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The Response of White Yam (*Dioscorea rotundata* Poir) Tuber Portions to Positive Selection for Quality Seed Yam Production

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Abstract

The production of yams is constrained by high cost and unavailability of clean planting materials, pests and diseases. Vegetative propagation has also caused a build of up diseases, reported to cause up to 80% yield reduction due to scarcity of quality declared seeds. Planting of disease-free material has been found to be effective in reducing disease problems in plants. This study was conducted to produce clean seed yam by reducing yam diseases through positive selection method. In this method, apparently healthy yam plants were identified, tagged (positive selection), assessed for yam mosaic virus incidence and severity using the scale 0 or 1 and 1-5 respectively. The harvested clean tubers from tagged plants were planted the following season so as to determine the rate of response of each genotype and tuber portion to positive selection. At the end of two cycles of positive selection, analysis of variance for percentage number of positive yam plants, YMV incidence, YMV severity and tuber yield shows significant difference ($p \leq 0.05$) between positive selection and no selection for both field and screen house plants. It was observed that positively selected plants performed significantly ($p \leq 0.05$) better than no selection plants. For field experiment, Number of positively selected plants was highest in positive TDr89/02665 with mean value of 75.00% while no selection Ogoja had the least number of positive plants (17.8%). A yield increase of 1.80 t/ha was recorded due to the application of positive selection method in two cycles. For screen house experiment, result followed a similar trend; however with a reduction in YMV incidence and severity. It is worthy to note that tail portions of yam were lest infected with YMV, hence more healthy plants were selected from the tail portion.

Keywords: Positive selection; Yam mosaic virus; Tuber portions

Introduction

Yam is a tropical tuber crop with many species and it belongs to the genus Dioscorea and family Dioscoreaceae. The crop is vegetatively propagated and millions of people in Africa depend on it for food (carbohydrate) each day [1]. Yam also plays a significant role in the socio-cultural lives of people in some producing regions like the celebrated New Yam Festival in West Africa [2,3]. Yams are grown on 5.36 million hectares in about 47 countries of the world with Nigeria as the leading world producer [1]. Yam production is endangered by many problems such as unavailability of quality planting materials, cost of seed yam, cost of labour, weeds, storage problems, pests and diseases [4]. Pest and diseases reduces tuber yield and quality up to 80%. Yam viruses also reduce tuber yield and quality. Yam mosaic virus (YMV) is one of the most economically important viruses infecting yam and is found in all yam growing regions with high prevalence in humid region reaching 80% [5]. Yam mosaic viruses have been reported to be widespread in all yam producing countries around the world [6]. As yams are cultivated through vegetative planting materials (whole yam tubers or setts) accumulate viruses over time, management of YMV is best implemented through the use of certified healthy planting material. Generally, the control of yam diseases has been extensively studied, and several control measures have been recommended but there has not been extensive work done on positive selection as a method of quality and healthy seed yam production.

Positive selection is a method in which healthy looking plants in the field are identified, tagged and tubers from identified plants are used for propagation [7]. Planting of disease-free plant materials has been found effective in reducing disease problems [8,9] and this method has been used to produce quality and disease free potato [10]. The objectives of this study is to determine the relative response of yam genotypes and tuber portions to positive selection for sustainable and quality seed production.

Materials and Methods

Research sites

The experiment was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan (7°26'N, 3°54'E) a rainforest-savanna transition zone.

Experimental design

A randomized complete block design (RCBD) with three replications and 24 treatment combinations was used. Yam (Dioscorea rotundata) genotypes that were used are Ogoja, Meccakusa, Danacha and TDr89/02665. They were obtained from the yam breeding unit of IITA. Each whole tuber was cut into three equal portions-Head, Middle and Tail. These portions were later cut into minisetts of 40 g and treated with lambda-cyhalothrin 5 EC at 2.5 ml/1 and dithiocarbamates at 7 g/1 of water, dried under shade for a day before planting on 6 m ridges at a spacing of 30 cm within rows and 1 m between rows. Weeding was done using hand held hoes and plants

