	1.012	6.750	88.887	1.636	10.909	88.887

Figure 4: Sprouted Renta yam bulbils ready for pot planting.

Table 5: Rotated Component Matrix.

Rotated Component Matrix[a]

	Component			
	1	2	3	4
av. Root length (mm)	.941			
tot. root length (mm)	.941			
root length 1*2	.831			
# roots	.823			
root length 2	.811			
root length 3	.786			
mass (g)		.889		
volume (cm3)		.871		
length (mm)		.749		.510
width (mm)		.702		
pre-sprouted*1			.934	
# sprouts			.925	
shoot length 1 (mm)		.534	.648	
total shoot length (mm)		.550	.643	
length/width				.921

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 5 iterations.

Table 6: Germination ANOVA Summary Table.

Source of Variation	SS	*df*	s^2	F
Main effect (treatments)	3539.262	3	1179.754	5.155*
Blocks (Environmental gradient)	951.3072	3	317.1024	1.386
Within	2059.683	9	228.8536	
Total	6550.252			

-* significant at P = 0.05

Figure 5: Germinated Renta bulbils *circa* 12 weeks post emregence.

Table 7 showed the germination rates for the four classes of mini-tubers (means ± St. dev). The computed Tukey test statistic was T = 33.36. Using the arc sin transformed data the only pairwise comparison of means that exceeded T was the comparison between the germination means of mini-tubers 0 - 5.5 g and the germination means of mini-tubers weighing 20.5 – 40.5 g (Figure 7). There was no significant difference in germination between 0 -5.5 g tubers, 5.5 -10.5 g and 10.5- 20.5 g tubers.

Table 7: Germination rates per weight classes (Mean ± St. dev).

Weight class (g)	Mean	St. dev
0-5.5	84.75	5.5
5.5 - 10.5	83.75	11.1
10.5 -20.5	91.75	16.5
20.5 - 40.5	100	0

Figure 6: Renta new mini tuber ad vestigial tuber at 12 weeks.

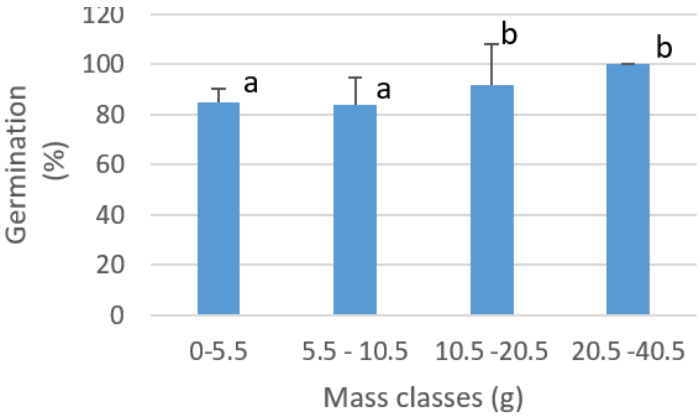

Figure 7: Differences in germination rates between mass classes.

In the significance test of the regression coefficient, $b = 0.605$ for germination rate plotted against mass, the calculated $t = 5.655$ exceeded the tabled value of 4.303 for 2 *df* at P=0.05. The linear relationship between germination and mass of tubers was highly significant (Figure 8).

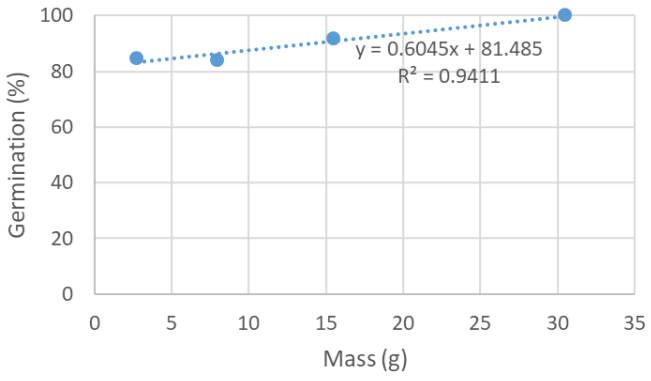

Figure 8: Regression of germination rate on mini-tuber mass.

There was no significant difference between the number of shoots emerging from the tubers with calculated F = 3.05, *df* 3, 9 (the tabled critical value was 3.86 at P = 0.05). Table 8 and Figure 9 however showed an increasing trend in the number of shoots with increasing mass despite F not being significant. The *t*-test for the significance of regression coefficient was $t = 4.89$ which exceeded the tabled value of 4.303 for 2 *df* at P = 0.05. The linear relationship between shoot number and mass of tubers was significant. The plot was similar to Figure 8 with the linear equation $y = 0.313x + 1.009$, $R^2 = 0.9$.

