

Effect of plantain barrier plants on potyvirus-associated diseases in yam cultivation

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Abstract

Yam belongs to a very diverse plant genus (*Dioscorea* L.), comprising more than 600 species, both wild and cultivated. In Cuba, different species are distributed in the central and eastern regions, where the most widespread cultivars belong to the species water yam (*Dioscorea alata* L.) and white yam (*Dioscorea cayenensis* subsp. *rotundata* (Poir.) J. Miège). Among global major constraints facing the yam production areas are those caused by viral diseases. In this sense, potyviruses have the greatest economic impact, since they can cause losses of more than 50% in agricultural yields. In Cuba, the presence of yam mosaic virus and yam mild mosaic virus has been detected in commercial plantations by Enzyme-Linked ImmunoSorbent Assay (ELISA) tests. The movement of propagules between plantations without phytosanitary certification has facilitated the distribution of the viruses throughout the producing areas. Disease management involves a continuous process of events consisting of the selection and use of techniques aimed at reducing plant diseases to a tolerable level. Knowledge about the existing relationships between the host, virus, vector, and ecosystem is fundamental to the implementation of successful management. In the present work, by using potyvirus-free propagation material of two commercial yam cultivars and the perimeter protection with a live barrier based on plantain 'Burro CEMSA', it was possible to reduce the field incidence of viral diseases (average infection decreased from 60 to 15%). Likewise, the barrier avoided losses in agricultural yields ranging between 50–57% for both cultivars, compared to the farmer's usual practices.

Introduction

Yam (*Dioscorea* spp.) ranks fourth worldwide in production among roots and tubers, only surpassed by potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* L.) (Ejikeme and Matthew 2017). According to FAOSTAT (2020), world production reached 72.5×10^6 t, where West Africa produces more than 90% (Aighewi et al. 2020), and Cuba ranks twenty-first in the world (Pérez-Camacho and Raz 2017). In this sense, the Food and Agriculture Organization does not record the productions of China and India, so world production must be higher than the statistical data consulted.

Yam tubers are an excellent source of carbohydrates and contain vitamins such as thiamine, riboflavin, niacin, ascorbic acid, and carotene. In addition, they possess many of the essential amino acids and minerals needed in the human diet (USDA NAL 2017), with low-fat contents, so it is considered an indispensable food for much of the population in tropical regions (Padhan and Panda 2020). In Africa alone, about 300×10^6 people incorporate more than 280 calories daily (Darkwa et al. 2020).

Viral infections decrease tuber yield and quality, thus threatening crop sustainability. Up to eight potyviruses have been described in yams (Dey et al. 2019; Luo et al. 2022). Potyviruses are transmitted non-persistently by many aphid species (Revers and Garcia 2015). This type of transmission is characterized by very short acquisition and inoculation periods because they are rapidly lost by the vector within a matter of a few hours after acquisition (Revers and Garcia 2015). These viruses are

acquired by the vector following exploratory tests on the epidermis by the aphids after landing in the crop, which lasts less than 1 min. They are also rapidly inoculated after an intracellular test in the epidermis (Fereres and Moreno 2009).

The application of chemical products (insecticides) has not been shown to be very effective in preventing non-persistent transmission of viruses, as aphids can spread them before they are eliminated (Hooks and Fereres 2006). However, aphids prefer the edges of plantations attracted by the contrast between their green color and the color of the soil and immediately begin to insert their stylet to determine whether or not the plant is their host (A'Brook 1968). If the aphid carries the viral particles on its stylet (typical of non-persistent transmission) and lands and tests on a non-host plant of the virus in question, it quickly inoculates it and also loses the virus, resulting in a virus-free aphid.

The use of barrier (non-host) crops has been suggested as a cultural control option to reduce the natural spread of non-persistent viruses in the main host crop. In this sense, Hooks and Fereres (2006) proposed four hypotheses to explain the effect of barrier plants *i.e.* virus sink (tall plants protect the main crop by acting as a natural sink for non-persistent viruses), physical barriers (plants slow the spread of the virus by blocking the arrival of the winged aphids to the main crop), host plant camouflage (high density of intercropped (non-host) plants hinders plant-to-plant transmission, reducing the spread of the disease), and trap crop (a secondary plant species is used that is more attractive to the target pest than the neighboring primary crop).