were staked 3-4 weeks after field planting, using the trellis method. Four months after planting (MAP), apparently healthy yam plants were identified and tagged using ribbons (positive selection-Figure 1a). The identified yam plants were assessed for yam mosaic virus (Figure 1b) using the scale of 1-5 [11] and YMV incidence on the scale of 0 or 1 [11]. Samples of yam leaves were screened in the laboratory to detect YMV and confirm visual/phenotypic assessment using polymerase gel resolution test. Eight MAP, tagged yam plants were harvested separately from untagged yam plants and the tubers harvested from selected yam plants were assessed for presence of nematode using scale 1-5 [12]. Infected tubers were discarded living behind apparently healthy tubers. The resulting clean tubers (positively selected) were planted the following season using the same experimental design. However, planting was done both in the field and screen house. The screen house experiment was laid out in Completely Randomized Design (CRD). Mean severity of yam mosaic virus was calculated as described by Seruwagi [11] in which case only diseased plants were considered.

Figure 1: (a) Selected (tagged) healthy yam plant: (Positive selection).

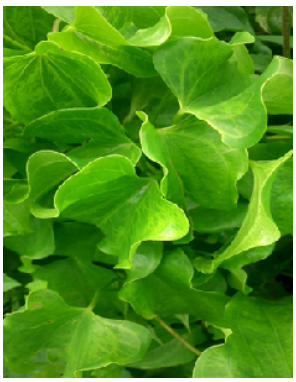

Figure 1: (b) Yam plant infected with yam mosaic virus.

Data collection

Data collected includes Days to 50% sprouting, Sprouting percentage, No of positive plants selected 4 MAP, Yam Mosaic Virus (YMV) incidence (scale 0 or 1), YMV severity (Scale 1-5), Tuber fresh weight, Weight of selected tubers, Weight of Non selected tubers, Tuber crack (Scale 1-5), Dry rot, Tuber gall.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) for individual experiments with the windows version of Statistical Analysis System (SAS). Where means were significant, they were separated with the least significant difference (LSD) at $p \leq 0.05$.

Results and Discussion

At the end of third generation, second cycle of positive selection, analysis of variance for percentage number of positive yam plants, YMV incidence, YMV severity and fresh tuber weight shows significant difference ($p \leq 0.05$) between positive selection and no selection field plants both for genotypes and tuber portions as shown in Tables 1 and 2 respectively. Days to 50% sprouting, sprouting percentage, tuber crack (Scale 1-5), dry rot, tuber gall were not significant. It was observed that positively selected plants performed significantly ($p \leq 0.05$) better than no selection plants. The population response of filed plants to selection as represented in Table 1 shows that positively selected plants were significantly higher in cycle 2 with mean value 55.00% than non-selection plants (28.06%) also, the mean value for no-selection in cycle two (28.06%) was slightly lower than its value in cycle one (30.28%). Fresh tuber weight for positive plants (7.05 t/ha) in cycle 2 was significantly higher ($p \leq 0.05$) and different from no-selection plants (5.25 t/ha).

	Cycle 1		Cycle 2		
Traits	NS	PS	NS	PS	LSD
% Positive plants	30.28a	44.58b	28.06a	55.00c	4.84
% YMV incidence	50.14b	34.72a	58.61c	30.56a	5.50
YMV Severity	2.94d	2.49b	2.75c	2.25a	0.17
Yield (t/ha)	4.99a	6.03b	5.25ab	7.05c	0.95
Nematode (Crack)	2.38a	2.31a	2.39a	2.25a	0.22

Table 1: Population responses of field plants to % positive plants, % YMV incidence, YMV severity, yield and crack in two cycles of positive selection. Means with the same alphabet along rows are not significantly different at p= ≤ 0.05 LSD (0.05). NS=No Selection, PS=Positive selection.

