State of Knowledge on Breeding for Quality Roots, Tubers and Cooking Bananas

Integrated End-user Focused Breeding for VUE (Variety, User, socio-economic Environment), WP4

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ABSTRACT

The main tropical root, tuber and banana crops addressed within RTBfoods project are: cassava (*Manihot esculenta*), yam (*Dioscorea spp.*), sweetpotato (*Ipomoea batatas*), potato (*Solanum tuberosum*) and the East African Highland banana (*Musa* AAA group): Matooke. These crops share common biological traits: they are vegetatively propagated, flowering of varieties is erratic, ploidy levels are variable, they are allogamous and highly heterozygous. Breeding starts with the selection of parents based on their individual value. Hybrids are evaluated for a few traits and undesirable genotypes are discarded as soon as possible (Lebot, 2013).

The national programs and CGIAR have been conducting RTB breeding programs for more than 30 years. A lot of progress has been made in the characterization of genetic resources, development of phenotyping tools, optimization of breeding schemes and lastly the integration of genomic tools in breeding programs. Genome Wide Association Studies and/or Genomic Selection are applied to all the crops concerned. Implementing of genetic and molecular approaches is contributing actively to the acceleration of the rate of genetic gains in the different on-going breeding projects. These efforts allowed the release of high yielding varieties and abiotic and/or biotic resistant ones. The quality is a trait, which is addressed from the beginning of the process or at the end when the breeding for the targeted traits was reached (Friedmann et al. 2018**)**. Breeders use basic quality parameters associated with consumer preference (high dry matter, color, oxidation, and various attributes of the roots, tubers, or fruit) as selection criteria. The heritability of roots, tubers and banana quality traits valued by processors and end-users is little known or completely lacking for certain crops.

Indeed, the means and subsequently the research investment on each crop, for quality and breeding, are not equal. We reviewed the statistics on the papers published on each crop by the international scientific community, recorded in the Web of Science. We applied the filter on the quality alone and in combination with the breeding filter. We found that research on quality in potatoes is far ahead with 3805 references recorded when the quality filter is applied alone and 273 when the quality and breeding filters are applied together. Cassava comes in second place (578, 57) followed by sweetpotato (154, 38) and yam (115, 10). For Matooke only 11 references were found without filter application. Although these statistics can be discussed and most likely are not as accurate, they reflect the scarcity of data and studies on breeding for the quality for these crops.

Although, the work on RTB breeding for quality is not exhaustive and did not encompass all the quality traits, it can be very instructive. Within an institute, due to the turn-over, different researchers might have worked on this field. Data are stored but not shared or mined for the targeted traits. In universities and institutes, students have produced theses. The results have not been published in peer-reviewed papers but they are still of interest and could be used. In this document, each institute has gathered the information they have access to, to report the state of knowledge on the targeted crop.

The objective of this review is to make an inventory of the previous activities on RTB breeding for quality, based on the papers published by the scientific community and on the activities conducted by research centers partners of the project. This review focuses on the breeding for product quality and is not intended to cover all breeding activities conducted within the different on-going projects. This review is a starting point, which will allow us to identify the gap analysis for each crop. Subsequently, within the RTBfoods projects, using the high throughput phenotyping (HTPP) methods developed, the breeding activities can be conducted in a way to fill up these gaps.

Key Words: state of knowledge, RTB, breeding, quality traits, phenotyping

1. BREEDING CASSAVA FOR QUALITY TRAITS

Introduction

Cassava (Manihot esculenta Crantz) is the most important crop among the tropical root and tuber crops (Pujol et al., 2002; Meireles da Silva et al., 2003). It originated in South America (Allem, 2002) and was domesticated less than 10,000 years ago (Elias et al., 2001). Cassava is a very rustic crop that grows well under marginal conditions where few other crops could survive. A large proportion of cassava varieties is drought-tolerant, can produce in degraded soils, and is resistant to the most important diseases and pests. The crop is naturally tolerant to acidic soils, and offers the convenient flexibility that it can be harvested when the farmers need it.

Cassava is a remarkable crop. Every tissue of the plant can be exploited. The most important commercial product of cassava is the storage root, which is full of starch. Mankind has learned to take advantage of the roots for many different purposes. Amerindians in the Amazon basin, the likely center of domestication of the crop, have selected varieties for specific end uses over the millenniums. There are many ways to process roots for human consumption, the simplest being boiling them. The requirements for the roots to be suitable for different processing techniques vary widely as described below. Roots can also be used for starch and flour production and for animal feeding (Ceballos and Hershey, 2017; Ceballos et al., 2017). Cassava is the second most important source of starch worldwide (Stapleton, 2012). End uses of cassava drastically define the traits that breeders have to take into consideration. For starch, ethanol or dried chips for animal feeding the key traits will be dry matter content (DMC) and fresh root yield (FRY). White parenchyma is preferred by the starch industry, but the enhanced nutritional quality of yellow roots would be preferable for animal feeding.

Stems cuttings are the most common source of planting material and are used for the commercial propagation of the crop. Cassava foliage is not widely exploited in spite of its high nutritive value, although consumption of leaves by human populations is relatively common in certain countries of Africa and Asia. Foliage is also used for animal feeding. Crude protein content in leaves typically ranges from 20 to 25% of dry weight (Babu and Chatterjee, 1999; Buitrago, 1990; Gomez et al., 1983), but levels as high as 30% have been identified (Buitrago, 1990). Exploitation of foliage in cassava is expected to increase because of the recent developments and testing of mechanical harvesters and alternative cultural practices to exploit it (Cadavid Lopez and Gil Llanos, 2003).

The great diversity of food uses of cassava was thoroughly described by Balagopalan (2002). Some regional and ethnic uses of cassava such as “farinha” and “casabe” (Amazon basin), “kokonte” (Ghana), “gaplek” and “krupuk” (Indonesia) or tapioca pearls (India) would also benefit from adequate DMC and FRY. However other ethnic uses require additional traits. Boiled cassava roots require low levels of cyanogenic glucosides, reduced boiling time and consumer preferred texture. On the other hand, African products such as fufu and gari require proper poundability or mealiness. It is not clear which are the anatomical or biochemical characteristics that define many of these characterisitics. There is ongoing research, however, to elucidate the histological and biochemical basis of these characteristics to ultimately facilitate the selection process made by breeders.

Cassava is a perennial plant species which is handled as an annual crop. It does not have a pre-established development such as that of the cereals where the plants germinate, grow, flower, fill the grain, mature and die. Cassava grows when conditions are favorable and, when they are not the plant drops the leaves and assumes dormancy until favorable conditions return. These characteristics have a profound effect on root quality traits that will be addressed in this report. A distinctive feature of the quality of cassava roots is that it fluctuates widely depending on the age of the plant and environmental conditions. Coping with this variation is a major challenge for several of the many chain values involving this crop.

The difference between root and tubers has profound implication beyond the botanical distinction. Roots, for example, cannot be used for reproductive purpose. The only evolutionary purpose of cassava roots is to serve as a reserve organ to the mother plant. Once roots are excised from the plant that

function ceases to exist. Roots have a very short shelf life due to a process known as post-harvest physiological deterioration (PPD). According to Montaldo (1996) up to 30% of roots arriving to the central market in Caracas (Venezuela) are lost to PPD. In addition to the drastic impact of PPD stored cassava roots undergo changes due to respiration, transpiration and biochemical changes such as conversion of starch into sugars and reduction of ascorbic acid (Montaldo, 1996; Sánchez et al., 2013). It has been estimated that cassava roots lose 1% of starch per day of storage (Sánchez et al., 2013).

1.1. Cassava Breeding for Quality Traits at CIAT: Colombia

1.1.1. Genetic variation among cassava clones adapted to different end uses

Amerindians in the Amazon basin, the likely center of domestication of the crop, have selected varieties for specific end uses over the millenniums. They were the first to recognize the differences in root quality traits, its variation through time and age of the plant, and to develop suitable processing techniques. They recognized, for example, that when roots are harvested too late or "over-matured", they turn fibrous and fail to soften regardless the time they are boiled. In the local language these roots are called "caulla" (Chirif, 2013). People such as Bora, Huitotos and Ocaina (eastern Peru) have recognized three main type of cassava (bitter, good and sweet) based on the cyanogenic glucoside and starch contents. The "good" cassava mentioned above is the one widely used for table consumption: it has average levels of starch or dry matter content and low levels of cyanogenic potential (HCN). High HCN levels, on the other hand, is the distinguishing feature of "bitter" cassava. "Sweet" varieties for these peoples are not the same as cool cassava (e.g. low cyanogenic potential). The former produce a limited amount of starch and is used in the preparation of indeed sweet beverages (called Manicuera in Brazil). It may be more appropriate to call them "sugary" cassavas. This type of cassava has been technically described by Carvalho et al. (2004). Sugary cassava is called "mandiocaba" in Brazil and the roots texture and taste resemble sugarcane stems. This type of cassava is not the subject of the RTB-Foods project and will not be described further.

The cultural differences among tribes in the Amazon basin define the way the consume cassava and this, in turn, define the type of cassava clones grown by them. Bora, Huitoto, Ocaina and Secoya people grow cassava clones that produce bitter roots, which need to be carefully processed to release the high HCN levels present in them. One common and popular product is "cassabe" in the Amazon basin. Another popular product widely spread even in urban centers is "farinha". Other tribes, however, grow good cassava and boil or roast their roots for consumption (Chirif, 2013). There is a generalized belief that bitter cassava has higher productivity; higher content and better quality of starch; enhanced resistance to pests and that they are better suited for the production of cassabe (Isendahl, 2011). However, there is also a generalized dissent regarding these beliefs. For example, Secoya people acknowledge that roots from good and bitter clones are equally suitable for the production of cassabe (Chirif, 2013). There is some scientific support to the idea that bitter cassava is more tolerant to pests, although there is no indication that high levels of cyanogenic glucosides prevent the attach by arthropods (insects and mites can prosper equally well in bitter and good cassava), it is possible that mammals avoid bitter ones (Chiwona-Karltun et al., 1998). There is also circumstantial evidence that the additional requirement of low cyanogenic potential for good varieties to the list of traits required reduces, to some extent, the probabilities of identifying genotypes that are also high yielding. This is the reason why the cassava breeding program at CIAT abandoned the idea of breeding for double-purpose cassava varieties (e.g. clones that are outstanding for both fresh consumption and industrial processing) by the year 2000.

This distinction in root quality traits, particularly HCN, is so important that it has even lead to the use of two different scientific names for such type of cassava: Manihot palmata and M. aypin. These two taxa, however, are no longer accepted and all cassava germplasm is now grouped into the M. esculenta gene pool. The distinction of bitter and sweet cassava genotypes is relevant for RTB-Food because cyanogenic potential is one key characteristic taken into consideration when boiling the roots for table consumption.

1.1.2. Quality traits required for boiling cassava and marketing considerations

The value chain of roots for boiling are relatively straightforward and, in some cases, require simple processing. Until few decades ago roots were sold in local markets and, more recently, in supermarkets. PPD is a major bottleneck in the marketing cassava roots for table consumption and the simple processing mentioned above is mainly directed at preventing it. Roots may be sold as they are harvested with just brushing and/or washing them to remove the soil from their surface (Figure 1.A). A second alternative is to peel the roots after brushing and washing, cut them in sections of suitable size and quick freeze them (Figure 1.B). This approach allows storing the roots for several months and is often used for the export markets to satisfy the ethnic markets in developed countries in North America, Europe and Asia. In addition to brushing and washing the roots may also be waxed with melted paraffin to extend their shelf life for up to 2-3 weeks (Figure 2). There are emerging technologies that all require roots with low cyanogenic potential and good cooking quality (Figure 3).

Regardless of the specific value chain roots for table consumption must have low HCN levels, adequate levels of DMC (roots suitable for boiling have usually intermediate levels) and excellent taste and texture after boiling. When roots are sold in local markets and supermarkets the external appearance (e.g. size, external and internal color of the peel, color of the parenchyma, etc.) is very important. Buyers (more often than not women) use these external traits as morphological markers that are traditionally used to identify roots from clones that are known to have good cooking quality. Although these morphological traits are not necessarily linked to good cooking quality breeders must be aware that they matter. Chiroza is the best-known variety for table consumption in Colombia. People are used to scratch the outer peel of the roots in search of a pinkish to purple coloration underneath the peel. This is a key marker associated with good cooking quality in Colombia. However, roots from many clones have the same characteristics but fail miserably to meet the standard for cooking quality. Similarly, other clones with excellent cooking quality fail to show that key pinkish to purple coloration.

Figure 1. Different value chains of cassava roots for table consumption. A. African local markets. B. Frozen sections of cassava roots sold in a Colombian supermarket.

The size and shape of the root is also important, not only when they are sold in local markets and supermarkets but also for the waxed roots value chain. Ideally roots should have intermediate (around 30 cm long) and diameter (8-10 cm) size, a conical or cylindrical shape. The peduncle should not be too short otherwise the parenchyma may be damaged while detaching the roots from the mother plant, thus promoting rapid onset of PPD. Length of peduncle and shape of the root and have been reported (Luciani, 1996) to have relatively high [broad sense] heritability (36 and 48%, respectively). Long roots break more easily and this also results in quick onset of PPD. Round or globular roots tend to suffer

more peel damage during transport and storage than those with a cylindrical shape (Aristizábal et al., 2007).

Figure 2. Illustration of simple processing technologies of cassava for table consumption. A. Selection in the field of roots with adequate size and shape. B. Brushing and washing roots. C. Treating roots with fungicide. D. Drying roots after fungicide treatment. E. Waxing roots with melted paraffin. F. Illustration of waxed roots (top) and non-waxed roots bagged and ready to delivery, for example, to restaurants.

Figure 3. A. Illustration of frozen cassava kept at temperatures below 0 °C. B. An emerging market for table consumption of cassava in China. C. Boiled and canned cassava roots.

