Adoption study of sweetpotato varieties in Malawi: DNA Fingerprinting Activity

Activity Report

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1. Introduction

Sweetpotato varietal identification is key for understanding the real impact of the CIP interventions in different African countries where most of the investment to reduce poverty and malnutrition has taken place. International Potato Center conducted a study on farmer adoption and utilization of orange fleshed sweet potato and its effects on household welfare in Malawi.

Recent research in Ethiopia has shown that identity confirmation by DNA fingerprinting is required in order to credibly estimate adoption of sweet potato varieties in SSA. This is because, since farmers' self-reporting of sweetpotato varieties they grown is not always reliable (Kosmowski et al., 2018). This emphasizes the importance of varietal identity confirmation for other root and tuber crops to avoid misclassification (Floro et al., 2017; Wossen et al., 2018; Ellis et al, 2018). In order to ascertain the genetic identity of the OSFP varieties grown in Malawi, this study included a component on DNA fingerprinting to assess the number of false positives and negatives from the farmer survey data. A varietal identification and verification activity were an important aspect for this our study, not only to validate what farmers were growing but also as robustness check for robustness from farmer-stated responses.

This study focused in a subsample from the household survey conducted at national level by CIP to estimate adoption and impact of OFSP varieties on health and economic outcomes. Therefore, the results will help to understand the extent of the misidentification of sweetpotato varieties in the different regions in Malawi.

2. Objectives

This activity has the objective to confirm that sweetpotato varieties identified by farmers' recall in household surveys match with the genotype of the varieties in the field. The specific objectives are:

1. To identify the sweetpotato varieties by genotypical matching based on a list of reference sweetpotato material.
2. To rank the most common OFSP varieties in Malawi based on DNA.
3. To estimate the error type I and II in the sub sample of the household survey.

3. Methodology

The methodology for genotypical identification of sweetpotato varieties is straightforward. It can be described in Figure 1.

Figure 1. Diagram describing the process from collecting leaf samples to analysis of data coming from DNA varietal identification.

3.1 Protocol definition

The leaf collection activity demands specific procedures, equipment and materials to properly get a sample with good quality DNA for extraction and subsequently the phenotypical identification based on reference material. This protocol was designed by the Social Science team based on information and suggestions from the Malawi CIP breeder Felistus Chipungu and CIP Genebank leader Noelle Anglin between February and May 2019, and it was further improved in the leaf collection training in Lilongwe in May 2019.

There are several critical points when collecting leave in order to get the quality needed for the varietal identification:

1. Leaves should be taken from healthy young leaves in order to get good quality DNA.
2. Those leaves should be properly labelled, if leaves are mixed, then work is ruined
3. The sample should dry fast and uniform to not get rotten or damage, and it should be done under field work conditions

The protocol developed for this purpose indicated step by step the way collectors should handle every step. The full protocol is in Appendix 7.1.

In order to make familiar the protocol to the enumerators, it was organized an eight day training to the leaf collectors in May 2019, with the support of Felistus Chipungu, CIP breeder in Malawi office, not only to select the candidates for this study, but also to support in the purchase of all the material needed for the leaf collection activity. Morevover, Daniel Van Vugt, Malawi country representative for CIP, gave technical backstopping about how to better perform the study in Malawi. The training was conducted at different locations according to the needs of the training, we used the Wankulu hotel in Lilongwe for training on general information and knowledge of the protocol, we traveled to different field for practice the protocol and we used CIP-Malawi office to prepare samples and trained in the drying of the leaves. Additionally, we selected a supervisor among the enumerators, Clementina Banata, who worked at the National Research Center and had extensive experience managing samples.

Two extra people were hired to help with the leaf collection as workload was very high for the two teams. The supervisor was in charge to train the new collectors. Then the three groups were organized as followed:

A. **Team A:** 1. Steve Saisi, 2. Baba Kayinga
B. **Team B:** 1. Gladys Chinthwango, 2. Mphatso Joshua
C. **Team C:** 1. Tonnex Simfukwe, 2. Bashir Kamwendo

The supervisor was in charge to support the three team of collectors (2 people per team), as well as to control quality in the collection and plot measurement. She also helped in introducing the collected information in a spreadsheet to be sent to activity leader (Wily Pradel). The supervisor collected a large container with the dry leaf's samples from the first month of collection and she was in charge of

completing the drying process with silica gel in Lilongwe. The supervisor moved with the teams according to car availability. She was in constant communication with DNA fingerprinting activity leader in order to inform of the advances and take decision when needed. The collectors were in charge to collect shoots and leaf samples with proper identification of those and filling the corresponding templates as detailed in the protocol. They were also responsible to take care of the collected samples in order to dry them without damages using silica gel.

At the end of the collection, the supervisor was in charge to take the second containers with dry samples and complete the process of drying them. After samples were dried, she supported the preparation of the sample to send to Australia using the corresponding plates.

For the DNA analysis, it was suggested to conduct the DNA extraction of the sweetpotato samples with Professor Wisdom Changadeya at Chancellor College, Zomba in Malawi, and the DNA fingerprinting at CIP laboratories in Kenya. However, after consulting pros and cons with CIP experts (Noelle Anglin, Felistus Chipungu and Dorcus Gemenet), it was decided to use the service of Diversity Arrays Technology Pty Ltd. Diversity Arrays Technology Pty Ltd is a private company based in Canberra, Australia with a unique business model developed by current Director, Andrzej Kilian, as a vehicle to deliver high throughput genotyping in any organism without the need for prior sequence information. The original platform for this used solid state arrays and DNA hybridization. The methodology has been adapted to take advantage of the very high data generation capacity of next generation sequencing platforms.