The use of barrier crops has made it possible to reduce the spread of diseases caused by insect-transmitted viruses in a non-persistent manner. In potyviruses, this practice has been successfully applied in potato, pepper (*Capsicum annuum* L.), squash (*Cucurbita pepo* L.), and papaya (*Carica papaya* L.) (Avilla et al. 1996; Manandhar and Hooks 2011; Cabera Mederos 2013), where sorghum (*Sorghum* spp.), maize (*Zea mays* L.), and sunflower (*Helianthus annuus* L.) have been used as barrier crops (Hooks and Fereres 2006). In yam, this practice has not been reported for potyvirus control, nor has the use of plantain barrier plants from seedlings. The objective of the present work was to determine the influence of plantain barrier plants cultivar 'Burro CEMSA' (ABB) on potyvirus transmission and yield of yam in the field.

Materials and Methods

Establishment of plantain barrier plants

To determine the influence on potyvirus transmission, a perimeter live barrier of plantain cultivar 'Burro CEMSA' was established in August 2015, using calibrated commercial propagules (B-caliber basic seed) (MINAG 2012), covering an area of 0.5 ha (Fig. 1). A planting frame of 1.5 m between rows and 1.5 m between plants was used, running the planting points 0.5 m between rows (false stagger). An entrance of 4 m was left with three rows 10 m long, acting as a trap directly in front of the entrance. Free tillering was allowed in the direction of the furrow. All cultural attentions were carried out according to the

technical instructions for the cultivation of bananas and plantains (*Musa* spp.) (MINAG 2012). In March 2016, mini-tubers of the cultivar 'Guinea' (*Dioscorea cayenensis* subsp. *rotundata*) were planted and seedlings of the cultivar 'Belep' (*Dioscorea alata*) were transplanted, both free of potyvirus by Antigen Coated Plate (ACP)-Enzyme-Linked ImmunoSorbent Assay (ELISA). Twenty-four plants (six groups of four plants) of each cultivar were used (T1: virus-free planting material plus protection inside the barrier and T2: virus-free planting material without barrier protection). As a control, the same number of plants of both cultivars were planted from seeds obtained by traditional methods from plants coming from production areas (T3: planting material without virus certification and without barrier protection). A completely randomized block design with four replications was used. The distance between yam plots and the plantain barrier was always greater than 3 m. Cultural practices were carried out according to the technical instructions for yam cultivation (MINAG 2012). A total of eight plots of each treatment (T1, T2, and T3) and two yam cultivars were used for each treatment (Fig. 1).

Detection of yam infected plants

To determine the infected plants in the field from potyvirus-free planting material (mini-tubers of cultivar 'Guinea' and seedlings of cultivar 'Belep'), symptoms were observed from 30 days after planting (dap) every two weeks. Symptomatic plants were marked for further evaluations and the status of plants that remained asymptomatic, throughout the crop cycle, was verified by ACP-ELISA analysis at 270 dap (January 2017). All yam crop evaluation was repeated between March 2017 and January 2018.

For the detection of potyviruses in the yam collected samples, an ELISA diagnostic system was used, with the ACP-ELISA variant, which uses a generic monoclonal antibody for potyviruses (DSMZ, Germany), following the manufacturer's recommendations, with two replicates per sample. After the enzymatic reaction and incubation periods with the aforementioned antibody, absorbance was determined at 405 nm on a Biotek® ELx-800 automated plate reader (BioTek Instruments, Inc., Winooski, Vermont, USA). The cut-off limit, for the determination of the presence of potyvirus, was calculated as twice the average of the absorbance values of the negative controls. This methodology was used throughout this investigation for all asymptomatic samples collected and for 25% of the samples showing viral symptoms. The percentage of infected plants was determined and statistically analyzed with Student's t-test for independent samples.

Identification of aphids

To capture aphids, five Moericke traps were placed at ground level inside and five outside the living plant barrier. Sampling was carried out every 15 days. The collected aphids were preserved in 70% ethanol and transferred to the laboratory for identification. The insects were rinsed in a 10% (m/v) KOH solution for 24 h and washed twice for 10 min in distilled water. An optical microscope (200x) (Olympus, Japan) was used to observe the morphological characters of the aphids, and species identification was performed according to Navarro and García-Marí (2014).