Sanitary issues are certainly a key requirement for cassava roots that will be sold for the table consumption. In addition to the non-infectious PPD process, roots may rot because of bacterial or fungal infections. Roots showing even mild symptoms should be discarded, otherwise the entire batch may eventually be affected. In some cases, symptoms may be difficult to detect. For example, symptoms of the subterranean burrower bug (*Cyrtomenus bergi* Froeschner) cannot be detected before peeling the

roots. The symptoms induced by this subterranean sucking insect are particularly damaging for the frozen cassava roots value chain (Figure 1.B).

1.1.3. Post-harvest physiological deterioration (PPD)

Cassava roots have a very short shelf life because of PPD. The visible symptoms of PPD in the roots are the blue-black discoloration that starts in the vascular tissue towards the periphery of the parenchyma (Han et al., 2001; Reilly et al., 2001; 2003; 2007; Wheatley, 1982; Wheatley and Gomez, 1985). Over time symptoms spreads extensively through much of the root. Fluorescence under UV light is detectable prior to and during the discoloration process (Liu et al., 2017). PPD rapidly renders the roots unpalatable and unmarketable. Consequently cassava roots need to be consumed soon after harvest (van Oirschot et al., 2000). The processes involved in PPD resemble changes typically associated with the plant's response to wounding. The symptoms result from the accumulation of phenolic compounds and their oxidation by reactive oxygen species (Djabou et al., 2017; Liu et al., 2017; Sánchez et al., 2013). Specific genes involved in PPD have been identified and characterized, and their expression evaluated. Several secondary metabolites, particularly hydroxycoumarins, such as scopoletin, accumulate in the process (Bayoumi et al., 2010; Blagbrough et al., 2010; Gnonlonfin et al., 2012; Liu et al., 2017; Reilly et al., 2007; Sánchez et al., 2013; Uarrota and Maraschin, 2015; Uarrota et al., 2015).

Shelf life of cassava roots can be extended by different treatments (e.g. waxing their surface to isolate the parenchyma from oxygen, freezing or drying). Recently the exogenous application of melatonin was reported to delay PPD (Hu et al., 2016). However, these methods are expensive or logistically difficult to implement. There are several reports on genetic variation for the reaction to PPD (Liu et al., 2017; Morante et al., 2010; Moyib et al., 2015). Genetic tolerance to PPD is envisioned as the most economical approach to overcome the problem. Vlaar and co-workers estimated in 2007 that extending shelf life of cassava roots to 45 days after harvest, would result in a benefit of approximately US $ 35 million for Thai cassava farmers and factory owners. However, breeding approaches have not proved successful due the polygenic nature of the trait and the unfortunate correlation between PPD with dry matter content (DMC). A major factor affecting research on PPD is the large influence of environmental conditions (particularly at harvest time), age of the plant, handling of the roots during harvest and thereafter, storage conditions, etc. (Cortés et al., 2002; Morante et al., 2010; Sánchez et al., 2006; 2013). Experimental errors, therefore, are typically large and this tends to mask true genetic differences that may exist. In spite of early promising results (Sayre, 2011) and huge financial efforts, genetic transformation did not contribute to alleviating the problem either. Regardless the difficulties associated with research on PPD in cassava few trends have been clearly established over the years: **a.** It is a process involving the genetic system of the plant along with reactive oxygen species; **b.** Environmental factors influence it greatly; **c.** There are genetic factors influencing it as well; **d.** Dry matter content in the roots is positively correlated with PPD; **e.** There is a negative association between PPD and carotenoids content; **f.** PPD has been linked to scopoletin in the roots; and **g.** Experimental errors associated with measuring PPD are very large.

Breeding for tolerance to PPD is difficult because proper assessment of the reaction to PPD requires relatively large number of commercial-size roots. Assessment is based on a destructive assay that is affected by large variation often resulting in high experimental errors (as some of the results presented in Table 1 illustrate). In addition, most of the germplasm is discarded at early stages of selection when only one or a few plants per genotype are available and, therefore, not enough roots can be harvested for properly quantifying PPD (Morante et al., 2005). Little progress, therefore, has been made so far to improve tolerance to PPD through genetic enhancement. There is growing evidence that yellow roots tended to have a delayed onset of PPD by one or two days. Dry matter content and PPD are known to be positively correlated (Morante et al., 2010). Pruning the plants a few days before harvest also delays PPD, but at the expense of a reduction in dry matter content of the root (van Oirschot et al., 2000). These alternatives do not offer a practical solution and PPD has remained an elusive problem to solve.

One of the main problems related to root quality traits, including reaction to PPD, is the variation related to the age of the plant and environmental conditions during its growth, particularly at the time of harvest. Studies related to PPD are particularly weak because of the strong influence of these factors. At CIAT

a lengthy study was carried out as a continuation of the research reported by Sánchez et al. (2013). It focused only in PPD, DMC and scopoletin, but through the analysis of roots from five different clones. There was no genotype involving yellow roots in this study. Analyses were conducted over three harvesting seasons from 2013 through 2015. Storage period was up to seven days after harvest (DAH). Data has not been yet formally published. Table 2 provides the results of the analysis of variance for PPD, DMC and scopoleting content (on freh and dry weight basis). All main sources of variation and their interactions were found to be highly significant (except for the interaction between year and duration of the storage period, which was significant only at 5% probability level). These results illustrate the complexity of the reaction to PPD and traits that influence it).

The averages for the three main sources of variation are presented in Table 3. The variation observed for the averages of the three years is particularly striking. As expected, PPD levels increased drastically after two days of storage. The lowest average PPD levels were observed in AM 206-5, which is also the clone with the lowest DMC. In general, the higher the DMC the higher the PPD. Scopoletin levels were particularly high in clone HMC-1. There was no apparent relationship between scopoletin and PPD levels looking at the average values presented in Table 3.

Table 1. Post-harvest physiological deterioration (PPD) quantified 5, 10, 20 and 40 days after harvest. Average total carotenoid content (TCC) and dry matter content (DMC) Source: Morante et al. 2010

Clone	Post-harvest physiological deterioration (%)					**TCC**	**DMC**
	5	10	20	40	**Mean**	(ug g^{-1})	**(%)**
Commercial checks							
CM 523-7	27.1(5)	40.7(5)	57.1(5)	64.1(5)	**47.2**	0.4	**44.8**
MCol 1505	25.7(5)	31.6(5)	71.6(5)	66.4(5)	**48.8**	0.7	**40.1**
MPer 183	5.4	4.0	5.3	9.2	**6.0**	0.5	**41.3**
Mutagenized (gamma rays irradiation) genotypes							
2G15-1	0.5(2)	0.0(1)	0.0(1)	6.9(3)	**1.9**	1.0	**44.0**
5G108-4	2.9(3)	3.7(3)	7.1(3)	7.3(3)	**5.3**	0.7	**45.4**
Back-crosses from crosses between *M. esculenta* and *M. walkerae*							
CW 429-1	12.5(4)	20.7(4)	23.2(4)	18.6(4)	**18.7**	0.6	**37.2**
BC284-42	16.8(4)	14.1(4)	16.0(4)	n.a.	**15.6**	0.7	**40.5**
BC284-49	4.7(3)	4.8(3)	23.3(4)	n.a.	**10.9**	2.5	**27.4**
BC289-30	0.0(1)	1.0(3)	0.0(1)	0.0(1)	**0.3**	0.5	**34.5**
Selected material with increased levels of carotenoids in the roots							
CB 7-9	3.6(3)	10.9(4)	0.0(1)	1.0(2)	**3.8**	10.2	**35.8**
CB 44-15	0.5(2)	0.0(1)	1.0(3)	1.0(3)	**0.6**	11.5	**29.5**
GM 905-66	0.0(1)	0.0(1)	0.0(1)	0.0(1)	**0.0**	11.1	**38.3**
MBra 253	1.5(3)	0.0(1)	2.9(3)	0.0(1)	**1.1**	9.5	**42.0**
MCol 2436	0.0(1)	26.3(5)	38.9(4)	n.a.	**21.7**	9.1	**34.6**
Waxy starch sources							
AM 206-5	0.0(1)	0.0(1)	0.0(1)	0.0(1)	**0.0**	0.7	**38.5**
Waxy 2	3.7(3)	8.0(3)	3.6(3)	3.6(3)	**4.7**	0.6	**35.6**
Waxy 3	0.2(2)	0.0(1)	3.7(3)	6.8(3)	**2.7**	0.5	**42.2**
Waxy 4	0.0(1)	0.0(1)	0.0(1)	0.0(1)	**0.0**	0.6	**36.2**
Waxy 5	0.0(1)	0.0(1)	4.1(3)	0.0(1)	**1.0**	0.9	**40.2**
Waxy 6	3.4(3)	1.4(3)	4.7(3)	2.2(3)	**2.9**	0.5	**36.1**
Waxy 7	18.2(5)	31.1(5)	30.4(4)	30.4(4)	**27.5**	1.0	**40.0**
Mean	**6.0**	**9.4**	**13.9**	**12.1**	**10.5**	**2.8**	39.9

Significance of reaction types: (1) Significantly lower than the average PPD of tolerant check (confidence intervals); (2) PPD values significantly lower than that of the tolerant check (LSD test); (3) PPD values similar to that of the tolerant check; (4) PPD values significantly higher than that of the tolerant check but lower than those of the susceptible checks; (5) values significantly higher than that of the tolerant check and similar to those of susceptible checks.

Table 4. Presents the results of step-wise regression analyses across genotypes (top of the table) and for each individual clone. The best model to explain PPD across genotypes included duration of the storage period as the first parameter. Duration of the storage period was also the most important independent variable when reaction to PPD was analyzed individually in each genotype, except for clone MCol 22. DMC was the second most important parameter in the model for the analysis combined across genotypes followed by scopoletin on a dry weight basis and finally scopoletin on a fresh weight basis.

However, DMC was not so important in the analysis for each clone. This makes sense as variation in DMC is mostly between the different clones, and there is reduced variation for this variable among roots from the same clone.

Table 2. Mean squares from the analysis of variance for PPD (transformed by the Arc sine function), DMC and scopoletin expressed on a fresh- and dry-weight basis.

Source of variation	df	PPD	DMC	Scopoletin (nmol/g)	
		Arc Sin (%)	(%)	FW	DW
Year	2	0.501**	1203.20**	20451**	94355**
Day	7	2.505**	4.97**	5964**	39710**
Clon	4	1.491**	444.95**	9245**	45355**
Year*Day	13	0.059*	8.97**	1110**	6603**
Clon*Day	28	0.075**	9.33**	491**	2724**
Year*Clon	8	0.300**	39.38**	1481**	5520**
Year*Clon*Day	51	0.055**	5.51**	221**	1185**
Error	563	0.034			
	194		0.03		
	114			8.7	48

*, ** Significant at the 5% and 1% probability level, respectively.

Table 3. Averages for the four variables analyzed in this study for the three most relevant sources of variation (years, duration of the storage period and clones).

Class	PPD[1]			DMC[1]			Scopoletin FW[1]			Scopoletin DW[1]		
	n	(%)		n			n	(nmol/g)		n	(nmol/g)	
Years												
2013	238	8.73	b	120	33.58	c	80	19.18	c	80	57.12	c
2014	209	15.53	a	71	40.30	a	70	52.16	a	70	127.65	a
2015	230	15.37	a	117	38.40	b	78	32.19	b	78	82.38	b
Duration of the storage period												
0	90	0.00	e	41	36.69	d	30	3.27	g	30	8.83	f
1	90	1.73	d	40	37.29	b	30	29.24	e	30	73.92	e
2	90	12.79	c	40	37.35	b	30	50.61	a	30	131.16	a
3	89	13.68	c	40	37.10	c	30	37.65	c	30	95.59	c
4	90	17.53	b	40	36.46	e	30	42.43	b	30	110.89	b
5	90	19.07	a b	40	37.17	c	30	35.63	d	30	92.30	d
6	89	22.59	a	40	37.45	a	30	41.78	b	30	106.65	b
7	49	21.06	a	27	35.78	f	18	26.59	f	18	74.57	e
Clone												
AM 206-5	131	7.10	d	60	33.40	e	44	23.11	c	44	67.88	d
MCOL22	134	8.69	d c	62	36.77	c	46	23.96	c	46	62.40	e
MPER183	136	9.65	c	62	35.36	d	46	31.76	b	46	86.94	b
HMC-1	138	14.72	b	62	39.24	b	46	58.04	a	46	140.95	a
CM523-7	138	24.80	a	62	39.91	a	46	31.44	b	46	78.08	c

[1] Duncan and LSD tests for contrasts among averages yielded the same conclusions regarding the statistical differences among them. For each category and response variable, averages with the same letter are not significantly different.

Results presented in Tables 2-4 are presented to highlight the complexities when analyzing PPD. The R^2 value in the analysis across clones was around 0.30. This clearly demonstrate that factors other than DMC and scopoletin have strong influence on PPD. The higher the average PPD, however, the better the model explained the variation on PPD. In the case of CM 523-7 the average PPD was the highest 24.8 (Table 3) and the R^2 value in the regression analysis (Table 4) was relatively high (0.43).

Table 4. Relevant results of the regression analyses conducted across clones and for each individual clone. The sequence of the parameters in the model has been respected in each case. The estimates are presented for each parameter and the respective sum of squares are also presented within parentheses. In some cases (clones AM 206-5 and MPER 183) the best model was obtained by using

the scopoletin quantification at day 0 either on a fresh weight (Sco. FW(0)) or dry weight (Sco. DW(0)) basis.