Traits	Cycle	Selection	TDr89/02665	Danacha	Mekacusa	Ogoja	LSD
% Positive Plant	1	NS	56.11a	20.56a	26.67a	17.78a	

		PS	70.56b	31.67b	41.11b	35.00b	
	2	NS	47.22a	21.67a	25.00a	18.33a	
		PS	75.00b	45.00c	55.00c	45.00c	
							9.67
% YMV Incidence	1	NS	30.00b	55.56b	53.33b	61.67b	
		PS	17.78a	41.67a	41.11a	38.33a	
	2	NS	45.00c	61.11b	58.89b	69.44b	
		PS	18.33a	37.22a	32.78a	33.89a	
							11.07
YMV Severity	1	NS	2.52c	3.49b	2.75b	3.01c	
		PS	2.15ab	2.82a	2.49ab	2.52ab	
	2	NS	2.35bc	3.26b	2.58b	2.79bc	
		PS	1.92a	2.56a	2.28a	2.25a	
							0.33
Yield (t/ha)	1	NS	6.05a	4.75a	5.18a	3.98a	
		PS	7.92ab	5.16a	5.18a	5.85ab	
	2	NS	6.19a	5.26a	5.17a	4.39a	
		PS	8.73b	6.05a	6.53a	6.91b	
							1.90

Table 2: Means for the response of yam genotypes (field plant) to % positive plants, % YMV incidence, YMV severity and yield in two cycles of positive selection. Means with the same alphabet along column are not significantly different at p= ≤ 0.05 LSD (0.05). NS=No Selection, PS = Positive selection.

Effect of selection within varieties for two cycle's (Table 2) shows that number of positively selected plants was highest in cycle 2 positive TDr89/02665 with mean value of 75.00% while no selection Ogoja had the least number of positive plants (17.78%). TDr 89/02665 positive plants had 75% healthy plants which are significantly different from TDr 89/02665 no selection with 47.22% of healthy plants. Yam plants were infected with yam mosaic virus (YMV) and percentage incidence was highest in no selection Ogoja (69.44%) and least in positive TDr89/02665 (17.78%), the severity of YMV was more in no selection Danacha with mean severity of 3.49. No significant different ($p \leq 0.05$) was observed for most variety between positive selection and no selection methods within species although tuber yield for positive selection were slightly higher than no selection. Tuber yield was highest in positive TDr89/02665 (7.92 t/ha) and least in no selection Ogoja (3.98 t/ha).

The effect of selection within tuber portions for two cycles also varied significantly ($p \leq 0.05$) with respect to percentage number of positive plants, YMV incidence, YMV severity and fresh tuber weight as represented in Table 3. Positive tail portion in cycle 2 had the highest number of positively selected yam plants with mean value of 62.08% while it was least in cycle 1 no selection head (26.67%). Generally, YMV incidence and severity were higher in head portion than other tuber portions.

Traits	Cycle	Selection	Head	Middle	Tail	LSD
% Positive Plant	1	NS	26.67a	29.58a	34.58a	
		PS	38.75b	40.42b	54.58b	
	2	NS	23.33a	26.25a	34.58a	
		PS	51.67c	51.25c	62.08b	
						8.38
% YMV incidence	1	NS	58.33c	42.92b	49.17b	
		PS	47.92b	34.58ab	21.67a	
	2	NS	67.08c	54.58c	54.17b	
		PS	35.83a	30.83a	25.00a	
						9.53
YMV Severity	1	NS	3.069	2.958	2.799	
		PS	2.681	2.5	2.299	
	2	NS	2.937	2.832	2.467	

		PS	2.446	2.328	1.982	
						0.29
Tuber weight (t/ha)	1	NS	5.39a	4.45a	5.13a	
		PS	6.52ab	5.68ab	5.87a	
	2	NS	5.78ab	4.25a	5.72a	
		PS	7.19b	6.13b	7.84b	
						1.65

Table 3: Effect of selection within tuber portions for two cycles. Means with the same alphabet along column are not significantly different at p= ≤0.05 LSD (0.05). NS=No Selection, PS=Positive selection.