Production per plant and field yield estimation

Tuber mass at harvest was determined using a handheld digital balance (Wei Heng, China). The percentage of losses avoided by using potyvirus-free seed and/or the protection provided by the live plant barrier was calculated, adapting the formula used by Lal and Lal Jat (2015).

$$\text{Avoided losses (\%)} = \frac{\text{MTM} - \text{MTSM}}{\text{MSTM}} \times 100\%$$

where MTM: the mean tuber mass with field management (potyvirus-free planting material inside (T1) and outside (T2) the living barrier) and MTSM: the mean tuber mass obtained from the planting material and cultivation method indicated in MINAG (2012), which not include any field management (T3).

Considering the plantation framework most commonly used by producers, the density of plantations in Cuba is approximately 8,300 plants ha⁻¹. From this density, yam plants grown according to traditional methods (MINAG 2012), and from the differences in production per plant determined, the yield per area was estimated for each of the treatments. Also, the nutritional value of the avoided losses was estimated from the composition of the crop. For the comparison of the percentage of infected plants, a t-test was performed for independent samples.

On the other hand, the comparison of production per plant (kg) was performed by simple rank analysis of variance and Tuckey's mean multiple ranks test. The experiment was repeated in the 2017–2018 season. In parallel, plantain bunches were harvested during the period of the experiment in the perimeter of the live plant barrier and their mass was determined with the use of a Roman balance.

Nutritional estimations

From the nutritional composition of yam tubers (Bekele y Bekele 2018; Mulualem et al. 2018), carbohydrates in g, proteins in g, dietary fiber in g, vitamin C in mg and the potassium in mg stopped producing due to potyvirus diseases were calculated.

Results

Detection of yam infected plants

No difference was detected between yam crop development inside and outside the barrier. The percentage and date of plant sprouting, leaf emission rate and number of vines were similar across barrier locations. The total number of infected plants in the field at 270 dap outside the barrier was 52 and 63 on the cultivar 'Guinea', and 62 and 64 on the cultivar 'Belep' for both seasons, respectively. However, when the crop was protected from infection inside the live barrier, the number of infected plants, according to ELISA results, decreased to 12 and 16 plants in cultivar 'Guinea' and to 13 and 9 in cultivar 'Belep', respectively (Table 1).

All symptomatic plants tested positive for the presence of potyvirus by ACP-ELISA. In the cultivar 'Guinea', the presence of the rolling and chlorosis symptoms was detected in 50 and 61 plants in both growing seasons, while the severe mosaic symptom was observed in 8 and 13 plants in the same period. Meanwhile, in cultivar 'Belep', the presence of yellow banding symptom was detected in 38 and 47 plants in both growing seasons, while the light mosaic symptom was observed in 25 and 17 plants in the same period. However, 41 asymptomatic plants tested positive for potyvirus at 270 dap by ACP-ELISA. With the application of this assay, 6.3% of potyvirus-positive plants were detected within the total number of asymptomatic plants.

Identification of aphids

The aphid species collected were *Myzus persicae*, *Aphis gossypii* and *Toxoptera citricidus*, both inside and outside the *Musa* sp. barrier, and it was not possible to collect individuals of other species. Statistical analyses showed no differences between the number of individuals collected inside and outside the barrier in the five traps installed.

Production per plant and field yield estimation

Figure 2 shows the results of tuber production per plant in the cultivars 'Guinea' and 'Belep', in the 2016-2017 and 2017-2018 seasons. The use of potyvirus-free planting material and the use of live barriers of cultivar 'Burro CEMSA' allowed increasing production per plant, regardless of the cultivar used. The lowest yields per plant were obtained with the use of planting material from traditional production systems, without confirmed criteria on the presence or absence of potyvirus and without the protection of the live barrier (T3). Under these growing conditions, more than 90% of the plants, of the total number planted in both seasons, showed symptoms typical of potyvirus-associated diseases for the two yam species under study.