Sum of s**quares**		**R^2**	**Parameters following the sequence in the best model**				
			Intercept	Day	DMC	Sco.	Sco.
Model	Residual			(n)	(%)	DW	FW
Regression across the five clones							
63562	159050	0.286	-58.162	3.253	1.552	0.185	-0.402
			(115961)	(38581)	(24093)	(887)	(989)
AM 206-5							
4462	11516	0.279	Intercept	Day	Sco.DW(0)		
			-7.281	2.377	0.782		
			(6605)	(3482)	980		
MCOL 22							
8845	22256	0.284	Intercept	Sco.FW	Day	Sco.DW	
			-1.166	2.564	2.870	-0.977	
			(10114)	(6032)	(1232)	(2351)	
MPER 183							
6405	15202	0.297	Intercept	Day	Sco.FW(0)	DMC	EscDW(0)
			118.302	2.189	2.240	-2.114	-4.826
			(12675)	(4046)	(1202)	(904)	(257)
HMC-1							
12597	27810	0.312	Intercept	Day	Sco.FW		
			-2.734	3.559	0.095		
			(29898)	(10740)	(1857)		
CM523-27							
37036	48303	0.434	Intercept	Day	Sco.FW	DMC	
			-90.599	5.537	0.313	2.165	
			(84846)	(18721)	(15338)	(2977)	

It is clear that there is genetic variation regarding PPD among different sources and they seem to be acting through different biochemical/genetic mechanisms. Further studies should concentrate on elucidating which of the genes involved in the cascade of reactions leading to PPD are related to the different sources of tolerance. For the time being, unfortunately, the only reliable way for determining the reaction to PPD of a given genotype is by testing at least ten roots in at least three different seasons. Storing roots for 5-7 days is adequate to maximize differences among genotypes (Ospina et al., 2018a: 2018b; Tran et al., 2018).

1.1.4. Dry matter content

DMC is an important root quality trait. It is critical for industrial uses of cassava roots (starch, animal feed and ethanol industries) but also influences cooking quality. A clear example of the relationship between DMC and cooking quality was published by Wheatley in 1991. Hongbété et al., (2011) supported these earlier findings. When DMC is low, the uncooked root looks “watery” and, after boiling, it assumes a “glassy” appearance (tends to be translucent and its texture is hard to penetrate). A reduction in the level of DMC (and a parallel increase in sugar contents) results in glassiness in the boiled root, which is highly undesirable. Wheatley linked the reduction of DMC to stresses suffered by the plants.

Table 5. Relationship between the undesirable glassy characteristic in boiled roots and dry matter, starch and total sugars contents. Source: Wheatley, 1991.

Glassiness[a]	**Dry matter content (%)[b]**	**Starch content(%)[b]**	**Total sugars content (%)[b]**
0.0	35.9ab	86.4a	1.82a
0.0	35.9ab	86.8a	2.72b
1.0	37.4a	87.3a	2.53b
1.3	34.4b	85.9b	3.62c
2.0	33.2c	82.3b	5.13d
2.3	33.4c	79.7b	4.97d

[a] Glassiness estimated using a score in which 0=not glassy; 1=slightly glassy; 2=glassy; and 3=strongly glassy
[b] Values within a column with different letters indicate significant differences at the 5% probability level

DMC varies widely in cassava. Sanchez et al. published in 2009 a study involving a large sample of cassava clones from the germplasm collection at CIAT (3272 accessions), 772 improved genotypes and 12 wild Manihot species. Table 6 provides a summary of the most relevant traits in this study, including DMC. Figure 4 presents a histogram with data for DMC showing the slight negative skewness (a tendency for longer tails to the left). Average DMC was 33.6%. As it is the case for PPD, DMC for a single genotype varies drastically with age of the plant and environmental conditions.

Table 6. Root quality traits from more than 4000 cassava genotypes (3272 landraces, including 12 wild relatives, from the germplasm collection at CIAT and 772 improved clones). Source: Sanchez et al., 2009.

	Dry matter	**Starch**	**Cyanogenic**	**Ease of**
Parameter	content	content	potential	**Cooking**
	(%)	(%)	(ppm)	**(min)**
Maximum	48,1	91,0	3274	**5,6**
Minimum	14,3	65,0	14	**1,1**
Average	33,6	84,5	327,4	**2,8**
Standard Deviation	6,47	3,34	397,7	**0,72**
Skewness	-0,40	-0,65	2,96	**0,33**
Count	**4051**	**4049**	**4050**	**4051**

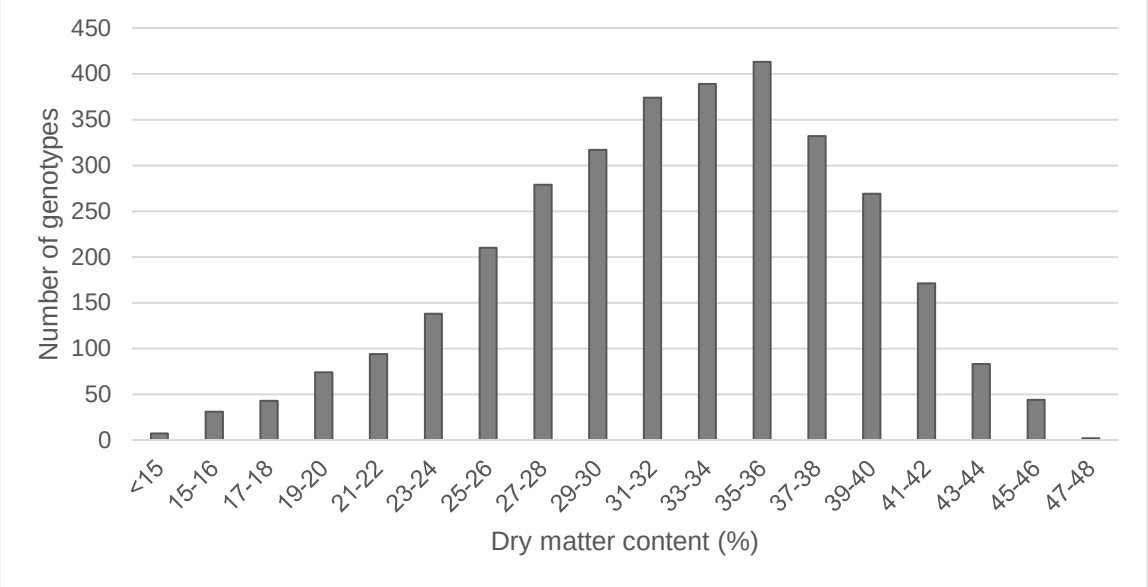

Figure 4. Histogram with frequencies for dry matter content in more than 4000 cassava genotypes (3272 landraces, including 12 wild relatives, from the germplasm collection at CIAT and 772 improved clones). Source: Sanchez et al., 2009.

There is considerable amount of information in relation to DMC in cassava roots. Cassava responds well to selection to increase DMC and many reports, particularly from SE Asia, demonstrate it (Kawano, 2003; Kawano and Cock., 2005; Kawano et al., 1998). DMC in the roots is highly heritable but also greatly affected by the environment and age of the plant. Broad sense heritability for DMC is intermediate (0.42) according to Kizito et al., 2007.Genotype-by-environment interactions, therefore, are usually significant (Benesi et al., 2004; 2008; Kawano et al., 1987; Morante et al., 2006). Cassava is generally grown in areas with a lengthy dry season. In these cases, farmers typically plant the stem cuttings soon after the arrival of the rains and harvest the roots (and the stems that will be used as planting material for the following cycle) just before the start of a new rainy season, around 10-12 months after planting (MAP). This system allows harvesting the roots when their DMC is at a maximum (around 35%) and results in a short storage period for the stems. During the dry period, cassava drops the leaves and enters a “dormant” state. With the arrival of the rains, starch from the roots is hydrolyzed for the mother plant to have the energy it requires to reinitiate growth. Therefore, when cassava is harvested after the arrival of the rains, DMC is reduced drastically to the point that industries often reject the roots (Bakayoko et al., 2009; Hongbété et al., 2011). Figure 5 illustrates this phenomenon. Data

comes from the harvest of a single row trial split into two harvesting dates (before and after the arrival of the rains). Wheatley indicated that variations in DMC affect cooking quality. Over-matured roots turn fibrous and fail to soften regardless the time they are boiled (Chirif, 2013).

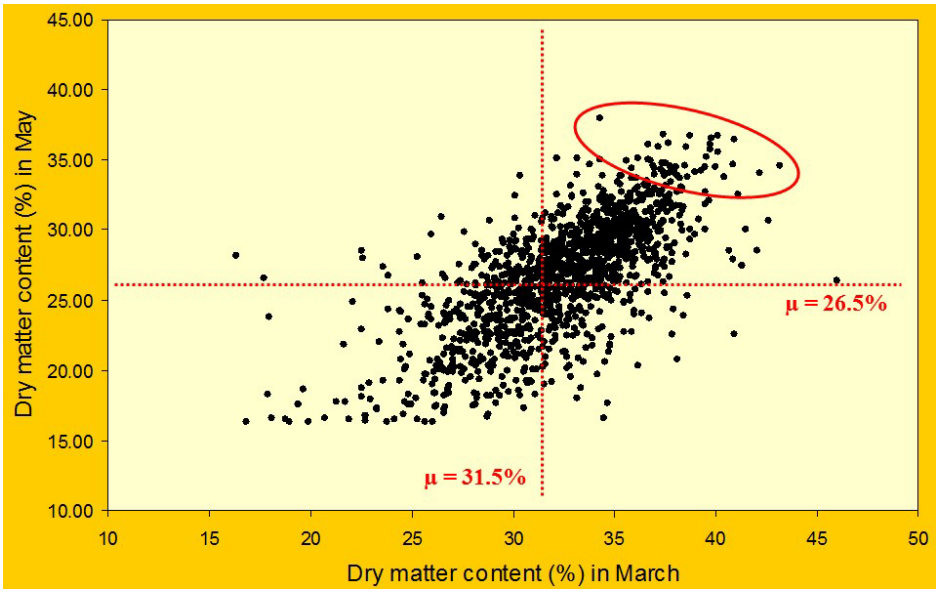

Figure 5. Dry matter content (%) of genotypes in a single row trial harvested at two different dates, before (March) and after (May) the arrival of the rains. In the later harvest, DMC fell drastically. A group of genotypes, however, showed excellent DMC at both dates.

In southern South America., cassava can be harvested just before the onset of winter (8-10 MAP) or, left in the field through the winter, and harvested after a second vegetative growth cycle (Kvitschal et al., 2009; Sagrilo et al., 2008). In this system productivity in late harvests increases considerably and DMC can be recovered to acceptable levels. CIAT, attempting to simulate such a system, harvested elite cassava germplasm 16-18 MAP in the seasonally dry environment of Colombia's northern coast in the Caribbean. However, elite germplasm failed to recover acceptable levels of DMC and increases in productivity were not attractive enough. In certain dry areas, cassava is harvested only at the end of the dry period of the second growth cycle. This is the case of NE Brazil (e.g. Pernambuco State) and many sub-Saharan locations where the rainy season is too short and productivity of cassava is acceptable only after 24 MAP. These extreme conditions are not the target environment of the main cassava-growing regions in the world, which is characterized by a relatively long rainy season with abundant rainfall and a dry period long enough to result in the seasonality of harvests.

The most relevant result of ongoing research at CIAT is the evidence that there is genetic variation for the capacity to recover DMC levels after the arrival of the rains and the appealing increase in FRY when the crop is left for additional 6-8 months in the field. However, these results were achieved in materials specifically bred for this kind of management. Table 7 presents the results from Advanced and Regional Evaluation Trials involving genotypes specifically bred for high and stable DMC. Results from this work suggest that optimum performance in delayed harvest cannot be expected (necessarily) from normal evaluation schemes. Other research arrived at the same conclusions (Bakayoko et al., 2009). However, evaluations involving extended growing periods allow the identification of promising genotypes. Commercial checks, as expected, did not perform sufficiently well to be suitable for this alternative cropping system.

A key learning lesson from these experiences is that harvesting at the end of the dry season increases heritability of DMC. Similarly, the reliability of phenotypic evaluations, after cassava reinitiates its growth with the arrival of the rains, decreases.

Table 7. Summary of the evaluation across 39 trials of 15 experimental clones and 5 commercial checks. The best five clones (across traits) are listed on top. Then the performances of four clones, outstanding for just one trait (e.g. among the best five for the trait) are presented. At the bottom are the

five commercial checks. Harvests took place from 9 to 18 months after planting. Source: Lenis et al., 2018

Clon	n	FRY[a]			DMC[a]			DMY[a]		
		t/ha[b]		Rank	(%)[b]		Rank	t/ha[b]		Rank
SM3134-5	169	27.06	a	2	35.08	ab	2	9.60	a	1
SM2834-31	177	24.73	bc	4	34.39	cde	5	8.59	bcd	3
SM2629-36	168	27.26	a	1	32.27	hi	16	8.79	b	2
GM579-13	168	25.17	c	3	32.54	hi	15	8.25	bcd	4
SM2828-28	170	23.51	c	10	34.36	de	6	8.12	cde	6
SM3110-15	176	24.41	bc	5	33.22	g	13	8.16	cd	5
SM3139-22	167	21.18	de	12	34.60	cd	4	7.43	ghf	10
SM3134-73	172	20.89	de	13	34.77	bc	3	7.35	gh	11
SM3144-42	167	14.82	h	20	35.25	ab	1	5.30	k	20
Tai	157	24.40	bc	6	29.72	l	19	7.28	gh	12
Costeña	160	20.38	de	15	31.71	j	17	6.57	ij	15
Verónica	121	19.94	ef	16	31.06	k	18	6.32	j	17
Ginés	116	24.19	bc	8	29.59	l	20	6.98	hi	14
Caiseli	96	17.80	fg	18	34.07	ef	9	6.17	j	18

[a] FRY: fresh root yield; DMC: dry matter content; DMY: dry matter yield
[b] Averages (for each trait) followed by the same letter are not significantly different at P ≤ 0.05

Figure 6 summarizes the analysis of DMC in roots from seven different clones harvested every other week for a period of 15 months. These clones had been planted also every other week thus roots were harvested always at the same age, but under varying environmental conditions. Figure 6 is presented to illustrate two main features: ***i)*** the differences in DMC in some harvesting dates were very wide (e.g. 26 to 39%) whereas few days later they shrank drastically (e.g. 35 to 41%); ***ii)*** only after several harvests at different times it becomes clear that clone CM523-7 tends to have higher and more stable DMC than, for example, roots from MPer 183. In many cases DMC in roots from CM523-7 was much higher than those from MPer 183. However, sometimes the differences became negligible and, in few cases, the tendency even reverted. As was the case for PPD (and perhaps because of the variation in DMC) there is wide oscilation in DMC and, therefore, only after many harvests at different times it is possible to reliably detect genotypic differences. This is very relevant because DMC, to some extent, influences cooking quality as demonstrated by Wheatley in 1991 and reaction to PPD (as demonstrated above).