Data collected from screen house plants were also analysed and analysis of variance for percentage number of positive yam plants, YMV incidence, YMV severity and fresh tuber weight shows significant difference between positive selection and no selection methods for genotypes and tuber portions as shown in Tables 4-6. The results obtained followed a similar pattern with that of field plants. Population responses of screen house plants to selection in two cycles (Table 4) shows that percentage number of positively selected plants (74.4%) in cycle 2 was significantly higher than no selection (40.0%) also, fresh tuber weight in cycle 2 had a mean yield of 1.49 kg/plots which was significantly ($p \leq 0.05$) higher than no selection mean yield of 0.79 kg/plot. Selection within varieties (Table 5) shows that cycle 2 TDr89/02665 had the highest number of clean plants (84.4%) and it was significantly different from no selection plants with 53.3% clean plants. Fresh tuber weight was observed to be highest in cycle 2 TDr89/02665 positive plants with mean value of 1.75 kg/plot which was significantly different from cycle 2 no selection TDr 89/02665 plants with value of 0.99 kg/plot.

	Cycle 1		**Cycle 2**		
Traits	NS	PS	NS	PS	LSD
% Positive plants	45.0a	62.2b	40.0a	74.4c	7.00
% YMV Incidence	50.6b	37.2b	55.6b	25.0a	7.06
YMV Severity	2.08c	1.77b	2.29c	1.41a	0.30
Tuber Weight (kg/plot)	0.88a	1.11b	0.79a	1.49c	0.10
Nematode (Crack)	2.32b	2.25ab	2.32b	2.07a	0.21

Table 4: Population responses of screen house plants to positive selection in two cycles. Means with the same alphabet along rows are not significantly different at p= ≤ 0.05 LSD (0.05). NS=No Selection, PS=Positive selection.

Traits	**Cycle**	**Selection**	**TDr89/02665**	**Danacha**	**Mekacusa**	**Ogoja**	**LSD**
% Positive Plant	1	NS	66.7a	31.1a	55.6ab	26.7a	
		PS	82.2b	48.9b	64.4bc	53.3b	
	2	NS	53.3a	35.6ab	44.4a	26.7a	
		PS	84.4b	71.1c	77.8c	64.4b	
							14.01
% YMV Incidence	1	NS	33.3b	62.2b	44.4bc	62.2b	
		PS	15.6a	51.1b	35.6b	46.7a	
	2	NS	46.7b	55.6b	55.6c	64.4b	
		PS	15.6a	28.9a	20a	35.6a	
							14.12
YMV Severity	1	NS	1.45ab	2.52b	1.72ab	2.67b	
		PS	0.90a	2.23b	1.95b	1.20a	
	2	NS	1.93b	2.33b	2.27b	2.65b	
		PS	1.20a	1.46a	1.32a	1.65a	
							0.59
Tuber weight (kg/plot)	1	NS	1.28b	0.69a	0.96ab	0.59a	
		PS	1.51c	0.85a	1.09b	0.99b	
	2	NS	0.99a	0.67a	0.83a	0.67a	
		PS	1.75d	1.39b	1.50c	1.35c	

							0.21

Table 5: Means for the response of yam genotypes (screen house plant) to % positive plants, % YMV incidence, YMV severity and yield in two cycles of positive selection. Means with the same alphabet along column are not significantly different at p= ≤ 0.05 using LSD (0.05). NS=No Selection, PS=Positive selection.

Traits	Cycle	Selection	Head	Middle	Tail	LSD
% Positive Plant	1	NS	41.70a	41.70a	51.70a	
		PS	60.00b	56.70b	70.00b	
	2	NS	35.00a	40.00a	45.00a	
		PS	75.00c	68.30b	80.00b	
						12.13
% YMV incidence	1	NS	51.70bc	53.30b	46.70b	
		PS	40.00b	43.30b	28.30a	
	2	NS	61.01c	53.30b	51.70b	
		PS	25.00a	30.00a	20.00a	
						12.23
YMV Severity	1	NS	2.08b	2.24b	1.95bc	
		PS	1.89ab	2.04b	1.37a	
	2	NS	2.64c	2.05b	2.19c	
		PS	1.42a	1.34a	1.46ab	
						0.52
Yield (kg/plot)	1	NS	0.74a	0.92a	0.97a	
		PS	1.12b	0.89a	1.34b	
	2	NS	0.70a	0.79a	0.87a	
		PS	1.49c	1.28b	1.73c	
						0.18
Nematode (Crack)	1	NS	2.51b	2.26a	2.18ab	
		PS	2.29ab	2.19a	2.26b	
	2	NS	2.57b	2.26a	2.13ab	
		PS	2.00a	2.35a	1.86a	
						0.35

Table 6: Means for the response of yam tuber portions (screen house plant) to % positive plants, % YMV incidence, YMV severity, yield and crack in two cycles of positive selection. Means with the same alphabet along column are not significantly different at p= ≤ 0.05 LSD (0.05). NS=No Selection, PS=Positive selection.