3.2 Household selection for leaf collection

Resources for this activity were restricted, not only financial resources, but also time and human resources. Therefore, it was decided that a sub sample of the total household. The total number of samples in the household survey was distributed in three groups:

- Group 1: Participants to CIP intervention projects in CIP intervention villages – 1500 surveys
- Group 2: Non-participants to CIP intervention projects in CIP intervention villages (Spillover group) – 500 surveys
- Group 3: Non-participants to CIP intervention projects in non-CIP intervention villages (Counterfactual group) – 500 surveys

The DNA fingerprinting study was designed to just collect samples from Group 1, 5 households per village in that group (from a total of 15 households per group). Leaf samples to be collected were OFSP, Kenya, and the most common white flesh sweetpotato in the District. However, due to different reasons including:

- Most plants in the central region of Malawi were attached by pests which affected collection of the desired part of the plants.
- In some areas though vines were distributed but the varieties were lost due to drought e.g. in Mangochi.
- Northern region we could hardly find OFSP, many farmers grow the local varieties unlike south and central. Many of varieties grown in the north are white fleshed.

- There were problems in sample collection where fields were already harvested as most of the leaves were found dry.
- Preservation of samples collected from “dambo” areas e.g. in Chikwawa led to poor quality of samples due to too humid conditions.

Therefore, in July, after one month of leaf collection activity, it was added the Group 2: Spillover group, to increase the number of samples to collect.

3.3 Sweetpotato leaves collection

Leave collection took place between June and July 2019. Initially there were two leaf collection teams, the third team joined after one month. The leaf collectors were accompanying the teams collecting household-level data. After the first round of interviews were completed, leaf collectors would follow farmers to their plots to take leaf samples and measure plot size with GPS handheld and measurement tape. This was challenging as not all plots were close by the farmer’s house and frequently, leaf collectors needed to walk (off-road) for some 1.5 hours one way.

Between 2-4 uppermost young leaves without necrotic areas or lesions were plucked using the tweezers. The leaves were put into paper towel and properly labeled. When moving to the next sample, the tweezers were disinfected with ethanol to avoid transferring DNA from one variety to another one, or an infected plant to a healthy one. When samples were dirty and there were no clean plants, water was used to clean the sample.

In the evening samples were transferred to the coffee filters with the label. The coffee filters were placed in a Ziploc containing orange silica gel. After a day, the moisture saturated silica gel was replaced with another orange silica gel (100g). The collected varieties were orange fleshed sweetpotato varieties, introduced varieties, and the most common white sweetpotato in the district. Initially leaf samples were collected to beneficiaries, in Mchinji its when non beneficiaries were also sampled when nearly have of the survey was completed. The teams also calculate the plots area from plots where samples were taken.

Leaf samples were collected from 388 households in most sampled Extension Planning Areas (EPAs)[1] resulting in a total collection of 1,039 leaf samples. Group A collected 434 samples, Group B collected 413 samples, and Group C collected 192 samples. Quality of samples were acceptable, with exemption of 20 samples from Group A (mostly in Chikwawa), and 28 from Group C (mostly in Rumphi) which were in not acceptable conditions for DNA extraction.

3.4 Sample preparation

At the beginning of August, after finishing the leaf collection activity in a subsample of the household survey; the supervisor and activity leader checked data quality, as well as preparing the samples to send the dried and crushed material to Australia for the DNA fingerprinting. Besides sending the collected

[1] Malawi is divided in 28 Districts and 187 Extension Planning Areas

material for DNA finger printing, we collected 23 samples for the reference plant material in the Bvmbwe Agricultural Research Station in Blantyre (Table 1)

Table 1. List of reference sweetpotato material collected for comparison with the samples collected in the field in Malawi.

	VARIETY NAME	FLESH COLOR	ORIGIN		VARIETY NAME	FLESH COLOR	ORIGIN
1	BABACHE	WHITE	LOCAL	13	CHIPIKA	ORANGE	IMPROVED
2	KAMCHIPUTU	ORANGE	LOCAL	14	BIE	PURPLE	MOZAMBIQUE
3	ZONDENI	ORANGE	IMPROVED	15	DEDZA PURPLE	PURPLE	LOCAL
4	KADYABWERERE	ORANGE	IMPROVED	16	CAECAN	PURPLE	MOZAMBIQUE
5	MUGAMBA	WHITE	IMPROVED	17	MWANZA PURPLE	WHITE	LOCAL
6	KAPHULIRA	ORANGE	IMPROVED	18	BITA	PURPLE	MOZAMBIQUE
7	MATHUTHU	ORANGE	IMPROVED	19	BERA	ORANGE	MOZAMBIQUE
8	SAKANANTHAKA	WHITE	IMPROVED	20	IREEN	ORANGE	MOZAMBIQUE
9	SEMUSA	WHITE	IMPROVED	21	DERVIA	ORANGE	MOZAMBIQUE
10	KENYA ADMARC	YELLOW	INTRODUCED	22	JANE	ORANGE	MOZAMBIQUE
11	MTHESANJALA	ORANGE	IMPROVED	23	AMELIA	ORANGE	MOZAMBIQUE
12	ANAAKWANILE	ORANGE	IMPROVED	24			

Another activity related to sending the material for DNA fingerprinting to Australia was to have the phytosanitary certificate, that it was provided by the Bvmbwe Agricultural Research Station.

After coming back to Lilongwe from Blantyre with the reference material, Chitedze Research Station kindly provided support to use their labs and equipment to prepare the material. It was selected dry material in good conditions, then we crushed them and filled eleven 94-well plate containing 991 samples plus the reference material. In the process of filling those tubes, it was required no more than 10 mg of dried from sweet potato per tube, which required the use of digital scale to get the right amount of material for micro array/sequencing and destructive analysis in the PC1 laboratory of Diversity Arrays Technology Pty Ltd. The leave material was sent in the beginning of September 2019, and results of the DNA extraction and DNA fingerprinting were provided mid-December 2019.