In the 2016-2017 and 2017-2018 seasons, yield per plant using potyvirus-free planting material protected from field infection with live barrier (T1), compared to that obtained without phytosanitary certification and grown without the use of live barrier (T3), increased by 50 and 55% (percentage of losses avoided) in the cultivar 'Belep' and by 57 and 55% in the cultivar 'Guinea', respectively. Similarly, yield per plant was higher when potyvirus-free planting material (T2) was used compared to the traditional cultivar without live barrier protection (T3). In the cultivar 'Belep', the percentages of avoided losses coincided at 22% for both campaigns, while for the cultivar 'Guinea' they were 33 and 28%, respectively. In the cultivar 'Belep', in the three treatments evaluated, the estimated yields were between 20.4 and 31.3 t ha⁻¹. Overall, in the experiments carried out in this research, the yield per plant achieved in the cultivar 'Guinea' was numerically higher than that achieved in the cultivar 'Belep'.

The use of the living barrier and the use of potyvirus-free planting material (T1) allowed estimating yields of 39.4 t ha⁻¹ and 31.3 t ha⁻¹ in the cultivars 'Guinea' and 'Belep', respectively, higher by 14.2 and 10.9 t ha⁻¹ than the agricultural yields achieved when the traditional form of the crop was used (T3).

On the other hand, a total of 590 banana bunches were harvested between July and August 2016, 1 150 between March and April 2017 and 1 100 approximately between November and December 2017, for a total of 2 840 banana bunches during the two campaigns of duration of the experiments with *Dioscorea* spp. Production per plant averaged 16.5 kg, a value that is within the potential yield ranges for this cultivar in our country (MINAG 2012).

Nutritional estimations

Table 2 shows the elements that ceased to be produced due to potyvirus infection. The greatest losses were in carbohydrates, vitamin C and potassium.

Discussion

The number of asymptomatic plants that tested positive by ACP-ELISA is numerically lower than reported by Njukeng et al. (2014) in two production areas in Cameroon, and Aihebor et al. (2017) in experimental areas of IITA in Nigeria. In this sense, serial sampling of symptoms expressed in the field contributes to the decrease in the number of asymptomatic plants observed when sampling a single symptom at the end of the crop cycle.

The similarity between the number of aphids found inside and outside the barrier suggests that the decrease in the number of infected plants obtained for both cultivars in the two seasons could be due to a sink effect of potyviruses in the living barrier, where virus-carrying insect vectors (viruliferous aphids) reduce their infective load after alighting on the barrier crop and before reaching the susceptible host (Manandhar and Hooks 2011). Therefore, the reduction in the number of potyvirus-infected plants is not due to a reduction in the number of aphids landing on yam, but to a reduction in the number of viruliferous aphids reaching the crop. According to Gadhav et al. (2020), *M. persicae* and *A. gossypii* are considered very efficient vectors of potyviruses and transmit 53.4% and 25.6% of total potyviruses, respectively. The control of vectors or their ability to spread the disease is fundamental in the disease management (DM) programs, as pointed out by Islam et al. (2020). In this regard, Boiteau et al. (2009) were able to decrease potato virus Y (PVY) incidence in susceptible cultivars by surrounding them with a resistant cultivar for three consecutive years. Similarly, Avilla et al. (1996) observed that a sorghum barrier was able to reduce PVY and cucumber mosaic virus incidence in bell pepper cultivars.

The use of planting material without sanitary certification of *Dioscorea* commercial cultivars in successive seasons leads to the accumulation of virus-infected tubers. In this sense, the use of live barriers contributes to maintaining the phytosanitary quality of the seed used by growers. Planting new areas with “healthy seeds” is a fundamental aspect of *Dioscorea* spp. development programs, which contribute to increasing the economic efficiency of the crop (Asala and Alegbejo 2016).

The cultivar ‘Burro CEMSA’ used as a living barrier in this research harbors neither the potyviruses reported in *Dioscorea* spp. nor their aphid vectors (ICTV 2020; Holman 2009). On the other hand, it has the advantage that it can be used for several years without replanting, unlike when using short-cycle or

annual crops. In this case, a rotation of crops such as cocoyam (*Xanthosoma* spp. and *Colocasia esculenta* (L.) Schott), sweet potato and papaya can be established within the barrier, taking advantage of the protection offered by the living barrier. In this case, these plant species are affected in Cuba by viruses of the *Potyvirus* genus transmitted in the field in the same way, non-persistent, and by aphids.