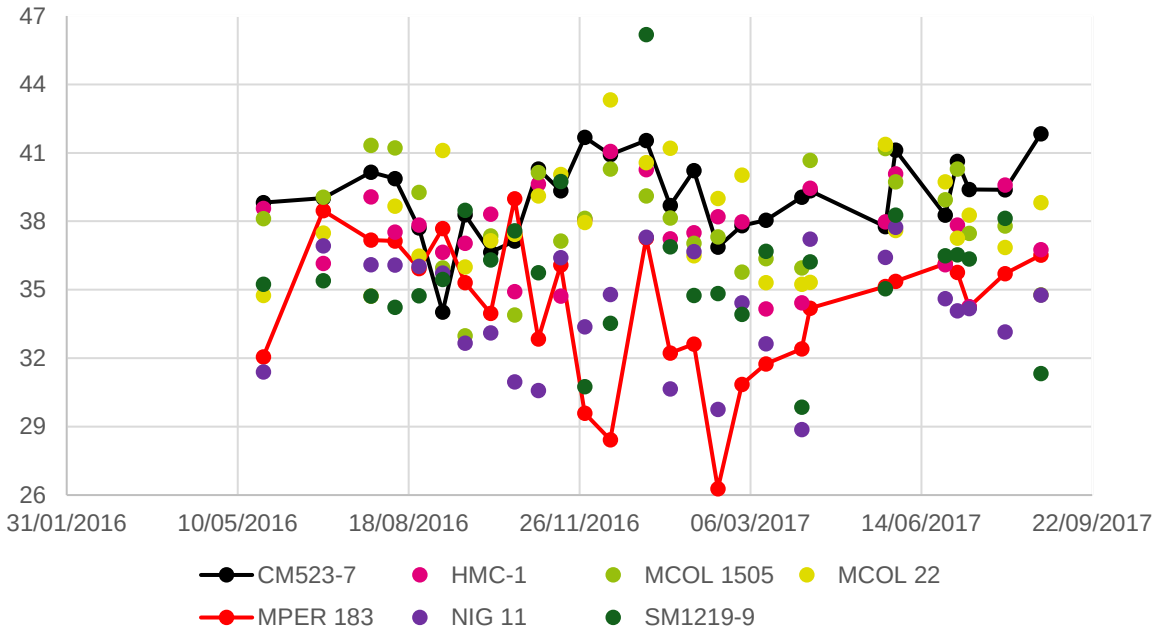

Figure 6. Dry matter content (%) from seven genotypes harvested every other week through 15 months of harvest in a single location (Palmira, Colombia). Source CIAT (unpublished data).

1.1.5. Cyanogenic potential

It was mentioned in the introduction that only "*good*" cassava (low cyanogenic potential) is suitable for boiling (Chirif, 2013). Farmers and folk culture are well aware of the differences between high and low cyanogenic cultivars and their uses (Mkumbira et al., 2003; Peroni et al., 2007). Interestingly, bitter cassava varieties tend to predominate in areas were cassava is an important crop. Good clones tend to occupy smaller areas around the households (Dufour, 1993; 1995). The distinction and separation between bitter and cool clones has led to a genetic differentiation and incipient creation of two gene pools (Peroni et al., 2007).

High levels of cyanogenic glucosides (HCN) affect the cooking quality of the roots because boiling does not remove all of them. An unacceptably high level of cyanogenic glucosides remain in boiled roots from bitter varieties which results in unpleasant taste. Aroma and taste differences may remain after different approaches to produce and consume cassava flour (Iwuoha, 1997). Although bitterness is mainly attributed to the cyanogenic glucosides in the roots other compounds in the parenchyma and cortex have been detected to contribute to the taste (King and Bradbury, 1995; Wheatley et al., 1991). Moreover, the remaining cyanogenic glucosides may reach toxic levels (Wheatley et al., 1991). Bitter cassava has been associated with several health problems (Oluwole et al., 200; Tylleskär et al., 1991). Taking Africa as a whole, the cyanogenic related Konzo disease may not be a major public health problem. However, for affected communities, the disease is a major burden. Increasing cassava production, declining production of other foods, global warming, more frequent droughts, wars and social unrest and population displacement have set the schene for Konzo to persist (Nzwalo and Cliff, 2011). Konzo is a distinct neurological entity with selective upper motor neuron damage, characterized by an abrupt onset of an irreversible, non-progressive, and symmetrical spastic para/tetraparesis. The disease is associated with high dietary cyanogen consumption from insufficiently processed roots of bitter cassava combined with a protein-deficient diet.

As Amerindians did thousands of years ago, cassava breeders today develop varieties with high, intermediate and low levels of cyanogenic potential. Each type of clone will serve specific purposes. The work published by Sanchez and co-workers in 2009 also involved measuring HCN. Table 6 shows that the range of variation was from 14 to 3274 ppm, with strong positive skewness. Figure 7 presents detailed information regarding the distribution frequencies of HCN in a large sample of genotypes. The skewness value in Figure 7 suggested the long tale to the right observed in Figure 7. Improved

germplasm tend to have lower levels of HCN but selection for it is required (Pizarro et al., 2018). Transgenic acyanogenic cassava has been produced but not grown commercially (Jørgensen et al., 2005)

Cyanogenic potential is controlled by quantitative inheritance genes and broad sense heritability values in five different trials ranged from 0.00 to 0.50 with an average of 0.25 (Dixon et al., 1994). According to Kizito and co-workers (2007), broad sense heritability for HCN is intermediate (0.43). The contrasts in these heritability values are likely due to the influence of environmental conditions: The evaluations by Dixon and co-workers included genotype-by-environment interactions, whereas Kizito's work was conducted in a single location. Transgressive effects have been reported for HCN (Whankaew et al., 2011) but a different study found the inheritance to be mostly additive in nature (Kizito et al., 2007). Traditional farmers are well aware that the level of cyanogenic potential varies with the environmental conditions (Chirif, 2013; Grace, 1977). These empirical observations have been validated through scientific research (Burns et al., 2010; Dixon et al., 1994; Vandegeer et al., 2013). Cultural practices and soil fertilization also influence cyanogenic potential (Omar et al., 2012). Molecular marker studies have been conducted for the development of QTLs. (Kizito et al., 2007; Mkumbiraet al., 2003; Whankaew et al., 2011) but they have not been used to support breeding efforts for reduced HCN levels. Marker-assisted selection, therefore, has not been yet implemented.

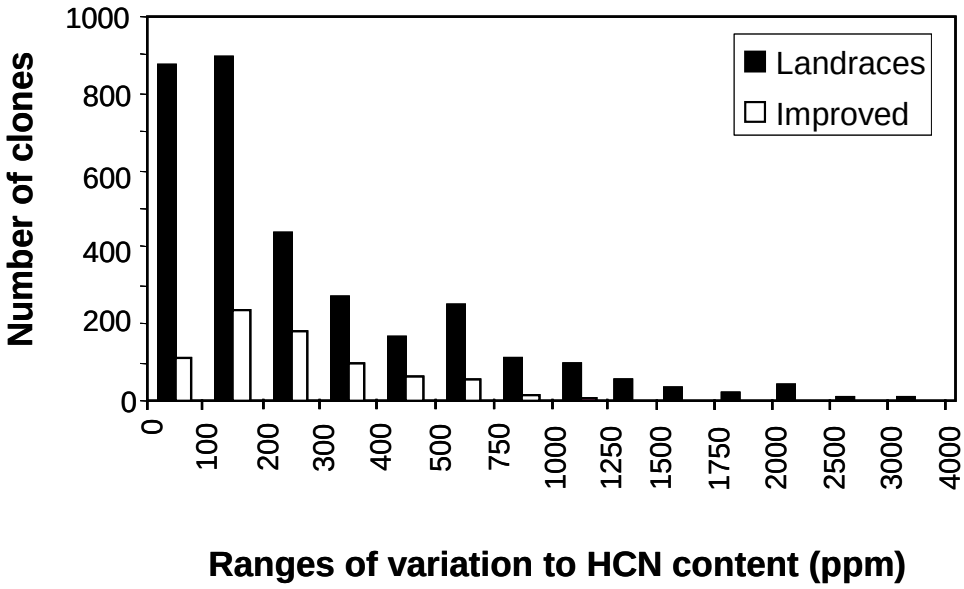

Figure 7. Histogram illustrating distribution of cyanogenic potential (HCN in ppm) of landraces and improved cassava. There is a clear asymmetry with a long tale to the right and the distribution of improved clones tends to be more concentrated around lower HCN values.

There are two main approaches to assess levels of cyanogenic potential. Total cyanide content can be measured quantitatively through a colorimetric protocol after coloration with 1,3-dimethyl barbiturate/isonicotinate reagent (Essers et al., 1993). This method requires hydrolysis with exogenous linamarase prepared in the lab from cassava peel extraction. A qualitative approach has been used while harvesting cassava in the field. A simple picrate paper kit method is useful for qualitatively assess cyanogenic potential while harvesting cassava in the field. Root samples are placed in glass test tubes and left to react overnight with the previously prepared reagents, including yellow picrate paper (Egan et al., 1998). The correlation of both methods is excellent (Borges et al., 2002). It has been postulated that NIRS can be used to predict cyanogenic potential in fresh cassava roots (Davrieux et al., 2016). Therefore, CIAT began building a database (> 3000 samples) in which NIRS spectra from fresh roots are taken along with the quantification of cyanogenic potential. Unpublished results suggest that NIRS can distinguish, at least, genotypes with low (< 200 ppm), intermediate (200-600 ppm) and high (> 600 ppm) of cyanogenic potential. Figure 8 presents actual HCN values in 3051 root samples and those predicted by NIRS. The R^2 value is 0.73. However, this data covers a wide range of variation for HCN. It would be important to improve the reliability of predictions to separate samples below or above a threshold (e.g. 200 ppm).

The cassava-breeding program at CIAT has established a threshold of 200 ppm in the selection for genotypes suitable for the table consumption markets (e.g. > 200 ppm is rejected). This is a rather high threshold. The main justification for this decision is that, given the large variation and environmental influence on HCN expression, it is acceptable to have a lenient approach in early stages of selection.

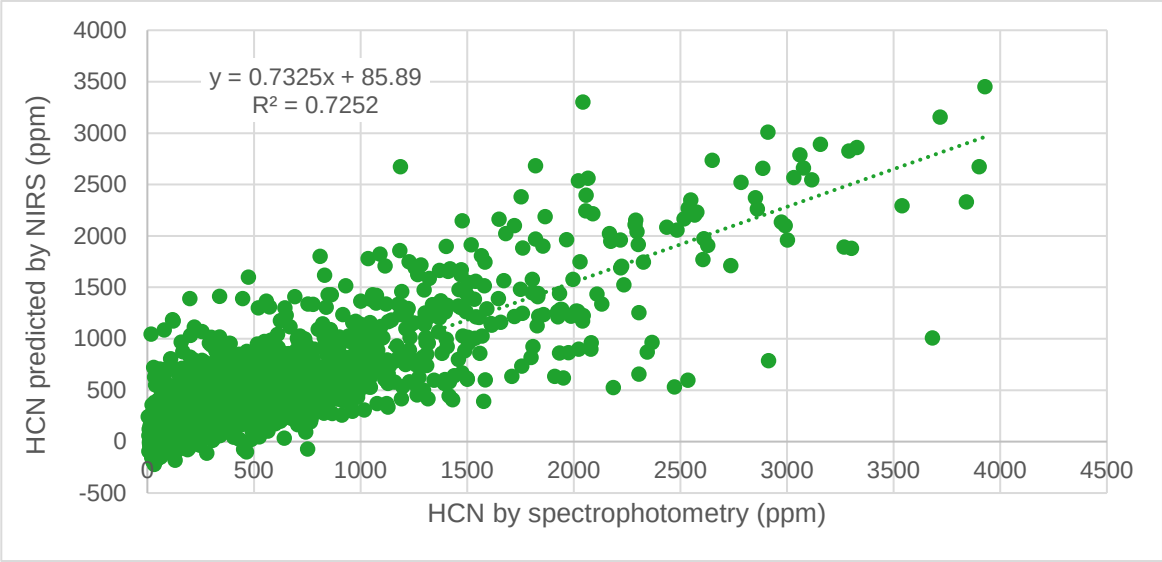

Figure 8. HCN values measured by spectrophotometry and predicted by NIRs in a large sample of more than 3,000 cassava roots.

1.1.6. Boiling time

Perhaps the most relevant trait to identify cassava roots suitable for table consumption (in addition to low cyanogenic potential) is that they soften upon boiling. Natives in the Amazon basin have a name (caulla) for those roots that fail to soften (Chirif, 2013). There is a large variation in the time required by the roots to get soft in response to boiling. In some cases, even after an hour of boiling, roots fail to soften. Similarly, there is large variability in how easy roots soften when they are submerged in water to promote fermentation (a critical step for many ethnic food preparations based on cassava). The biochemical basis for softening in response to boiling or fermentation have not yet been established. The degree of association between these two response variables has not been determined either. What is clear is that boiling results in a gradual and consistent reduction of starch and cyanogenic glucosides in the root (Ezeigbo et al., 2015).