4. Results

4.1 DNA fingerprinting

DNA extraction and fingerprinting were done through Diversity Arrays Technology, whom provide a cost-effective genotyping technology detecting all types of DNA variation (SNP, indel, CNV, and methylation sites). The technology was invented by Andrzej Kilian and his group (Kilian et al, 2012), to overcome some of the limitations of other molecular marker technologies such as RFLP, AFLP, and SSR. Their prices are significantly less expensive than other alternative service providers. Further, this project required using the same protocol as employed in previous studies so that data generated in could be directly compared. Some of these studies are genotyping of the ex situ accessions, the 100 best bets of Africa, and varietal adoption of different crops in Africa). This assisted in varietal identification.

The use of varietal identification through fingerprinting technologies is not only useful to correctly identify the variety, but also can estimate the errors in the varietal identification, both, false positives where farmers believe they have a specific variety when they do not (also known as error type I) and false negatives where farmers believe they do not have the specific variety, when indeed they have it (also known as error type II). Error type I and Error type II was calculated based on the genotyping identification and the farmer knowledge/recall of their varieties planted. Those errors commonly occur in these types of studies. Some examples include Wineman et al. (2019) in maize and rice, Kosmowski et al. (2018) in sweetpotato, Floro et al., (2017) in cassava, Maredia et al. (2016) in cassava and beans, Rabbi et al. (2015) in cassava, Labarta et al., (2015) in rice. Those studies calculated the extent of varietal misidentification. Reasons for misidentification of varieties by farmers can be attributed to incorrect information on the varieties they are growing, not remembering correctly, variety has been renamed, or mixtures of varieties have occurred over time. All of these reasons have made it important to validate the survey information by fingerprinting a subsection of samples found in the field.

Results from the DNA fingerprinting work at Diversity Arrays Technology Pty Ltd could extract good quality DNA for 98% of the 991 household samples (969 samples) for the varietal confirmation. From those, 422 didn't match any of the reference material (163 single unknown varieties were identified). In Figure 2 we observed the frequency of the most common varieties cultivated in the different

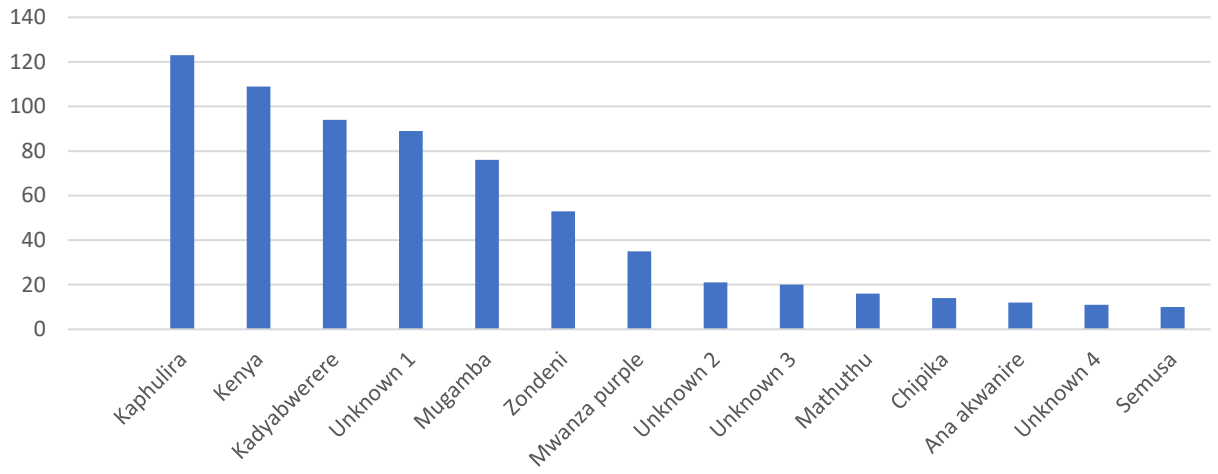

Figure 2. Count of the samples matched with the most common varieties sent from the Malawi Research Station, and the 100 best bets project to DarT P/L.

The most important varieties are Kaphulira, Kenya, and Kadyaubwerere, with 12.7%, 11.1% and 9.8%, of the samples collected, respectively. These results show the importance of Kaphulira and Kadyaubwerere as the most important OFSP present in the country. Kenya (SPN/O), a yellow fleshed variety also appeared to be an important variety in the samples from participating villages, closely followed by an unknown variety (named as Unknown 1). The latter variety was present in 89 samples but it received 28 different names in the leaf collection database, and one third of farmers recognized they didn't know the name of the variety (naming it as other OFSP or unknown). It would be important to identify this variety as it seems to be OFSP. Finally, Mugamba, an improved variety, released in 1999, was considered as the most important variety in several districts.

Kaphulira's main attributes are that this variety is an early maturing variety (3-4 months) and have high yield (35 t/ha), this seems to be highly desirable for Malawian farmers. Kadyaubwerere, even tough, it takes one month longer than Kaphulira, the taste is sweet, and farmer enjoy the taste of this variety, also it is rounder than the other OFSP, and have high yield, similar to Kaphulira.

From the data coming from Dart P/L, also helped to understand the distribution of the different varieties across the country (Table 2). The varietal identification by DNA showed that fewer percentage of OFSP with respect to all sweetpotato varieties were found in the Northern regions in comparison to Central and Southern regions. The districts with higher adoption of OFSP were Nsanje, Mangochi and Mwanza in the South, and Dedza, Lilongwe and Salima in Central region. Kenya variety was more important in Central region, especially in Ntchisi, Dowa, and Mchinji. Finally, local varieties are important across the country, being more important in Northern region with 56% of total sweetpotato samples collected in the region. Places with higher percentage of local varieties are Karonga and Rumphi in the north, Ntcheu and Ntchisi in Central region and Balaka and Blantyre in the south.