Phytosanitary certification of planting material should be the starting point of any field DM program to reduce losses due to viral diseases (Islam 2017). In this regard, Aihebor et al. (2017) found yield increases in *Dioscorea* spp. due to the use of potyvirus-free planting material. Similarly, Adeniji et al. (2012) reached differences between 50 and 65% in commercial cultivars of *D. rotundata* when comparing yields obtained by using yam mosaic virus (YMV)-free and YMV-inoculated planting material, while Séka et al. (2014) reported losses of about 40% by using YMV-inoculated planting material. In the cultivar 'Belep', in the three treatments evaluated, the estimated yields were between 20.4 and 31.3 t ha⁻¹, similar to those achieved in five *D. alata* cultivars developed by the breeding program of the Central Tuber Crops Research Institute at its Regional Center in Thiruvananthapuram, India, with values between 23–30 t ha⁻¹ (Sivakumar et al. 2009). In all the experiments conducted in this research, the production per plant achieved in the cultivar 'Guinea' was numerically higher than that achieved in the cultivar 'Belep', similar to what occurs in producing areas in the central and eastern regions of Cuba. As proposed by Udiarto et al. (2023), the use of live plantain barrier and the use of potyvirus-free planting material (T1) showed a favorable influence on yields of the main crop (yam), due to protection against the incidence of viral diseases. Similarly, there is a plantain production that is economically favorable for farmers who adopt this management strategy.

The amount of vitamin C left unproduced per hectare, in the cultivar 'Guinea', is equivalent to the daily requirement of 53 960 boys or girls between 7 and 13 years old (USDA NAL 2017), while the potassium not produced, would mean the amount needed to be consumed daily by 34 080 or 44 560 adults (males or females, respectively) to reduce the risks of arterial hypertension, according to recommendations of the National Academies of Sciences, Engineering and Medicine of the United States of America (Greer et al. 2020). This analysis should be taken into account for the design of agricultural strategies aimed at achieving the food and nutritional sovereignty pursued by the Ministry of Agriculture of Cuba. In this sense, development programs should be favored for those crops that, due to their biochemical composition, contribute to improving the quality of the population's diet.

The perception of risks and damage caused by viral diseases is subjective and is limited to diseases that have marked significant advances in plant virology research or that have been devastating in economically important crops (Velásquez et al. 2018). However, the results obtained in this research demonstrate that it is necessary to evaluate in each agricultural system viral diseases that may go unnoticed due to tolerant plant-host interactions, as is the case of potyviruses in the economically important yam cultivars used in this research.

The association of *Dioscorea* spp. plants (main crop) with cultivar 'Burro CEMSA' (barrier crop) involves a redesign of the agricultural ecosystem to the detriment of potyvirus diseases in *Dioscorea* spp. by

reducing their dissemination in the field and reducing the economic losses they can cause. In this case, the perimeter living barrier would fulfill the main function of protecting the host crop from potyvirus infection and, in addition, of producing food. On the other hand, the use of non-“biorational” agents (insecticides) is avoided, thus preventing contamination of the environment (Matyjaszczyk 2018). This approach would allow the establishment of environmentally friendly, long-lasting and economically sustainable DM methodologies.

Declarations

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Author contributions

Conceptualization was done by JEGR and OP; methodology was done by JEGR and VVC; validation was done by AF and OP; formal analysis was done by JEGR and OP; investigation was done by JEGR and VVC; resources were done by JEGR, AF and OP; data curation was done by JEGR, AF and OP; writing - original draft preparation were done by JEGR and OP; writing - review and editing were done by JEGR, AF and OP; visualization was done by AF; supervision was done by AF and OP; project administration was done by JEGR; and funding acquisition was done by JEGR.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Tables

Table 1 Infected plants in the field on cultivars 'Guinea' and 'Belep' during 2016-2017 and 2017-2018 seasons