An ongoing evaluation of accessions from the germplasm collection at CIAT is screening roots for their dry matter content, cyanogenic potential and boiling time. A total of 392 genotypes haven been analyzed. However, in some cases it was possible to analyze samples from the same genotype grown in different seasons. Three genotypes offered five different root samples. Four root samples were available in the case of 11 genotypes. There were 27 and 113 genotypes with three and two root samples available, respectively. Finally, there was just one root sample available for the remaining 238 genotypes. In total, 546 root samples were screened for the three variables mentioned above. Table 8, presents a summary of this ongoing study. The average differences between the minimum and maximum values (within the same genotype) for DMC, HCN and cooking time were respectively 4.04%, 167.34 ppm and 16.56 min. This type of information provides a general idea of how variable information from samples obtained in different growing seasons may be.

Figure 9 presents the relationship between DMC and cooking time across the 546 samples. There is no obvious trend and both variables seem to be independent from each other. Figure 10 presents the averages (when more than root samples was available) or single values (when only one root sample per genotype was available) for boiling time and cyanogenic potential in the 392 accessions from the germplasm collection screened. This Figure has been included to highlight the relatively low frequency

of accessions in the germplasm collection that meet the requirements of quick boiling (e.g. < 20 min) and low cyanogenic potential (e.g. < 200 ppm).

Table 8. Consistency in repeated measurements (from 2 to 5 samples per clone) on roots from the same genotypes (a total of 154)

Parameter	DMC (%)	HCN (ppm)	Cooking Time(min)
Average across 151 repeated genotypes	37,43	266,89	36,66
Average difference between minimum and maximum	4,04	167,34	16,56
Minimum difference between minimum and maximum	0,02	0,59	0,00
Maximum difference between minimum and maximum	12,48	796,99	47,00
Average St. deviation in data from same genotype	1,91	79,87	8,28

Sajeev and collaborators reported in 2010 on the thermal softening behavior in roots from nine cassava clones. They used linear regression and fractional conversion techniques, rheological properties of the gelated starch by Maxwell and power law models. The results showed that textural, rheological and gelatinization properties varied considerably among the varieties and besides the physico-chemical properties, interaction between them and structural make up of the tuber parenchyma had a great influence on cooking quality and rheological properties. Figure 11 reproduces the original data presented by Sajeev et al., in 2010. There is a remarkable difference in the softening or root tissue during boiling. Roots from Koliakodan and Sre Prabha showed a reduction in firmness above 80% after only five minutes. By ten minutes of boiling roots from all clones (except Sree Rekha and Venjaramoodan) had lost firmness above 80%. Very relevant in this data is that there is no major changes through time. Roots that softened quickly kept softening faster through time. On the other hand, roots that were initially slow in their softening were consistently slow through time. In practical terms, therefore, it is reasonable to propose to assess response to boiling at a standard time (e.g. after 10 minutes of boiling) uniformly across all genotypes under evaluation.

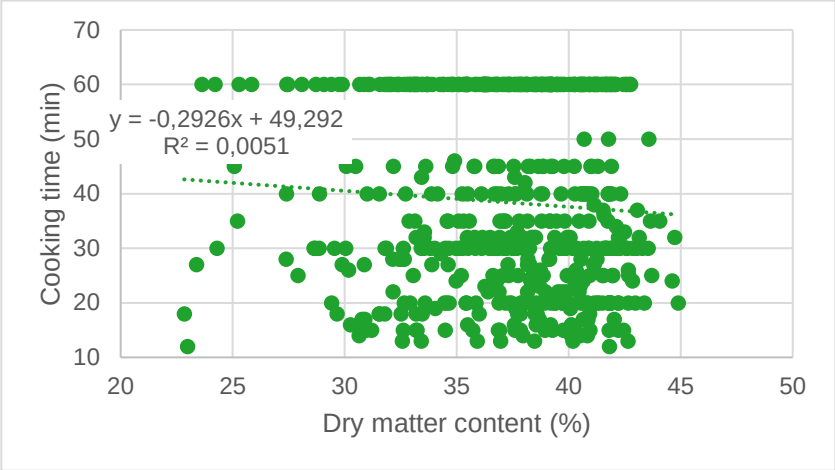

Figure 9. Relationship between dry matter content (%) and cooking time (minutes) in a sample of 546 cassava roots from 392 genotypes (in some cases more than one root sample per genotype was available).

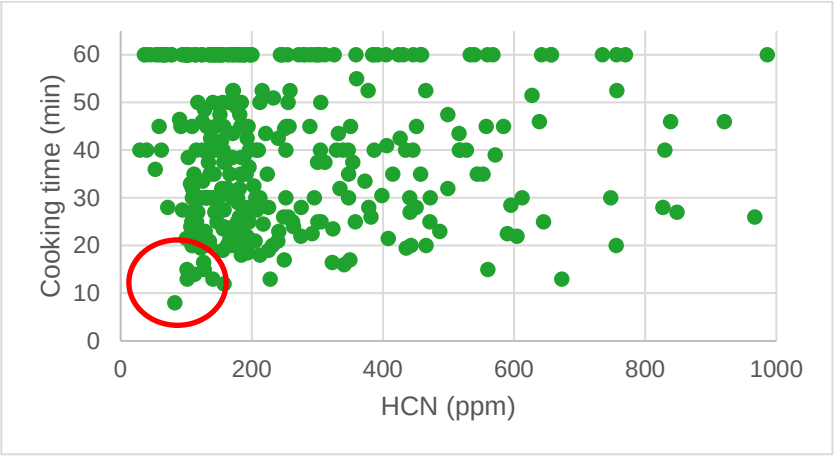

Figure 10. Relationship between cooking time and cyanogenic potential (HCN) in root samples from 392 genotypes. The frequency of materials suitable for boiling (red circle) is very low.

Sajeev et al. (2010) not only assessed the loss of firmness through boiling of the roots from nine cassava genotypes, but also the texture profile parameters of these cooked. Table 9 reproduces the results published by these authors. Cooking time and texture profiles can be linked to root physico-chemical and starch gelatinization properties presented respectively in Tables 10 and 11.

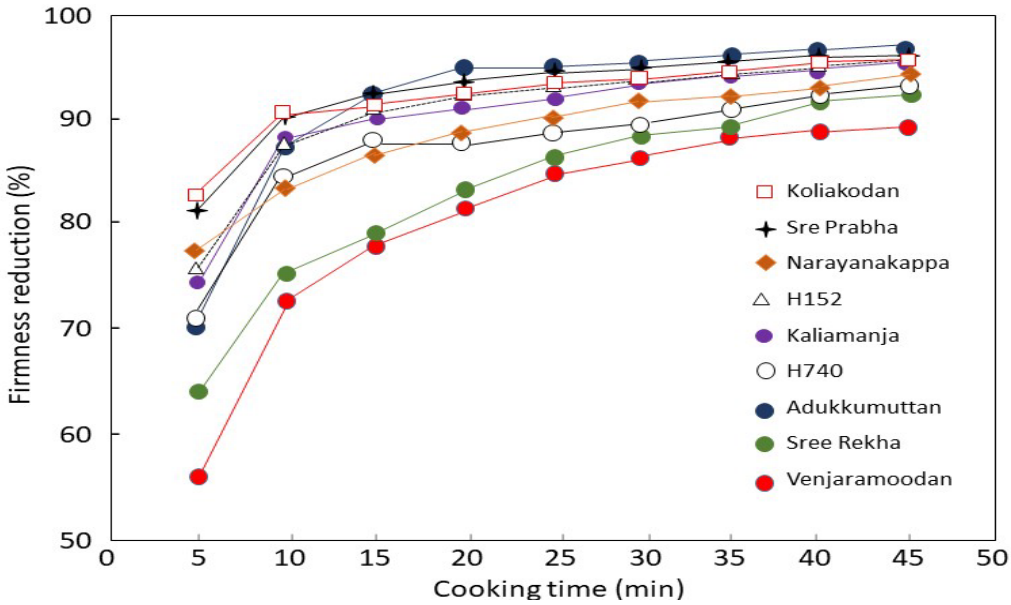

Figure 11. Reduction of firmness (e.g. softening) in roots from nine Indian cassava clones through boiling. Source: Sajeev et al., 2010.

Table 9. Texture profile parameters of cooked tuberous roots of cassava varieties. Source: Sajeev et al., 2010.[a]

Clone	Hardness, N	Adhesiveness,Ns	Springiness	Cohesiveness	Chewiness
Kaliamanja	55.1 [ab]	−0.49 [a]	1.23 [a]	0.260 [a]	13.9 [a]
Koliakodan	58.2 [ab]	−1.83 [b]	1.23 [a]	0.332 [a]	21.7 [a]
Adukkumuttan	84.2 [a]	−1.25 [ab]	0.82	0.340 [a]	23.3 [a]
Narayanakappa	43.0 [b]	−0.58 [a]	1.10 [ab]	0.321 [a]	14.0 [a]
Sree Prabha	90.2 [a]	−0.31 [a]	0.89 [ab]	0.317 [a]	24.5 [a]
H152	66.2 [ab]	−0.38 [a]	1.02 [abc]	0.320 [a]	18.7 [a]
Venjaramoodan	78.4 [ab]	−0.94 [a]	1.02 [abc]	0.282 [a]	22.7 [a]
Sree Prabha	59.7 [ab]	−0.34 [a]	0.85 [bc]	0.332 [a]	15.1 [a]
H740	72.0 [ab]	−0.43 [a]	0.76 [c]	0.305 [a]	14.8 [a]

[a] Means followed by same superscripts in a column are not significantly different ($p<0.05$)

Table 10. Physico-chemical properties of raw tubers of different varieties of cassava. Source: Sajeev et al., 2010. [a]

Clone	DMC	Starch	Sugars	Fiber	Ash	Amylose (%)	
	(%)	(% wb)	(% db)	(% db)	(% db)	Apparent	Total
Kaliamanja	68,6[c]	27,6 [a]	2,0 [e]	1,01 [d]	2,0 [b]	18,2 [b]	19,6 [bc]
Koliakodan	62,0 [e]	27,5 [a]	1,9 [f]	0,46 [f]	1,8 [c]	15,6 [de]	19,1 [c]
Adukkumuttan	67,0 [d]	25,2[c]	1,6 [b]	0,97 [d]	1,9 [bc]	16,8 [bcd]	21,3 [b]
Narayanakappa	70,6[b]	24,9 [cd]	1,9 [f]	0,95 [d]	2,0 [b]	17,7 [bc]	19,8 [bc]
Sree Prabha	72,4 [a]	20,9	2,4 [c]	1,28 [b]	2,4 [a]	14,5 [ef]	16,7 [d]
H152	66,1[d]	26,5[b]	2,8 [a]	0,97 [d]	1,5 [d]	20,6 [a]	23,4 [a]
Venjaramoodan	67,1 [d]	23,2 [e]	2,3 [d]	1,19 [c]	1,8 [c]	16,3 [cd]	19,8 [bc]
Sree Prabha	72,5 [a]	21,4[f]	1,8[g]	0,78 [e]	2,3 [a]	15,3 [de]	18,8 [c]
H740	67,5 [cd]	24,3[d]	2,5 [b]	1,57 [a]	2,4 [a]	13,4 [f]	15,6 [d]

[a] Means followed by same superscripts in a column are not significantly different (p<0.05)

Table 11. Gelatinization properties of cassava starches by the DSC thermograms. Source: Sajeev et al., 2010. [a]

Clone	Enthalpy	Gelatinisation temperature (°C				Peak height index	Swelling Volume (ml/g)	Solubility (%)
		Onset	Peak	Endset	Range			
Kaliamanja	11.6 [bc]	64.9 [bc]	68.3 [c]	79.4 [bc]	14.5 [abc]	3.5 [ab]	56.2 [a]	14.4 [a]
Koliakodan	12.3 [a]	66.1 [a]	70.5 [a]	80.8 [ab]	14.7 [abc]	2.8 [ab]	43.8 [e]	20.8 [b]
Adukkumuttan	12.2 [ab]	66.7 [a]	70.1 [ab]	80.1 [bc]	13.4 [bc]	3.7 [ab]	50.0 [e]	21.5 [b]
Narayanakappa	12.2 [ab]	64.1 [c]	68.7 [bc]	76.3 [d]	12.2 [c]	2.6 [b]	36.9 [e]	15.2 [cd]
Sree Prabha	12.0 [ab]	65.6 [ab]	69.3 [abc]	79.4 [bc]	13.7 [abc]	3.3 [ab]	52.5 [b]	16.9 [cd]
H152	11.1 [c]	64.2 [c]	68.7 [bc]	77.9 [cd]	13.6 [abc]	2.5 [b]	47.5 [d]	18.4 [bx]
Venjaramoodan	11.9 [ab]	65.7 [ab]	68.7 [bc]	80.5 [ab]	14.8 [ab]	4.1 [a]	47.5 [d]	27.3 [a]
Sree Prabha	12.1 [ab]	66.7 [a]	70.4 [a]	82.7 [a]	16.0 [a]	3.2 [ab]	43.7 [e]	14.4 [d]
H740	11.2 [c]	65.6 [ab]	69.2 [abc]	78.9 [bc]	13.4 [bc]	3.1 [ab]	50.0 [c]	25.8 [a]

[a] Means followed by same superscripts in a column are not significantly different (p<0.05)

Table 12. Pasting properties from starches of more than 4000 cassava genotypes. Source: Sanchez et al., 2009

Parameter	Pasting Temperature (oC)	Maximum Viscosity (cP)	Breakdown (cP)	Consistency (cP)	Setback (cP)	Ease of Cooking (min)
Maximum	71,2	1505,0	859.0	626.0	273.0	5,6
Minimum	58,8	146,0	28.1	0.0	-702.0	1,1
Average	65.3	777,5	298.1	155.8	-144.5	2,8
St. Dev.	1.75	165,03	107.1	57.8	96.2	0,72
Skewness	-0,13	0.22	0.81	0.94	-0.38	0,33
Count	4051	4051	4051	4051	4051	4051

According to the information published by Sanchez et al., in 2009 (Table 12) there was a positive correlation between easy of cooking and amylose content (0.12). As expected, the regression of easy of cooking on amylose content was positive but with very small R^2 value (0.015). The correlation between dry matter content and easy of cooking was small and negative (-0.08). The R^2 value of the regression of easy of cooking on dry matter content was also negligible (0.006).