Table 2. *Distribution of sweetpotato categories identified by DNA fingerprinting in Malawi by District and Region in Malawi, 2019*

	N	OFSP*	Kenya AdMarc	Dominant WFSP**	Other local varieties
Northern Region	**131**	**14%**	**8%**	**22%**	**56%**
Chitipa	19	0%	21%	11%	68%
Karonga	24	8%	0%	4%	88%
Mzimba	54	22%	11%	28%	39%
Nkhata Bay	32	13%	0%	34%	53%
Rumphi	2	0%	0%	0%	100%
Central Region	**467**	**33%**	**15%**	**12%**	**40%**
Dedza	42	48%	19%	10%	24%
Dowa	42	24%	24%	10%	43%
Kasungu	6	33%	17%	0%	50%
Lilongwe	72	43%	19%	10%	28%
Mchinji	106	31%	22%	5%	42%
Nkhotakota	49	12%	10%	33%	45%
Ntcheu	32	25%	6%	6%	63%
Ntchisi	18	11%	28%	6%	56%
Salima	100	41%	3%	19%	37%
Southern Region	**371**	**39%**	**8%**	**9%**	**44%**
Balaka	19	21%	0%	5%	74%
Blantyre	14	21%	7%	0%	71%
Chiradzulu	13	15%	15%	8%	62%
Machinga	62	27%	5%	11%	56%
Mangochi	24	63%	0%	0%	38%
Mulanje	85	31%	12%	9%	48%
Mwanza	5	60%	0%	0%	40%
Neno	13	38%	0%	0%	62%
Nsanje	3	67%	0%	33%	0%
Phalombe	13	38%	8%	31%	23%
Thyolo	80	59%	14%	10%	18%
Zomba	40	43%	0%	10%	48%

* OFSP: Orange Fleshed sweetpotato, ** WFSP: White Fleshed sweetpotato.

4.2 Sample matching with household survey

4.2.1 Sample matching with household survey

From 969 samples that DNA analysis could produce good quality results, from them, 955 could match the information of 369 households from the household survey (Table 3). The importance to match the household adoption survey relies on the possibility not only to use additional data from the household but also to extrapolate the results to the larger survey.

Table 3. Summary of data produced in the leaf collection activity with respect of number of farmers planting OFSP, and varieties planted in farmers plots in Malawi.

Column1	TOTAL NUMBER OF FARMERS	FARMERS CULTIVATING CIP OFSP	NUMBER OF TOTAL SP VARIETIES	NUMBER OF CIP OFSP VARIETIES	PROPORTION OF FARMERS WITH CIP OFSP
Northern Region	**51**	**15**	**131**	**17**	**29%**
Central Region	**174**	**99**	**456**	**151**	**57%**
Southern Region	**153**	**95**	**368**	**142**	**62%**
Grand Total	**378**	**209**	**955**	**310**	**55%**

The data shows that more than half of the farmers were planting OFSP varieties produced by CIP breeding efforts. However, regional differences were found, and much lower farmer ad option was detected in northern regions with respect to Central and Southern regions; 29% of farmers had at least one OFSP variety in their fields, in comparison to 57% and 62% of farmers in Central and Southern regions, respectively. Besides, farmers that cultivate OFSP, in average, they had just 1.1 OFSP varieties per farmer in the Northern region, while in Central and Southern Region, farmers had 1.5 OFSP varieties per farmers. The results on the variation by region can be explained. CIP started interventions on OFSP in 2009 in three Districts in the Southern Region, namely, Phalombe, Chikwawa and Zomba and one District in the Central Region, Dedza. Further CIP projects are concentrated in the Southern and Central Regions, and a few Districts in the South.

We collected information from two groups of farmers, Participants to CIP intervention projects and non-participant to CIP intervention projects but from those who live in the same villages. When compared data from those groups, there were differences in the two groups (Table 4).

Table 4. Difference in adoption of OFSP within regions of Malawi, according to the participation of the CIP intervention projects.

	CIP project participant (PG)			Spillover group (SG)		
	PG Farmers with OFSP	Number of OFSP varieties	% of PG Farmer w/ OFSP	SG farmers with OFSP	Number of OFSP varieties	% of SG Farmer with OFSP
Northern region	14	15	33%	1	2	11%
Central region	90	134	65%	9	17	35%
Southern region	90	136	63%	5	6	56%

The main difference is that Farmers from the intervention groups has a higher percentage of farmers adopting OFSP with respect to the reference group. The different is higher between the two groups in the Northern region, in comparison to Central region, and the difference is much lower in Districts in Southern region where spillover households have similar percent