Treatments	Symptoms	ELISA	Cultivar 'Guinea'		Cultivar 'Belep'	
			2016-2017	2017-2018	2016-2017	2017-2018
No barrier	Symptomatic		47	60	54	58
	Asymptomatic	ELISA +	5	3	8	6
		ELISA -	44	33	34	32
	Infection (%)		54.2 a	65.6 a	64.6 a	66.7 a
Barrier	Symptomatic		11	14	9	6
	Asymptomatic	ELISA +	1	2	4	3
		ELISA -	84	80	83	87
	Infection (%)		12.5 b	16.7 b	14.5 b	9.4 b

Different letters in the columns differ according to the T-test for $p < 0,05$

Table 2 Nutritional elements lost per hectare in the yam cultivars 'Guinea' and 'Belep'

Elements	Cultivar 'Guinea'	Cultivar 'Belep'
Carbohydrate (g)	3 958 960	3 038 920
Protein (g)	217 260	166 777
Dietary fiber (g)	582 200	446 900
Vitamin C (mg)	2 428 200	1 863 900
Potassium (mg)	115 872 000	88 944 000

Figures

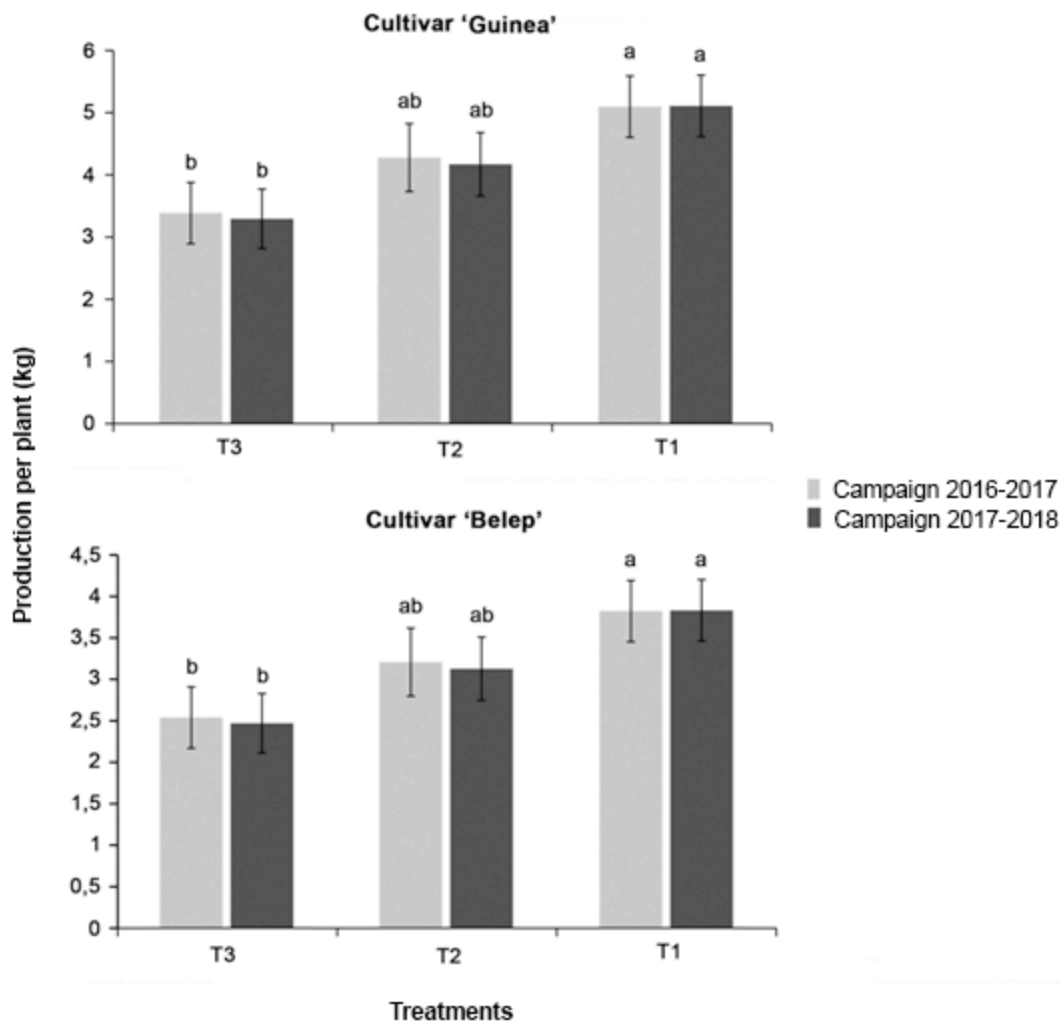