Table 8: Number of shoots per aerial tuber (Mean ± St. dev).

Weight Class (g)	Mean	St. dev
0-5.5	1.0	0.05
5.5-10.5	1.3	0.65
10.5-20.5	1.6	0.80
20.5-40.5	1.9	0.95

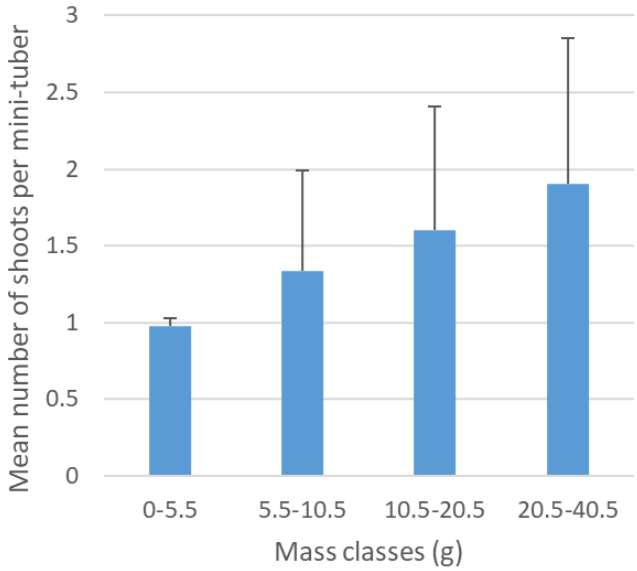

Figure 9: Differences in shoot numbers between mass classes.

The difference in vine length between the four tuber mass classes (Table 9) was shown to be not highly significant, with calculated F

= 1.301. The bar graph however showed a trend similar to Figure 9. The regression graph was similar to Figure 8 with y = 2.535x + 21.704, R^2 = 0.98. In the significance test of the regression coefficient, *t* = 9.32 at 2 df exceeded the critical tabled value 4.303 at P = 0.05. The linear relationship between vine length and mass of tubers was highly significant.

Table 9: Vine length (cm) (Mean ± St. Dev).

Weight class (g)	Mean	St. dev
0-5.5	24.08	12.16
5.5 - 10.5	43.93	22.50
10.5 -20.5	66.58	44.28
20.5 -40.5	96.10	35.08

There was no significant difference in SFW between the four weight classes. The calculated F = 3.34 at 3, 9 *df*. The fresh weights are illustrated (Table 10) with a visible trend in the bar graph similar to Figure 9. The significance test of the regression coefficient showed that *t* = 21.05. This exceeded the tabled critical value of 9.93, P = 0.01. The linear relationship between SFW and mass of tuber was highly significant. Similar to Figure 8 the linear equation was y = 0.354x + 3.460, R^2 0.996.

Table10: Shoot Fresh Weight (g) (Mean ± St. Dev).

Weight class (g)	Mean	St. Dev
0-5.5	4.35	1.76
5.5 - 10.5	6.63	2.99
10.5 -20.5	8.60	5.69
20.5 -40.5	14.35	4.65

There was no difference in the number of mini-tubers harvested between the four weight classes. The calculated F = 1.97 at 3, 9 *df*. The number of mini-tubers from lowest to highest weight class were 0.85 ± 0.1, 1.25 ± 0.44, 1.58 ± 0.56 and 1.9 ± 0.95 respectively. The bar charts mirrored the trend in Figure 9. The other trend in Figure 8 where the relationship between number of mini-tubers and mass was highly significant. The linear equation was y = 0.036x + 0.887, R^2 = 0.92.

In the assessment of post emergence data (Table 2), removal of data fields for density and ID ensured that all the remaining elements had an increased extraction value of ≥ 0.9 (Table 11). Three principal components explained 90.2 % of the differences between germinated tubers in the shade-house (Table 12).

The rotated component matrix showed that PC1 correlated strongly with the leaf to shoot ratio. This described all the factors related to foliage. PC2 correlated to the number of emerged shoots and number of mini tubers. This describes *de novo* regeneration. PC3 correlated with the mass of tubers formed (original and newly formed). These are the determinants of yield. Figure 10 illustrates the component plot in rotated space.

Communalities

	Initial	Extraction
mass (g)	1.000	.920
# emerged shoots	1.000	.929
total. Sh length (mm)	1.000	.858
# tubers	1.000	.911
tot. # nodes	1.000	.948
#nodes/shoot	1.000	.881
tot. # leaves	1.000	.939
#leaves/shoot	1.000	.910
tot. FW (g)	1.000	.865
av.sh FW	1.000	.871
original tuber mass (g)	1.000	.897
tot. new tuber mass (g)	1.000	.819
tot. tuber mass (g)	1.000	.981

Extraction Method: Principal Component Analysis.