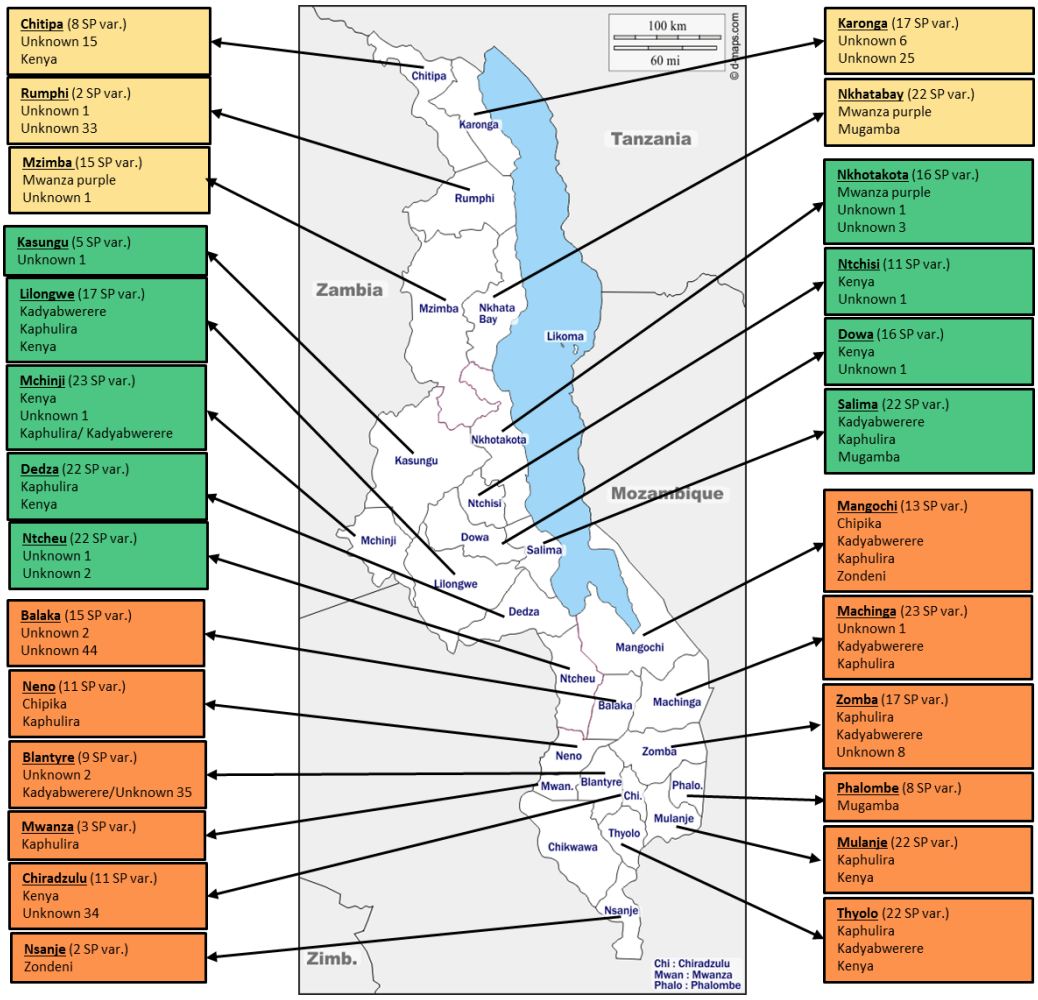

Figure 3. Main sweetpotato variety and number of varieties found in the DNA fingerprinting study in Malawi.

4.2.2 Identification of Error type I and Error type II in the sample.

Error type I and Error type II are the most common methods to identify misidentification of farmers recall of sweetpotato varieties based on DNA information of those varieties.

Six varieties were promoted by CIP and its partners. Apart from Zondeni, OFSP which was promoted in early CIP intervention, then other five varieties were promoted in the more recent intervention strategies (e.g. SUSTAIN, MISST, or RTC-Action, from 2014 onwards) to promote health related outcome through adoption of improved OFSP. Those varieties were: Kaphulira, Kadyaubwerere, Chipika, Ana-akwanire, and Mathuthu. Table 5 shows the error that affected the identification of error in identifying those varieties.

Table 5. True positive, Error type I and Error type II per OFSP variety and Kenya variety in Malawi (✕ means that it was identified by the specific method, ✕means that it was not identified by the specific method)

	Number of varieties identified in Survey	Number of varieties identified by DNA	True positive Survey ✕= DNA✕		False positive Survey ✓≠ DNA✕		False negative Survey ✕≠ DNA✓	
Variety	(#)	(#)	(#)	(%)	(#)	(%)	(#)	(%)
Kaphulira*	52	121	45	35	7	5	76	59
Zondeni*	33	53	16	23	17	24	37	53
Kadyabwerere*	87	94	47	35	40	30	47	35
Chipika*	44	14	5	9	39	74	9	17
Anaakwanire*	42	12	10	23	32	73	2	5
Mathuthu*	32	16	11	30	21	57	5	14
Kenya	131	106	64	37	67	39	42	24
CIP-promoted OFSP (6 OFSP varieties)*	289	310	192	47	97	24	76	29

Notes: True positive: farmers reported in the household survey they do have the specific variety, and the DNA fingerprinting demonstrate they do have it; False positive: farmers reported in the household survey they have a specific variety when DNA fingerprinting shows they do not (also known as error type I); False negative: farmers reported in the household survey they do not have the specific variety, when DNA fingerprinting shows they have it (also known as error type II). OFSP varieties are marked with (*)

Through DNA fingerprinting method it was further investigated that the direction of the error included: did farmers report to have a specific variety while they actually do not have it (false positive: error type I)? And if this was the case, what did farmers grow instead (false negative: error type II)? Error I was frequently the case for Chipika (74%), Anaakwanire (73%), Mathuthu (57%), and Kenya (39%). Not surprising that these are the varieties we earlier mentioned to be over-reported. Instead of having had one of these over-reported varieties, farmers likely had one of the following under-reported varieties instead: error II was most frequently the case for Kaphulira (59%), Zondeni (53%), and Kadyaubwerere (35%). In sum, while farmers to a large extent misidentified the specific variety they were growing, it was observed that, first, all promoted varieties, jointly, were under-reported which means that in some 29% of the sampled cases respondents unknowingly cultivated a promoted OFSP variety. Second, non-OFSP variety Kenya was found to be over-reported by 15%.

Misidentification was not even across Malawi nor different groups. Table 6 shows differences in error type I and error type II in the regions by the different intervention groups. Error type I and error type II were similar in the northern region, while in Central region error type II was larger than error type II and in southern region it was the opposite, and error type I was larger than error type II. Most errors type I and type II are concentrated in certain districts, such as Salima (19.5% of error type II), Mchinji (14.4% of error

type II) in Central region; and Machinga (16.5% of error type I), and Mulanje (12.4% of error type I) in Southern region.

Table 6. Error type I and error type II for OFSP varieties in CIP participant and spillover group in the three regions in Malawi

	CIP project participant group				Spillover group			
Variety	% TRUE NEGATIVE	% FALSE NEGATIVE	% FALSE POSITIVE	% TRUE POSITIVE	% TRUE NEGATIVE	% FALSE NEGATIVE	% FALSE POSITIVE	% TRUE POSITIVE
Northern Region	80%	7%	7%	6%	79%	6%	8%	7%
Central Region	59%	15%	8%	18%	55%	15%	10%	20%
Southern Region	48%	11%	14%	28%	48%	10%	13%	28%

To contextualize the results, in Kosmowski et al. (2018) 20% of farmers identified a variety as improved when in fact it was local and 19% identified a variety as local when it was in fact improved. According to this author, the variety names given by farmers delivered inconsistent and inaccurate varietal identities.