In the screen house experiment, positive tail portion in cycle 2 gave the highest number of clean plants (80.00%) and it was significantly different from no selection tail portion with 45.00% clean plants. Percentage virus incidence and severity were highest in head portions and least in tail portions.

Field plants were compared with screen house plants to determine which environments will fast track positive selection (healthy plants) if planting materials is from the same source (Table 7). A significant difference ($p \leq 0.05$) was observed in percentage number of positive plants, YMV incidence and severity. From the experiment, it was observed that screen house plants performed significantly better than field plants. YMV incidence and severity were lower in screen house plants and hence more positive plants been selected from the screen house than were selected from field plants. It is worthy to note that the tail portion still gave the highest number of healthy plants.

		Field		Screen house		
Traits	Selection	Cycle 1	Cycle 2	Cycle 1	Cycle 2	LSD
% positive plants	NS	30.28a	28.06a	45.00a	40.00a	
	PS	44.58b	55.00b	62.22b	74.44b	
						6.73
YMV incidence	NS	50.14b	58.61b	50.56b	55.56b	
	PS	34.72a	30.56a	37.22a	25.00a	
						6.93
YMV severity	NS	2.94b	2.75b	2.09b	2.29b	
	PS	2.49a	2.25a	1.77a	1.36a	
						0.25

Table 7: Comparison of Field and screen house plants for the response of plants to % positive plants, % YMV incidence and YMV severity in two cycles of positive selection. Means with the same letter along columns are not significantly different ($p \leq 0.05$) using LSD (0.05). NS=No Selection, PS=Positive selection.

Correlation coefficient analysis in Table 8 showed the relationship between YMV incidence, % positive plants, YMV severity, yield (t/ha) and tuber crack. It was observed that number of positively selected plants were negatively correlated with YMV incidence and severity at $p \leq 0.01$ (-0.850^{**}, -0.622^{**}) while it correlate positively with yield (0.322^{**}).

	YMV incidence	% positive	YMV	Wt(t/ha)	tuber Cracks
YMV incidence	1				

% positive	-0.850**	1			
YMV severity	0.583**	-0.622**	1		
Yield (t/ha)	-0.118	0.322**	-0.261**	1	
Tuber Cracks	0.142	-0.197*	0.110	-0.203*	1

Table 8: Correlation coefficient for YMV incidence, % positive plants, YMV severity, yield and tuber crack. **. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level.

In addition to conventional/phenotypic assessment of disease incidence and severity, a novel real-time quantitative Polymerase Chain Reaction (PCR) assay (TaqMan technology) was used for the detection of yam mosaic virus and was also used to assess and confirm the result of phenotypic evaluation of disease incidence on experimental yam plants. Generally, phenotypic assessment agreed with PCR assessment up to 92.30%. On visual bases, six plants were assessed to be positive (healthy) and nine plants virus negative (diseased) but PCR showed that only five out of six plants were virus free which represents an accuracy of 83.33% (i.e., 5/6) for visual assessment. This show that about 16.67% of plants that were visually assessed to be free from virus may be diseased, that is at least 16.67% suffers from YMV latent infection. From the PCR result, it can be inferred that all plants visually assessed to be diseased were actually diseased since visual and PCR evaluation for disease were the same.