Table 11: Table of Communalities.

Rotated Component Matrix[a]

	Component		
	1	2	3
#leaves/shoot	.927		
av.sh FW	.915		
#nodes/shoot	.912		
total. Sh length (mm)	.769		
tot. FW (g)	.750		
tot. new tuber mass (g)	.691		.557
# emerged shoots		.948	
# tubers		.932	
tot. # leaves	.630	.690	
tot. # nodes	.637	.680	
original tuber mass (g)			.918
tot. tuber mass (g)			.851
mass (g)			.846

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 5 iterations.

Table 13: Rotated Component Matrix.

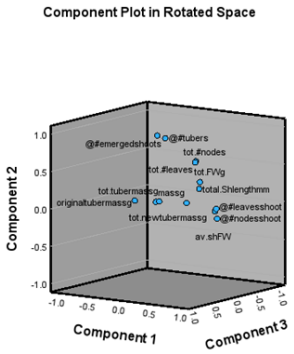

Figure 10: Component Plot in Rotated Space.

Table 12: Total Variance Explained.

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	8.329	64.070	64.070	8.329	64.070	64.070	5.350	41.151	41.151
2	2.119	16.303	80.373	2.119	16.303	80.373	3.202	24.631	65.782
3	1.280	9.846	90.218	1.280	9.846	90.218	3.177	24.436	90.218

Discussion

The Biotechnology Department at the Jamaica Scientific Research Council (SRC) annually produces rooted and weaned tissue culture yam plants of mostly local *D. alata*, *D. rotunda* and *D. cayenensis* varieties. The common observance is that regardless of photoperiod tissue culture plants form mini-tubers in the shade-house if kept for more than three months in pots. Where yam vines run along the surface of the potting substrate and roots establish, tuberization may occur. The findings from the germination experiment with Renta akam is useful in the understanding mini tubers.

Viability of mini-tubers whether less than or greater than 0.5 g appears not to be an issue. The findings validated that the only difference in germination rates comes with significantly larger tubers due to their additional resources. It is interesting that despite no significant difference in parameter means between tuber mass classes, these all have a linear relationship with mass. This is consistent with literature reports where larger size setts exhibit better sprouting, shoot growth and yield [13,14].

The PCA identified tuber mass as significant in the success of germination. It was an oversight that measurements were not recorded for the three zones on tubers as described by Ezeike [14]. More useful information may have been provided in the PCA if here was an estimation of weight loss during storage.

Intuitively there are differences between the subterranean mini-tubers normally encountered at SRC in pot cultures and the aerial bulbils investigated from Renta yam. Among them is an outer physical protective structure on akam that mitigates desiccation. There are therefore implications for engineering a coating for mini-tubers. Coating in artificial substrates comprising a biodegradable polymer may reduce drying out and enhance viability of the mini-tubers. Similar polymers like alginate matrices, hydrogels and polyoxyethlene are described in literature [16,17].

The research findings from the PCA indicate a correlation between root formation and tuber viability. There is lots of research on use of auxins in rooting vine cuttings but the correlation of storage of tubers with root formation suggests a role for external applications of auxins in enhancing mini-tuber storage viability. The results in literature may be species specific with contradictory reports as well. The reports speak specifically to inducing sprouting [18,19]. Once sprouting occurs in yams, root formation naturally follows. It is a well-established idea that the growth cycle of yams is under hormonal control. As such, any chemicals that negate the effects of GA3 and ABA may be useful to apply to tubers [20].

Dormancy has a role in the organoleptic quality of the yam [21]. This was not a consideration in the activities investigated. While *in vitro* micro-tubers reportedly do not exhibit dormancy, it is uncertain if mini-tubers in the shade-house show dormancy. There is speculation that in field grown yams, tubers may be dormant well before harvest and hence ready to sprout earlier than anticipated [21].

Conclusion

Whereas there may be physiological, morphological and most importantly size differences between the aerial tubers investigated and subterranean tubers normally encountered at tissue culture plants *in vitro* and in the shade-house, certain similarities exist. Most importantly the capability to achieve 80 - 100% germination in mini-tubers without chemical amendments is notable. The difference in germination rates are not significant between tubers less than 5.0 g up to 20 g. Differences are only visible with masses over 20 - 40 g. Observations are consistent with current literature where many of the growth parameters show a significant linear relationship with tuber mass.

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