There was not only misidentification of variety name, but also in smaller percentage, there was error in flesh color of the variety. Some reasons of farmers can confuse flesh color is that farmer confuse the type of planting material they have in the fields, but also a language and/or cultural restrictions, as Chichewa (the local language), does not have a the word to represent orange color in their dictionary, and the closest color is "red" to describe "orange". This demonstrates how culture and language can often impact the answers to survey data.

Results showed that 11% of farmers planting OFSP varieties didn't name those varieties as orange flesh, similarly, 18% of farmers that cultivated Kenya ad-marc (yellow flesh) indicated that it was an orange variety. Finally, farmers that have planted Mugamba (cream fleshed and improved) and Mwanza Purple (Cream fleshed and local) indicated that they were orange in 28% and 20% respectively (Table 7).

Table 7. List of OFSP color of sweetpotato varieties stated by farmers with respect to varieties identified by DNA in Malawi

Varieties identified by DNA	OFSP	No OFSP	Percentage of error
Ana akwanire (OFSP)	12	0	0%
Chipika (OFSP)	13	1	7%
Kadyabwerere (OFSP)	82	12	13%
Kaphulira (OFSP)	113	8	7%
Mathuthu (OFSP)	15	1	6%
Zondeni (OFSP)	40	13	25%
CIP OFSP (OFSP)	275	35	11%
Kamchiputu (OFSP)	3	2	40%
Kenya (non-OFSP)	19	87	18%
Mugamba (non-OFSP)	21	55	28%
Mwanza purple (non-OFSP)	7	28	20%
Semusa (non-OFSP)	1	9	10%
Unknown	161	252	

5. Conclusions

There are important contributions of CIP effort to OFSP varietal adoption in Malawi. We found that proportion of farmers growing those varieties in CIP implementing villages varies from region to region. The region with less adoption of OFSP varieties is the Northern region where adoption in Spillover group is 11%, and in the CIP participant villages is 33%. In Central region, percentage of farmers Spillover groups is 35%, nearly half of the farmers adopting OFSP in CIP intervention groups 65%. In Southern region, both groups, CIP project intervention group and spillover group have similar percentage of farmers adoption OFSP (63% and 56%, respectively).

The most important OFSP planted in Malawi is Kaphulira followed by Kadyaubwerere. The first one is high yielding and early maturing, attributes to be determinant of increasing income and producing under water deficit years. On the other hand, Kadyaubwerere, is also high yielding, but even though has more cultivation time until harvest, it has sweet taste, researchers even name this variety "you cannot stop eating it after you taste it".

43% of the sample couldn't match any of the reference list we had. That value is high in comparison with other studies, such as Kosmowski et al. (2018) that found 11% of cases that couldn't match their reference material for sweetpotato in Ethiopia, or the case of Rabbi et al. (2015), that found that 22% of the sample in cassava in Ghana couldn't match any of the varieties in the DNA analysis. Similar to Wossen et al. (2018) and Wiseman (2019), our data suggests that there is misinformation of proper variety naming, as well as dissemination efforts that do not focused on varietal knowledge also play a role in the observed high misclassification rates of improved varieties.

We found regional difference in the distribution of error type I and error type II in the country, being more important error type II in Central region and error type I in Southern region. Results of error type II being larger than error type I can be found in Tizale et al. (2015), and Labarta et al., (2015) in wheat and maize in Ethiopia, and rice in Bolivia, respectively; while error type I being larger than error type II can be found in Wineman et al. (2019) for maize varieties in Tanzania.

Error type II is larger than error type I in the sum of CIP OFSP indicate that farmers in Malawi tend to under-report their OFSP in Central region, while in Southern regions, farmers tend to over report their adoption of OFSP.

Flesh color mistake was also found in the analysis, in average, 11% of the samples related to CIP OFSP varieties were recalled as non-OFSP. Several factors could have affected the misidentification of flesh color such as confusing the variety they have in the field, or as "orange" not being a word in the Chichewa language (local language).

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7. Appendix

7.1 Appendix 7.1. Protocol of leaf collection developed for this study

Adoption study of sweetpotato varieties in Malawi

Protocol to follow by enumerators to confirm genetic integrity of putative sweetpotato field plants

1. Introduction

This activity has the objective to confirm that sweetpotato varieties identified by farmers' recall in household surveys match with the genotype of the varieties in the field. We will collect leaves samples of the varieties identified by farmers from the cultivated plots, followed by drying and crushing of the leave samples. DNA will be extracted from each sample and the genotype will be compared with the reference library for the respective varieties. We expect to collect one sample for each cultivated variety from a subsample of around 600 households.

2. Materials

The materials needed for this activity include. (1) for the field: gloves, tweezers, alcohol, water, squeeze bottle, masking tape, paper towel, pens, blocks, small container, measurement tape and measurement rope (2) for the sampling preparation place: gloves, alcohol, tweezers, silica gel, coffee filters, Ziploc bags, labels, permanent markers, template for sample identification and plot measurement, pencils and big containers for sample storage. (3) For silica gel drying: Microwaves, gloves for handling hot silica gel, bowls, stir. (4) For cleaning: Soap and Vaseline.

3. Protocol for collection and preparation of leaves samples

3.1 Coordination

- Before field work starts, enumerators will be trained on the sampling protocol, and plot measurement protocol.
- The selection of the households where to collect the samples from will be defined in advance in coordination with the household survey.
- Fill the template with the information of the household, plot and variety. Area will be calculated using measurement tapes or measurement ropes.
- The enumerators responsible to collect the leaves samples (two per household survey group) will assure that gloves, scissors, alcohol, silica gel, envelops or coffee filters, Ziploc bags, labels, permanent marker and containers (or buckets) are available and in good conditions.