Talma and co-workers reported in 2013 the responses to boiling in roots from 15 cassava clones. There was significant correlation between the shear strength of the cooked pulp and the cooking time (0.62), but the correlation between cooking time and shear strength of fresh roots was considerably lower (0.35). Figure 12 presents the relationship between shear strength in the raw roots failed to predict cooking time. The average CV for cooking time was acceptable (7.57 %). However, higher values were obtained for the coefficient of variation of the texture measurements, highlighting the heterogeneity of the roots. Cooking time in this study ranged from 17 to 31 minutes.

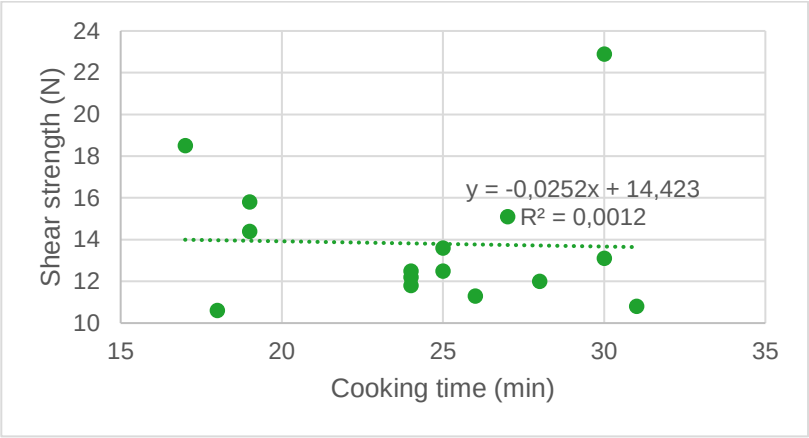

Figure 12. Relationship between shear strength in uncooked cassava roots and boiling time. Source: Talma et al., 2013.

There is a contradiction between the perception of Amerindians regarding the effect of age of the plant and cooking time and more recent studies. Azevedo Miranda et al. (2008) reported on the evaluation of roots from six different cassava clones grown in two different environment and harvested 8, 10, 12, 14 , 16, 18 and 20 MAP. There were significant differences among the six clones for cooking time. In general, there was a good correlation (0.70) between average cooking time (across harvesting dates) of each clone at the two different locations. These results clearly indicate that boiling time is under genetic control. Interestingly, cooking times increased with age of the plant reaching a maximum (at both locations) in plants harvested 16 MAP and then decreased sharply.

Lorenzi reported in 1994 differences in boiling time in different types of comparisons for a single genotype. Within a root proximal, intermediate and distant section of the roots boil increasingly faster (31, 24 and 19 minutes, respectively). In agreement with Azevedo Miranda (2008) boiling time increased in roots from plants harvested after 12 months of age. Differences were not large for roots from plants harvested at of before 12 months of age. Boiling time of roots from 15 months-old plants showed a wider range of variation. Lorezni also provided evidence that type of soil has a strong effect on cooking time when roots from five clones were evaluated monthly from the 7th through the 15th month of age (53 vs 36 min in two contrasting soils).

Other reports have also found an effect of age of the plant on boiling time (Pereira et al., 1983; 1985; Fukuda & Borges, 1988; 1990; Fukuda et al., 1988). An earlier report (Borges et al., 2002) on 26 cassava clones evaluated two consecutive years at Cruz das Almas (Brazil) and harvested 8, 10 and 12 MAP indicated an increase of fresh root yield with age of the plants which was unrelated to starch and dry matter content in the roots. More importantly, there was no much influence of years of harvest or age on the plants on boiling time. However, this early work included only harvests between 8 and 12 MAP. Azevedo Miranda and co-workers found the effect of age of the plant mostly at harvest beyond 12 MAP.

1.1.7. Cooking quality

Cooking quality is associated with boiling time. According to Lorenzi (1994) roots that soften quickly upon boiling tend to offer better culinary quality. An interesting study by Hongbété et al. was published in 2011. These researchers analized roots from seven genotypes harvested 10, 12 and 14 months after planting and in three different seasons. Age of plant and environmental conditions during growing affect texture (friability) and taste of boiled cassava roots. Sensory taste (sweet or bitter) of boiled cassava root could not be correlated with sugar content and/or cyanide potential, which both interfere with taste perception. The authors concluded that bitterness is not a good indicator of the poisonous character of cassava roots. Wheatley had reported in 1991 the presence of many volatile compounds (different from HCN) having a sensory effect whose concentration increase upon boiling the roots. Improved cultivars generally showed lower friability scores, independently of plant age (10–14 months) or season. Rainfall before harvest directly lowers dry matter and mealiness of boiled roots. Pectins (higher content for

improved cultivars) are suspected to be the major biochemical cause of vegetable mealiness or friability. A puncture test can confidently be used for evaluating cassava friability; it correlates with both texture sensorial evaluation and disintegration visual appearance.

Culinary quality of cassava roots depends of many factors (DMC, HCN, boiling time) as described in the previous sections. Some of these factors have a direct or indirect effect. There is a strong influence of the environment, genotype and genotype-by-environment interactions in cooking quality. Cooking quality also depend strongly on age of the plant and season in which harvest takes place. Ultimately, a panel of experts is required for properly assessing quality of boiled roots (Wheatley, 1991).

1.2. Cassava Breeding for Quality Traits at NaCRRI: Uganda

This year 2018, cassava marks its 158th anniversary since its introduction in Uganda. As of this writing, seven aspects characterize the cassava value-chain in Uganda: 1) cassava is grown by ~7.4 million people majority of whom are women on smallholdings that ~850,000 hectares in total; 2) the crop is popular among the populace owing to its flexible harvesting times and diversity of food and non-food products to which its starchy roots and/or leaves can be subjected to; 3) upon harvest, inefficient cassava processing and storage methods are commonplace; 4) the crop suffers from acute and variable on-farm productivity owing to an array of biotic and abiotic constraints; 5) drudgery is commonplace during, production, processing and marketing; 6) the crop has opened up new vistas for income generation arising from sale of stems and/or new cassava-based products; and 7) existence of inefficient extension services to support cassava production, processing and marketing.

It's apparent from the foregoing that cassava's landscape in Uganda has had, and continues, to witness both exciting opportunities and acute development challenges. Research interventions over the past decades have attempted to address some of these challenges. Indeed, we acknowledge three significant research interventions. Firstly, the Colonial Government, which made first attempts in 1941. These efforts led to the release of 13 varieties, some of which are still surviving today and thus referred to as "local varieties". Secondly, interventions in the late 1980s by the Government of Uganda and development partners; notable of these interventions were the intensive selections from introduced germplasm from the International Institute of Tropical Agriculture (IITA). These efforts led to the release of 12 high-yielding varieties. And thirdly, interventions initiated in 2004; these were largely responding to: 1) the outbreak of cassava brown streak disease that caused substantial reduction in production in the 2000s; 2) the need for nutritious cassava (with beta-carotene); and 3) growing need of high starch varieties for the highly competitive and growing cassava starch industries.

Through these various intervention, experience has taught us that end-user preferences are critical for success. For instance, as cassava virus incidences and severities decreased owing to breeding interventions, farmers reluctantly cultivated these improved varieties, and resorted back to their locally adapted varieties, for which, they have had a long historic association. This was partly attributed to the notion that many of the released varieties lacked desirable root quality attributes (taste, mealiness, texture and aroma) as compared to locally adapted varieties. This reversion by farmers to local susceptible varieties after the control of major disease pandemics motivated NaCRRI to initiate cross-functional cassava breeding schemes guided by breeding objectives tailored towards: 1) increased and stable yields; 2) improved nutritional value and organoleptic properties; 3) improved root quality; 4) improved disease and pest resistance; and 5) improved cassava agronomics; and 6) improved tolerance to abiotic stresses. In all these interventions, our ultimate desire is to benefit cassava farmers, processors and consumers.

Our desire is to have cassava varieties that have "*must-have-traits*" and/or "v*alue-added traits*" The "*must-have-traits*" ensure that cassava optimally yields despite the prevailing pests, diseases and abiotic stresses in farmers' fields. Of keen interest for WP 4 is to focus on the "v*alue-added traits*" most of which deal with root quality and use. Accordingly, our immediate target traits for 2018 are: 1) root dry matter content; 2) softness; and 3) cyanogenic potential. For 2019, our target traits will be: 1) root dry matter content; 2) beta carotene content; 3) softness; 4) cyanogenic potential; and 5) starch content. Beyond 2019, we may add fibre content to these aforementioned five root quality traits. Prioritization of this trait list will be informed by information collected from end-user surveys conducted under WP1. Hereafter we provide a brief assessment procedure on each of the target traits

1.2.1. Assessment for root dry matter content

Done for all breeding trials at harvest (12 months) using two methods; specific gravity or the oven dry method. For oven dry method, roots (3-5 per plot) should be sampled randomly from several plants within the plot and peeled. Root samples weighing 100 – 200g of fresh roots per plot are cut into thin slices using a knife (<1 cm thick) and then weighed. Drying in a forced air oven (Leader engineering, Cheshire, England) is done at 60oC until constant weight. The DMC is expressed as a percentage of fresh root weight as: DMC (%) = ("Dry sample weight" /"Fresh sample weight)" ×100. DMC when multiplied by fresh root yield provides estimates of root dry yield of a clone. For the specific gravity method, root samples weighing 3–5 kg are sampled/plot. These samples were weighed in air (Wa) and in water (Ww) using a suitable balance. Thereafter, the specific gravity (Sg) is computed as a ratio of Ww and the difference between Wa and Ww. DMC content is then computed using the formula: DMC = (158.3 * Sgl) – 142. Heritability of DMC ranges between 0.14 to 0.75.

1.2.2. Cassava total carotenoid determination

Total carotenoid content is analysed both on fresh and processed samples, using an icheckTM carotene kit (BioAnalyt Laboratory, Germany) (www.bioanalyt.com). Fresh samples are analysed within four hours of harvest, in a dark room, to minimize losses resulting from photo oxidation. Opposite quarters of sectioned roots are selected, chopped into small pieces and pooled. About 5 g of the finely chopped root samples are pounded and ground into a smooth and fine paste using a mortar and pestle. To aid grinding of the sample, 20 ml of distilled water is often added gradually and the resulting solution is transferred into a 50 ml calibrated tube. The tube content is shaken thoroughly and 0.4 ml of the solution is injected into the iExTM CAROTENE vial using the syringe and needle provided with the kit.

Vials are placed on a solid surface for approximately 5 min, shaken again and allowed to stand until two solution phases appear inside the vial: a clear upper phase and a turbid lower phase. At this point, the absorbance of the vial content (the upper solution phase) is measured at a wavelength of 450 nm using the iCheckTM CAROTENE device. Total carotenoids content is calculated as:

$$\text{TCC (Ppm)} = \frac{\text{Ws}}{\text{Vs}} \times \text{A}$$

Where Ws = weight of sample, Vs = volume of solution transferred to the tube and A = absorbance of the iExTM CAROTENE vial contents. The processed samples are treated in a similar manner for carotenoids content, and percentage retention calculated using the formula: retention (%) = $\frac{c_1 \times w_b}{c_2 \times w_{b'}} \times 100$ where; c_1= carotenoids content in processed roots, c_2 = carotenoids content in fresh roots, w_b = weight of processed roots, w_b, = weight of unprocessed roots.

1.2.3. Determination of cyanide content

Cyanide content is determined using the method of Howard et al., 1994. Fifty grams (50g) of fresh cassava root is extracted by 160 ml of 1M orthophosphoric acid by homogenization using a waring blender (Waring, New Hartford, USA). The homogenate is centrifuged for 30 minutes at 12000 rpm, 4oC using a refrigerated centrifuge (Hermle Z300K, Wehingen, Germany). The supernatant containing the extracted linamarin (0.1 ml) is buffered with 0.4 ml of phosphate buffer (pH 7.0) and hydrolysed to cyanide using *linamarase* enzyme. The released cyanide is chlorinated using 0.1 ml of 0.4 % (w/v) Chloramine T solution, followed by reaction with 0.6 ml of 0.2M Isonicotinic/ barbituric acid solution (pH 6.5). Cyanide is quantified spectrophotometrically (Biowave ii+, Cambridge, England) by measuring absorbance of the colored complex at a wave length of 605 nm, against standard potassium cyanide. The cyanide content is expressed in parts per million (ppm).