Figure 1

Diagram of plantain barrier plants preestablished and the treatment arrangements for the two yam cultivars under study ('Guinea' and 'Belep'). T1) virus-free ACP-ELISA propagation material + inside the barrier; T2) virus-free ACP-ELISA propagation material + outside the barrier; T3) virus-uncertified propagation material + outside the barrier

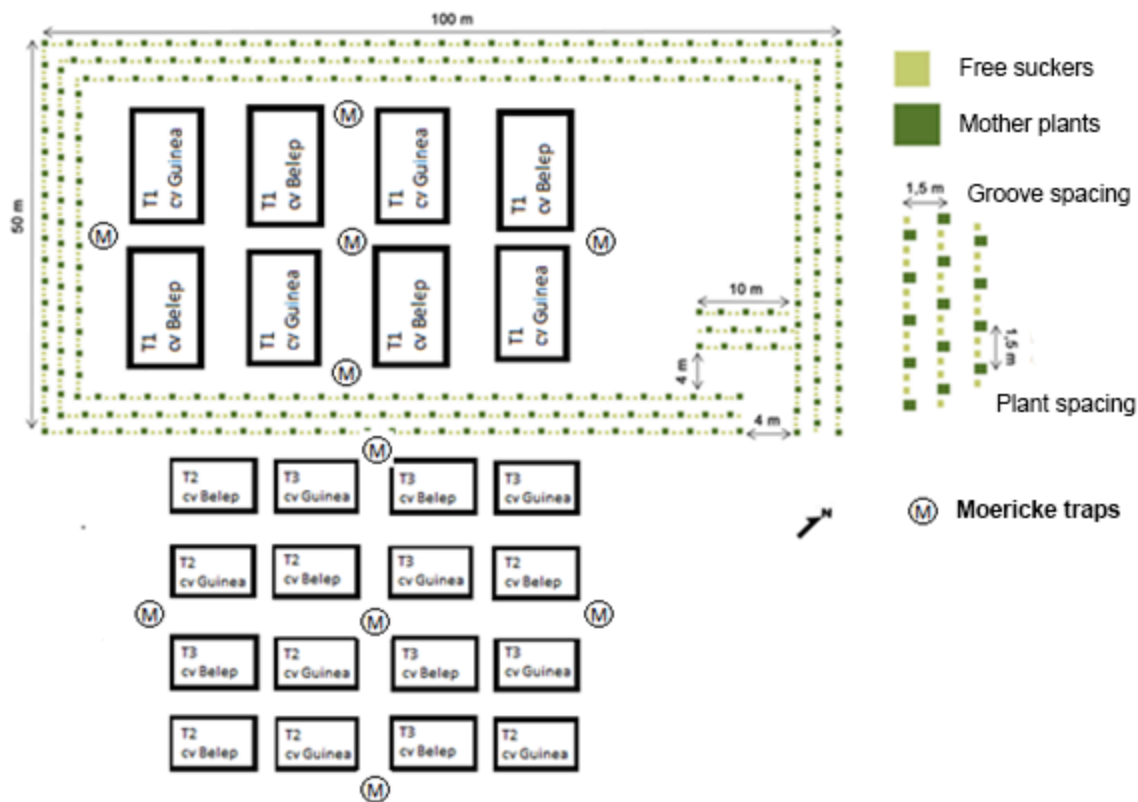


Figure 2

Yield of yam per plant obtained in two commercial cultivars of *Dioscorea* spp. T1) potyvirus-free planting material inside a live barrier of cultivar 'Burro CEMSA'; T2) potyvirus-free planting material without live barrier; T3) propagation material without virus certification + outside the barrier. Different letters differ according to Tuckey's test for $p < 0.05$