3.2 Leaves samples collection

- Enumerators will review the data in the household survey to identify the sweetpotato varieties identified in the questionnaire and the specific plots. The varieties to be collected are: OFSP, Kenya Admarc, and the most important white sweetpotato in the

district (The names and characteristics of the released OFSP, Kenya Admarc, and other white sweetpotato varieties is found in Appendix 1).

- Fill the template with the information describe above and the identifying notes on labels to ensure future identity of the leaves samples and matched to the survey questionnaire.
- Enumerators fill template (Appendix 2a) and labels (Appendix 3) with the information of the collector's group, district, household survey code, plot identification number and variety name.
- stick it into the paper coffee filters.
- Walk with the farmer with your container with the needed material to the selected plots and ask them to point out the sweetpotato varieties identified in the questionnaire.
- Collectors should untangle the vine of the selected plant to be sure you are taking the leave for the desired plant.
- It is preferable to pick the uppermost young leaves without necrotic areas or lesions (Figure 1.A), although older leaves which are not senescent may be used (B), diseased and dry leaves should not be collected (C).

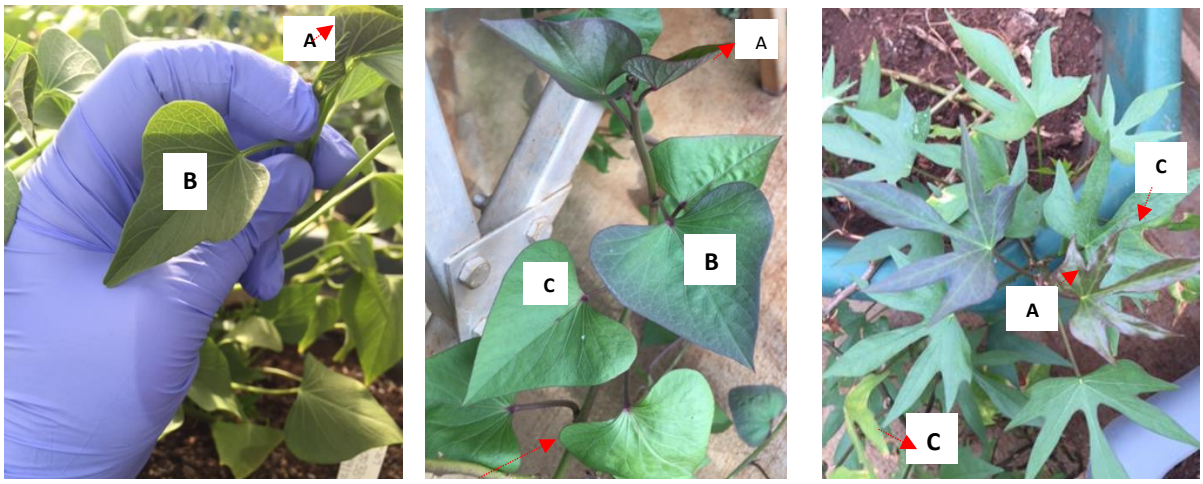


Figure 1. Different types of sweet potato leaves in the plant and preferences for leave collection

- Pick between 2-3 shoots or leaves, depending on what is available on the plot, using the tweezers to not touch the sample with hands (no need to change or clean gloves after each collection).
- Do not bend or fold collected leaves.
- When moving to the next sample, collectors should always disinfect the tweezers with ethanol. This is to avoid transferring DNA from one variety to another one, or an infected plant to a healthy one.
- If samples are dirty and there is no clean plants, clean the sample with water using the squeezer

- Put the leaves into paper towel and properly identified before moving to a place where to put shoots and/or leaves in the final coffee filter and Ziploc.
- If leaves are wet, use paper towel to dry them before transferring to the corresponding coffee filters with the identifier.
- Mark with pencil, the corresponding number from the template and the date of collection.
- Place the coffee filters/paper envelop in a Ziploc bag containing approx. 100 g orange silica gel.
- Before closing the Ziplock, take most of the air out of it.
- It is possible to keep from 6 to 9 coffee filters/paper envelopes in a Ziploc bag.
- Keep the Ziploc bags in an airtight plastic container in a dry place.
- After one or two days, replace the moisture saturated silica gel (change from orange to green when saturated) with another 100 g of orange silica gel. During the drying process, preserve the leaves as green as possible, and the best way to keep them green is to dry the leaves quickly.
- Leaves that are less dry should go to the bottom of the ziplock bag, to be closer to the silica gel.
- Moisture silica gel can be dried using a microwave (3 minutes). Then can be reused.
- Leaves can dry in silica gel between 2-7 days. Once leaves are dried, they can stay on the Ziplock with fewer amount of silica gel (10-20 grams).
- When the leaves are dry (crunchy), replace the moisture saturated silica gel with a small amount of the non-saturated with moisture ones (orange).
- Put 1 kg of silica gel in the big container for keeping low moisture in the large container.

The following pictures are an example of how to store the collected leaves in the Ziplock bags with silica gel and in the respective containers:

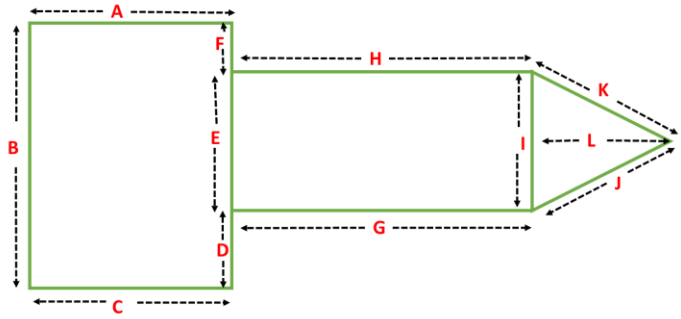
Figure 2. Orange silica gel with the leave sample inside a coffee filter into Ziplock bags

Figure 3. Plastic container to put 9 Ziplock bags filled with 9 leaves samples each.