Table 13. Performance of parents and their respective F1 progeny across two locations in Uganda during 2014-2015

Parent/Family	Number[a]	TCC[b]	DMC[c]
NASE 3 (P1)	-	0.2	37.3
CPCR24B-10 (P2)	-	4.4	32.3
MH05-2870 (P3)	-	4.3	33.4
MH05-0233 (P4)	-	4.9	29.0
CPCR15B-26 (P5)	-	5.3	30.7
MH02-073HS (P6)	-	10.4	22.2
P1 x P2	17	2.1	34.4
P1 x P3	18	3.1	31.0
P1 x P4	19	3.1	31.3
P1 x P5	20	2.4	33.9
P1 x P6	16	2.6	28.1
P2 x P3	15	2.7	32.4
P2 x P4	15	3.1	32.1
P2 x P5	20	3.2	32.1
P2 x P6	17	3.7	29.6
P3 x P4	20	2.3	33.0
P3 x P5	16	3.2	32.9
P3 x P6	20	3.7	29.7
P4 x P5	20	4.3	33.6
P4 x P6	20	4.4	29.0
P5 x P6	19	5.8	22.2
Mean		3.8	30.9
SE[h]		0.062	0.150
$LSD_{0.05}$[i]		0.127	0.304

[a]Number of genotypes evaluated per F_1 family; [b]Total carotenoid content (µg g^{-1}); [c]Dry matter content of roots (%);[g]Values based on all F_1 genotypes evaluated; [h]Standard error; [i]Least significant difference at 5% confidence level; [j]Number of F_1 genotypes evaluated: reduction from the total population (272) indicates proportion of genotypes whose roots were not sufficient for measuring TCC and/or DMC. Adopted from Esuma et al 2016; https://doi.org/10.1270/jsbbs.15159

Table 14. GCA effects of cassava parental lines used in a 6x6 half-diallel analysis of five traits

Parent	TCC[a]	DMC[b]
NASE 3	-1.60***	1.75***
CPCR24B-10	-0.38**	0.87**
MH05-2870	-0.07	0.82**
MH05-0233	0.03**	-0.17*
CPCR15B-26	0.28**	0.44**
MH02-073HS	1.93***	-3.72***
$LSD_{0.05}$[f]	0.253	0.607
SE[g]	0.041	0.097

[a]Total carotenoid content (μg g^{-1}); [b]Dry matter content (%);[f]Least significant difference at 5% confidence level; [g]Standard error. *, ** and *** significant at P < 0.05, P < 0.01 and P < 0.001, respectively. Adopted from Esuma et al 2016; https://doi.org/10.1270/jsbbs.15159

Table 15. Genetic parameter estimates for five traits of 6x6 half diallel F_1 families evaluated at two locations in Uganda

Component	TCC[a]	DMC[b]
δ^2_A[f]	6.47	12.01
δ^2_D[g]	4.46	11.94
δ^2_A x E[h]	0.01	0.87
δ^2_D x E	0.00	3.71
h^{2i}	0.48	0.41
H^{2j}	0.94	0.82

[a]Total carotenoid content (μg g^{-1}); [b]Dry matter content (%);[f]Additive genetic variance; [g]Dominance genetic variance; [h]Environmental effect; [i]Narrow sense heritability; [j]Broad sense heritability. Adopted from Esuma et al 2016; https://doi.org/10.1270/jsbbs.15159

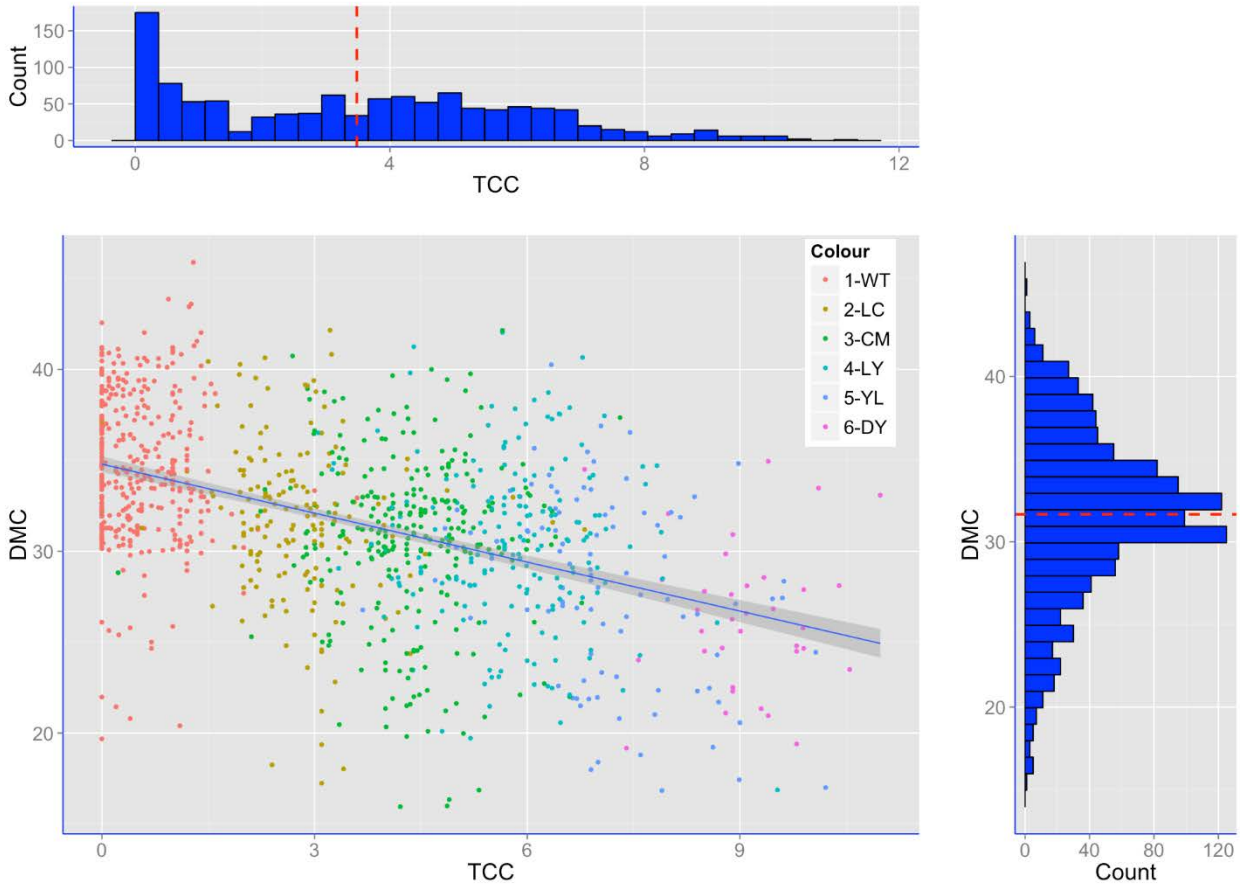

Figure 13. Scatter plot and histograms of TCC vs. root dry matter content. Color represents pigmentation of the root parenchyma such that 1-WT = white; 2-LC = light cream; 3-CM = cream, 4-LY = light yellow; 5-YL = yellow; 6-DY = deep yellow; red lines on histograms indicate means. Adopted from Esuma et al 2016; https://doi.org/10.1270/jsbbs.15159.

1.2.4. Assessment of softness of cooked roots

Assessed on either fresh or waxed root samples; waxing is appropriate when analyzing many root samples, as it maintains sample integrity for up to one month. Two roots sampled/plot are peeled and sliced into four 3cm sections. Next, the four sections are loosely wrapped in perforated aluminum foil and heated (cooked) in a water bath set at a constant near boiling temperature of 90°C. Boiling is undertaken for 45 minutes. Once this is completed, a 7.9 mm diameter tip of a digital penetrometer (Model number: FHT-1122, Vetus Industrial Company Limited, Hefei, China) is pushed to a depth of 1 cm into three different sides of the cooked root section (i.e., three technical measurements taken per root section). Softness is recorded as the maximum force used to penetrate the root section. The only study that has quantified heritability was one by Paula Iragaba (unpublished data), which documented that heritability for softness ranged from 0.17 for samples boiled for 60 minutes to 0.37 for samples boiled for 45 minutes.

Table 16. BLUPs and heritability estimates of cassava root softness following four different cooking durations.

Cooking time (min)[a]	No. cassava genotypes[b]	BLUPs[c]			Broad-sense heritability	
		Mean (N)	**SD[d] (N)**	**Range (N)**	**Estimate**	**SE[e]**
15	268	3.33	0.155	2.94 - 3.76	0.22	0.087
30	268	2.78	0.154	2.45 - 3.27	0.28	0.081
45	267	2.47	0.205	1.94 - 3.21	0.37	0.076
60	267	2.20	0.096	1.89 - 2.49	0.17	0.092

[a]Data based on analysis across two locations; Arua (west Nile region) and Mubende (central region); Back-transformed BLUPs are reported for the 15 min time point; [b]Only 267 genotypes were evaluated at the 45 and 60 min cooking times, because one of the genotypes did not have enough roots for phenotypic evaluation; [c]BLUPs, best linear unbiased predictors; [d]SD, standard deviation of the BLUPs and [e]SE, standard error of heritabilities. *Adopted from Iragaba et al Unpublished data*.

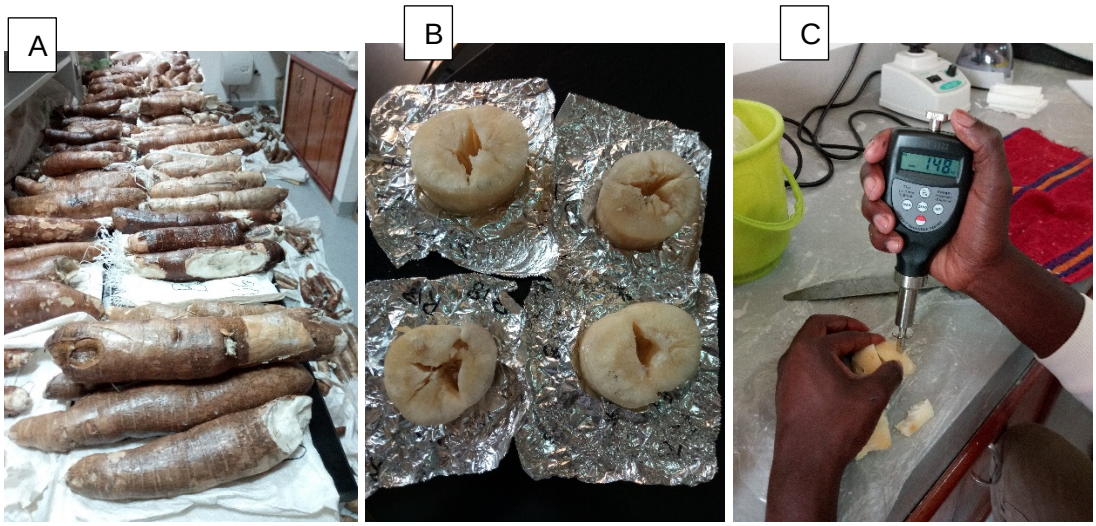

Figure 14. Step by step process of assessing softness of boiled cassava roots: a) roots harvested from the field waxed and stored at room temperature; b) root samples boiled in a water bath maintained at 90^0C; and c) softness assessed using a penetrometer; upto 4 root sections sampled per clone.

1.2.5. Genetic variability in NaCRRI breeding program

At NaCRRI, we have different cassava populations and/or clones at different evaluation stages. It's these populations and/or trials that we shall use to constitute the target population for WP 4. First, for 2018, our target population was the NextGen C_1 population that comprised of ~730 clones established at Namulonge (NaCRRI). This trial was harvested in September 2018, roots sampled, waxed and shipped to the laboratory for trait analyses.

Second, we specifically established a WP 4 trial comprising of 73 clones (52 elite and 21 local). This trial was established in August 2018 at two sites: Namulonge (central region) and Serere (eastern region), and will be due for harvesting in August 2019. Third, if resources permit, we shall also target a portion (~400 clones) of the NextGen C_2 cassava seedling population that was established at Namulonge in October 2018; this trial will be due for harvesting in October 2019.

1.3. Cassava Breeding for Quality Traits at NRCRI: Nigeria

The Nigerian cassava sector is unique and quite massive. It has maintained its place as the leading cassava producing country in the world at an estimated 57 million MT (FAO, 2016) and contributing over 20% of the world's annual production. However, domestic production is not meeting the national need for cassava root for food security and several industrial needs.

The National Root Crops Research Institute (NRCRI) Umudike is charged with a national mandate of genetic improvement of root and tuber crops in Nigeria to enable farmers, processors and commercial producers maximize their output per unit input, improve their livelihoods and wellbeing and meet their daily food needs. Even though NRCRI and her partners (IITA and CIAT) together have officially released 46 cassava varieties, recent studies by the Cassava Monitoring Survey (CMS) showed the top most popular 10 varieties in the different growing areas of Nigeria (Wossen et al., 2017). A lot of attention is given to the productivity traits such as fresh root yields per unit area and resilience to pests and diseases. Even though consideration is usually given root quality parameters, it has hardly occupied the priority list because a lot of scientists believe that much of the outcome of the quality of gari or fufu depends on the processing methods and the duration of fermentation of fresh roots. This is gradually changing and there are prospects for including boiled roots as an avenue to convey micronutrients since it is retained due to minimal processing.

Cassava production and processing is a core livelihood activity in Nigeria, a major source of income for over 4 million farmers in production and providing food for over 100 million persons (IITA 2012, FAOSTAT 2016). Nigeria's cassava industry is growing with diverse end-users and growing industrial use. However, small-scale farming and processing represents by far the largest cassava food product agribusiness cluster in Nigeria (Forsythe et al., 2016; Onyenweaku and Simonyan 2014). Gari and Fufu are most widely consumed products of cassava root and a component of daily diets of over 60% of Nigeria's 190 million people.

Gari is most preferred because of its convenience, long shelf life and its easy to eat form either as a snack or a meal, *eba* (Ernest *et al.,* 2000; Onabolu, 2001; Ajala *et al.*, 2000) Cassava breeding has been aimed at developing improved varieties that are more acceptable and nutritious to the end-users in terms of its sensory attributes such as mouldability, adhesiveness, drawability, colour and general acceptance (Chijioke *et al.,* 2018). Oparinde *et al.* (2012) and Tumuhimbise *et al. (*2012) also described sweetness, dry matter content, carotenoids and its influence on the colour, appearance, taste, smell, and texture among the attributes and criteria that influences acceptance of this product.