Discussion

It was observed that YMV incidence for landraces, no selection field plants (Ogoja, Danacha and Meccakusa) used for this experiment ranges between 53.33 to 69.44%. This agrees with the findings of [13]. In their study they found out that out of 300 D.rotundata leaf samples collected, 184 (61.3%) tested positive for YMV. This finding buttresses previous report of YMV as one of the most important yam viruses occurring in very high incidence and it is also widely distributed in yam fields throughout the world [14,15]. The high incidence of YMV observed in this study can be attributed to unselected farmers planting material that have accumulated viruses and diseases over several cycles of vegetative propagation and the exchange of yam germplasm between field and screen house plants accounts for YMV incidence in the screen house plants. These infected planting materials have continued to be distributed because of low awareness about the potentials of positive selection in reducing viral and disease load in yam and also the absence of sensitive and probably hand held reliable field diagnostic tools for yam viruses [16]. However, lower level of YMV incidence in screen house plants could be attributed to the fact that the movement of aphis that spreads YMV were restricted in the screen house. Danacha showed more YMV severity than other yam species while it was low in TDr89/02665. This is similar to what Agbaje [17] observed when they reported that the reaction of TDr89/02665 to leaf mosaic virus was low but severe in Danacha.

It was observed that the head portions were more infected with YMV hence shows higher percentage for incidence and severity. This probably suggest that as photosynthate flows down to the tuber, they carry along virus particles transferred to the plant by aphids and more of these particles settles in the head region of the yam tuber. Thouvenel and Fauquet [18] observed that YMV virus is transmitted mechanically by several aphid species in a non-persistent manner. The detection of these YMV viruses on non-symptomatic leaf samples (latent infection) shows that laboratory diagnosis serves as a more sensitive and conclusive way of affirming the health status of planting materials.

The observed positive correlation of percentage positive plants with yield is expected since healthy plants produces optimum assimilate which is translocated to the root and stored in tubers as starch. Positive and significant ($p= \leq 0.01$) relationship of yield with positive plant suggested that the tuber yield can be increased by simple selection of healthy plants. YMV incidence did not significantly reduce yield although it was negatively corrected but YMV severity reduced tuber yield significantly at $p= \leq 0.01$. This clearly shows that mild infection is not detrimental to plant yield but severe infection will reduce yield. Therefore, more work should be done in positive selection as an affordable and adaptable means of reducing YMV incidence and severity. Adeniji [19] observed that tuber yield in white yam (D. rotundata Poir.) could be reduced up to 92.8% if it is inoculated with YMV. Yam plants infected with YMV become unhealthy and chlorotic [11] and these plants will not produce optimally because of distorted chlorophyll content.

Positive selection may become a valuable technology for increasing and sustaining yield by yam producers. Yam yields in the trials plots were increased as a result of the use of positive seed yam selection. Based on the results from field and screen house trials, a yield increase under field conditions in cycle 1 is 1.04 t/ha (6.03 t/ha for positive selection-4.99 t/ha for no selection) and screen house condition is about 0.234 kg/plot (1.112 kg for positive selection-0.878 kg for no selection) while in cycle 2 is 1.80 t/ha (7.05 t/ha for positive selection-5.25 t/ha for no selection) and screen house condition is about 0.70 kg/plot (1.49 kg for positive selection-0.79 kg for no selection). This represent a yield gain of 0.76 t/ha (1.8 t/ha-1.04 t/ha) for every cycle. If positive selection is maintained, yam yield could be increased from its current production of 12 t/ha to its potential yield of 24 t/ha in 15 years. These yield increase could be attributed to increased number of healthy plants (positive selection) and more healthy plants were obtained from the tail portions of yam. As such, the technology may become effective notwithstanding the variation in circumstances. The effectiveness of positive selection in giving higher yields was observed in potato plots by Peter et al. [20]. They noted that positive selection plots gave an average yield of 14.2 t/ha which was significantly higher than the 11.8 t/ha for the farmer seed selection plots (no selection). It is important to note that yield increase could be obtained without additional financial investment, and this is of vital importance for poor smallholder farmers. The additional labour required for identifying and tagging the healthy yam plants is negligible.

Conclusion

In conclusion, farmers should consistently continue positive selection from the tail portions of yam over a number of seasons in the same yam plant population in order to assess the full potential of this technology to further increase the yield, reduce pest and diseases incidence and severity over several generations.

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