- Once the large container is full of all leaves and/or shoot samples from the southern region, inform the supervisor that it is full, and it is needed to ship to the identified storage (either the CIP-Malawi office or the rented house in Lilongwe). Then a new large container will be used for central and northern region.
- Make prevision when any of the needed material is going to run out of stock (calculate when the material will last 7 days) to get more stock before is too late and there is no material to do the work properly.

4. Additional protocol for plot measurement.

- Plot measurement will be done to ALL plots where shoots and/or leaf collection was done. It means just plots that have OFSP, Kenya Admarc or the most important white sweetpotato in the District.
- After shoots and/or leaf samples are taken, the two collectors will prepare the material needed for the measurement: Blocks, pencils and measurement tape or measurement rope.
- *The selected farmer will show the limits of the plot where the variety was collected.*
- *The collector will draw the shape of the plot following the closest standard shape known (triangle, rectangle, square, circle).*
- *Put letters to each side that the drawn shape has (See example below)*



- *Start the measurement in one corner of the plot and make a mark (which is the point where the shape will start and finish).*

- *Measure each side of the shape, following the LETTERS included in the drawn shape.*, with the measurement tape or measurement rope.
- Write the measure in the template given by the trainer, together with the shape and the household and plot information (Appendix 2b).

7.2 Appendix 7.2. Main released varieties in Malawi

1. Anaakwanire

3. Kadyaubwerere

2. Chipika

4. Kaphulira

5. Mathuthu

7. Kenya

6. Zondeni

8. Lunyangwa

9. Mugamba

11. Sakananthaka

10. Nyamoyo

12. Semusa

13. Sungani

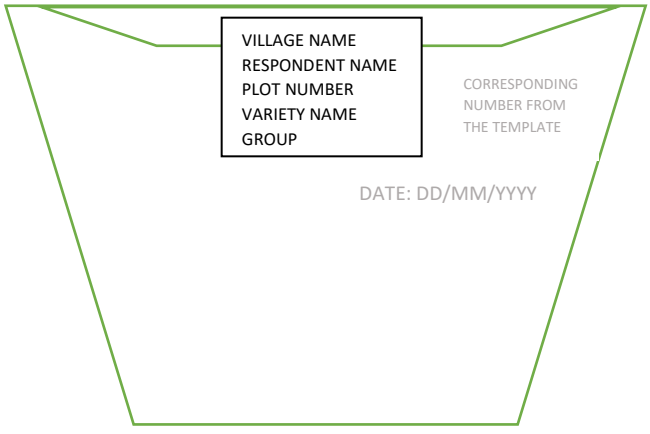
7.3 Appendix 7.3. Template for registering leave and/or shoot samples

Format to fill leaf collection samples from the adoption and impact OFSP study in Malawi, 2019						Group : _____
N	DISTRICT	HH Nr.	RESPONDENT NAME	PLOT	VARIETY NAME	COMMENTS
1						
2						
3						
4						
5						

7.4 Appendix 7.4. Template for registering plot measurement.

Format to fill plot measurement for area calculation of OFSP study in Malawi, 2019				Group : _____
N	HOUSEHOLD IDENTIFIER	PLOT #	PLOT SHAPE	SIDE MEASURMENTS
1	District: _____ Village: _____ Household Number: _____ Respondent name: _____			

7.5 Appendix 7.5. Template of information to be written in labels stick in coffee filters containing samples.



7.6 Appendix 7.6 Picture collection of the training and field work

Picture 1. Training on using protocols to maintain DNA integrity of the sample, Lilongwe, Malawi, May 2020..

Picture 2. Training on identification of suitable leaf material for DNA, Lilongwe, Malawi, May 2020..

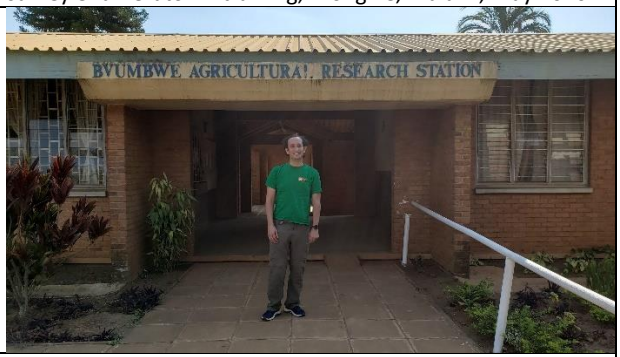
Picture 3. Training on proper leaf sample collection for DNA varietal identification, Lilongwe, Malawi, May 2020..

Picture 4. Field training on plot area measurement using tape, Lilongwe, Malawi, May 2020.

Picture 5. Training on correct handling of leaf material for proper drying using silica gel, Lilongwe, Malawi, May 2020.

Picture 6. Interaction of leaf collection group and household survey enumerator in training, Lilongwe, Malawi, May 2020..

Picture 7. Selection of leaf collectors and supervisor after leaf collection for DNA fingerprinting training, Lilongwe, Malawi, May 2020.



Picture 8. Visit to the Bvumbwe Agricultural research station for reference material collection, Blantyre, Malawi, August 2020.

Picture 9. Personnel from Bvumbwe Agricultural research station support collection of reference material, Blantyre, Malawi, August 2020.

Picture 10. Collection of reference material in Bvumbwe Agricultural research station, Blantyre, Malawi, August 2020.

Picture 11. Support of Chitedze Agricultural research station to process dry leaf material, Lilongwe, Malawi, August 2020.

Picture 12. Dry material of sweetpotato samples ready to be prepared, Lilongwe, Malawi, August 2020.

Picture 13. Weighting dry leaf material following Dart instructions, Lilongwe, Malawi, August 2020.

Picture 14. Preparation of plates following Dart instructions, Lilongwe, Malawi, August 2020.

Picture 15. Plates ready to be sent to Australia for the DNA extraction fingerprinting, Lilongwe, Malawi, August 2020.

Picture 16. Road market, Lilongwe, Malawi, August 2020.