End-user preference is cardinal to all production activities among smallholder farmers and processors in Nigeria. Therefore, the higher the demand for specific products from a specific processor there is increased business opportunity. This entails that certain product characteristics define its market viability. Such deal-breaker traits has over the years been viewed from the processing end of the value chain thus leaving the consumers needs unmet and in turn, market opportunities are not maximized. The Cassava breeding team at NRCRI, has identified this need and has over the past few years, focused on consumer preferred traits as key to defining their breeding objectives. The higher the demands for certain product trait characteristics, the more the derived demand for cassava varieties with such quality traits increases leading to higher adoption rates and better yields for farming families and smallholder cassava processing agribusinesses.

Cassava breeding has over time evolved with the inclusion of consumer preferences in traits identified through participatory studies useful in mapping out traits of top quality that drives market demand for cassava. These include colour, taste, consistency for products like garri and fufu.

1.3.1. Phenotyping for quality traits at NRCRI, Umudike

Cassava breeding programmes have explored a wide spectrum research and development interventions through innovative partnerships towards increased resistance to pest and diseases, improved nutritional quality, best agronomic traits for cassava stems and roots, drought tolerance in changing climate and most of all increased yields to benefit smallholder farmers and processors who hold the highest stake in the Nigerian cassava sector.

Furthermore, of topmost priority is genetic improvement of cassava varieties for identified quality traits based on diverse end-user preferences. These traits of preference include root dry matter content (relevant for root mealiness), product consistency (for garri and fufu) associated with cassava starch content, colour (especially with respect to beta carotene content), aroma and taste. Chijioke *et al.,* 2018 evaluated the sensory attributes and consumer acceptance of eba prepared from some cassava clones at the uniform yield trial (UYT) breeding stage. This study assessed seven cassava genotypes at UYT and 2 controls [1 national check white (TMS30572) and a biofortified variety (UMUCASS36)] harvested from NRCRI's HarvestPlus trial fields planted at NRCRI Umudike experimental plots. Gari was processed from the different cassava varieties using the a standard processing method. Dry matter content of gari was determined using the method of AOAC (2005). *Eba* was prepared from the 9 varieties and method of preparation was optimized by quantifying volume of hot water needed to reconstitute 100 g of dry *gari* granules. The weight of resultant dough was determined. Sensory perception and consumer acceptance of some selected sensory properties of *eba* was determined by 50 semi-panelist trained using a 5-point hedonic scale: 5= like extremely, 3= neither like nor dislike, 1= dislike extreme. The datasets (Table17-19) showed the ranking of the newly developed varieties compared to the check variety, TMS30572, and that some were better preferred.

Table 17. Dry matter content of gari granules from a Uniform Yield Trial (UYT) grown and processed at NRCRI, Umudike.

Sample	Dry matter (%)
NR120103	90.30a
TMS30572	81.30d*
NR120118	88.85c
NR120212	87.50c
NR120122	90.80a
UMUCAS36	83.50d*
NR120047	89.00b
NR120220	89.10b
NR120214	89.40b

Table 18. Some cooking characteristics of the gari granules from a Uniform Yield Trial (UYT) grown and processed at NRCRI, Umudike.

Sample	Volume of water absorbed/100 g gari (mL)	Weight of Eba/100 g gari
TMS30572	209.32	514.79
UMUCASS36	238.97	312.76
NR120122	209.94	316.43
NR120220	209.99	301.33
NR120118	349.70	407.87
NR120103	280.11	351.72
NR120047	280.07	356.01
NR120212	280.19	344.07

Table 19. Mean scores of sensory perception and consumer acceptance of *Eba* prepared from selected cassava varieties in a Uniform Yield Trial (UYT) grown and processed at NRCRI, Umudike.

Sample	Mouldability	Adhesiveness	Colour	Drawability	General Acceptability
NR120103	$4.16^{a}\pm0.69$	$3.92^{a}\pm0.76$	$3.80^{bc}\pm0.91$	$3.72^{ab}\pm1.02$	$20^{ab}\pm0.71$
TMS30572	$3.80^{abc}\pm0.87$	$3.80^{a}\pm0.65$	$3.12^{d}\pm0.93$	$3.48^{b}\pm0.92$	$3.80^{bc}\pm0.82$
NR120122	$3.68^{bc}\pm0.95$	$3.72^{ab}\pm0.84$	$4.28^{a}\pm0.54$	$3.52^{b}\pm0.71$	$4.04^{ab}\pm0.73$
NR120118	$4.12^{ab}\pm0.73$	$3.88^{a}\pm0.73$	$4.36^{a}\pm0.76$	$4.20^{a}\pm2.45$	$4.28^{a}\pm0.79$
UMUCASS36	$3.44^{c}\pm0.87$	$3.32^{bc}\pm0.80$	$3.04^{d}\pm0.68$	$3.44^{b}\pm0.77$	$3.52^{c}\pm0.71$
NR120212	$3.44^{c}\pm0.87$	$3.16^{c}\pm0.99$	$4.08^{ab}\pm0.64$	$3.28^{b}\pm0.84$	$3.80^{bc}\pm0.65$
NR120047	$3.68^{bc}\pm0.90$	$3.52^{abc}\pm0.82$	$3.84^{b}\pm0.69$	$3.44^{b}\pm0.92$	$3.80^{bc}\pm0.58$
NR120214	$3.88^{abc}\pm0.73$	$3.60^{ab}\pm0.65$	$3.40^{cd}\pm0.87$	$3.52^{b}\pm0.77$	$3.80^{bc}\pm1.00$
NR120220	$1.88^{d}\pm0.73$	$2.32^{d}\pm0.75$	$2.56_{e}\pm0.87$	$2.28^{c}\pm0.54$	$2.28^{d}\pm0.74$

Chijioke et al., 2018

In 2011, NRCRI evaluated a set of clones from a national pre-release trials including the first wave of 3 biofortified cassava varieties for root quality traits (Table20). Average mean dry matter contents of varieties range from 30-35%, for starch 16-22% and for gari was 18-21%. Further studies include the determination of the proximate composition of key biophysical parameters as shown in Table21.

Table 20. Means of dry matter and starch contents from fresh roots of cassava varieties evaluated at Ibadan and Umudike both in Nigeria.

Clone	Dry matter content (%)		Starch content (%)		Gari yield from 20kg tubers (kg)
	Ibadan	Umudike	Ibadan	Umudike	Umudike
AR182	30.85	34.00	22.10	20.90	3.50
AR37108	34.45	26.50	21.80	16.60	3.00
CR1245	32.40	32.00	19.70	19.70	3.70
UMUCASS40	38.50	39.50	22.40	22.80	4.00
UMUCASS39	29.45	33.50	21.50	22.20	3.50
UMUCASS38	28.90	32.50	17.50	20.50	3.70
UMUCASS37	30.45	31.00	16.20	20.20	3.85
UMUCASS36	30.50	36.30	21.90	18.80	3.80
TMS30572	37.25	37.00	18.90	21.50	3.50
*Antiota/Nwaibibi**	35.80	39.50	21.60	28.20	4.00

*Antiota and Nwaibibi were local checks at Ibadan and Umudike, respectively.

Table 21. Proximate composition of selected cassava varieties from a national variety pre-release trial in Nigeria in 2011.

Clone	Root Pulp Colour	Amylose	Crude Fiber (%)	Ash (%)	Reducing Sugar (%)	HCN (mg/100g)
UMUCASS36	Yellow	14.5	1.7	0.50	4.09	10.78
UMUCASS37	Yellow	14.4	2.25	1.05	4.12	8.08
UMUCASS38	Yellow	15.8	2.45	0.85	3.75	11.11
UMUCASS39	Cream	18.9	1.95	0.45	4.18	10.2
UMUCASS40	Cream	19.8	1.25	0.75	5.32	19.7
TMS30572	White	24.74	1.26	2.81	1.90	10.76

Current breeding focus through several breeding programmes is focused on evaluating sensory properties on consumer-preferred traits with product specification for profiling. This is aimed at determining the correlation between root biophysical properties and product sensory properties that drive market demand for its products. Current emphasis for Nigeria is on gari and fufu as the most widely consumed in Nigeria. There exists an opportunity for a boiled biofortified cassava root food

product, as this will be important for nutrient retention. Currently, Kelechi Uchendu (PhD candidate at University of Ghana) is conducting a genome wide associated study of mealiness of biofortified cassava varieties using a panel of 150 individuals. The work will involve phenotyping for root mealiness of boiled roots and the quantification using spectral reads from portable NIRS (Qualispect) equipment. In Nigeria, two local landraces (Isunikakiyan or TMEB117 and TMEB693) represent standard checks for good root mealiness.

Ongoing research has identified clones from new cassava varieties of derived from NextGen Cassava and HarvestPlus projects using TMEB419 as a national check for experimental populations for biophysical and sensory evaluations. The RTBFoods project targets such goals as developing product profiles from selected cassava varieties. This involves gender-responsive participatory research and is aimed at developing descriptors so as to enable high throughput phenotyping and matching these with biophysical traits that are important for food products derived from the varieties. A majority of product studies have focused on processing there is a dearth in literature on the correlation between roots biophysical properties and consumer preferred traits. One class of traits that have limited information and their causative effects on food products are the textural traits and presents an opportunity for further studies.

Through the NextGen Cassava project, NRCRI and her partners developed and validated a rapid assay for root quality parameters for traits such as dry matter, beta carotene and starch contents (Ikeogu et al., 2017). It represents a game-changing technique for high throughput phenotyping as the accuracy of genetic predictions are dependent on the quality and reliability of the phenotypic data. The study also involved a genome wide association mapping which determined the genomic regions that control the accumulation of the different carotenoids in cassava roots.

1.3.2. Participatory quality evaluation of cassava by farmers and consumers

In Nigeria, cassava value chain actors are being engaged in participatory research to identify the traits of quality, of high demand, market value and market drivers in terms of product quality. These evaluations cascade down the best agronomic traits for farmers in production and the optimal production levels possible in the prevailing variations in climatic elements. Participatory quality evaluation is cardinal to the entire cassava value chain to help with product profiling as it defines what the farmers produce, drives demand and sketches the trajectory for consumer patronage and agribusiness activities within the cassava value chain. This is the core of the objectives of NRCRI cassava breeding unit. To meet consumers needs and preferences, increase market value and empower smallholder agribusinesses within the value chain which in this case is a female-dominant sector. These activities are currently being implemented under the NextGen Cassava – RTBFoods collaboration. Gender mainstreaming is a global phenomenon and it's impact in all aspects of life cannot be over emphasized. Gender differentials in every cassava value chain that influence the levels of adoption, use, market participation or inclusion and opportunities for agribusinesses are embedded in the varied roles and values of individuals from diverse cultures, religion and ethnic groups in Nigeria (Teeken et al., 2018). It regulates demands, practices, choices, opportunities, aspirations and actions according to different needs, preferences and use of cassava by men, women and children (male and female). In the long run, it defines the entirety of their economic empowerment in cassava business, general wellbeing, food security and existence. Nigeria is a multicultural and multi-ethnic, country with a relatively huge population- home to over 190 million[1] people who consume cassava as a major staple in their daily meals.

As a pre-requisite for a variety to be released, it must have been evaluated both in research stations representing a diverse agro-ecological conditions and by farmers in those areas. In Nigeria, a minimum set of 50-100 cassava farmers are usually engaged in the trials. In 2011, 104 cassava farmers each tested a set of 5 varieties in a design that regarded each of the 13 states used a trial unit whereby 8 farmers were regarded as replicates. The acceptable food quality (for gari – eba and fufu) attributes of the varieties as tested and ranked by most of the farmers distinguished these varieties for the

[1] http://www.worldometers.info/world-population/nigeria-population/

appropriate food forms and thus the basis for their necessary release to farmers (Tables 22 and23). These varieties were preferred over the national or local checks for the gari and fufu products.

Table 22. Gari (Eba) quality assessment of cassava varieties under on-farm pre-release trial in some states of Nigeria during 2010/2011 cropping season

State	Cassava Variety						
	UMUCASS36	UMUCASS37	UMUCASS38	UMUCASS39	UMUCASS40	TMS30572	Local check
Benue	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	N.T.
Cross River	V. Good (2)	V. Good (2)	Excellent (1)	V. Good (2)	V. Good (2)	V. Good (2)	V. Good (2)
Delta	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	Good (6)	Good (6)
Enugu	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)
Imo	Excellent (1)	V. Good (2)	V. Good (2)	V. Good (2)	V. Good (2)	Good (6)	Good (6)
Nasarawa	V. Good (2)	V. Good (2)	V. Good (2)	Excellent (1)	V. Good (2)	V. Good (2)	V. Good (2)
Ogun	Poor (5)	Good (4)	Poor (5)	Good (4)	V. Good (1)	V. Good (1)	V. Good (1)
Mean*	1.85 (3)	1.85 (3)	1.85 (3)	1.71 (2)	1.42 (1)	2.71 (6)	3.0 (7)

The figure in parenthesis is the rank on a scale of 1–7, where 1 = most preferred and 7 = least preferred. N.T = Not tested. * = mean ranking

Table 23. Fufu quality assessment of cassava varieties under on-farm pre-release trial in some states of Nigeria during 2010/2011 cropping season.

State	Cassava Variety						
	UMUCASS36	UMUCASS37	UMUCASS38	UMUCASS39	UMUCASS40	TMS30572	Local check
Benue	Good (3)	V. Good (1)	Good (3)	Good (3)	Good (3)	V. Good (1)	N.T.
Cross River	Excellent (1)	V. Good (5)	Excellent (1)	Excellent (1)	Excellent (1)	Poor (7)	Good (6)
Delta	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)	V. Good (1)
Enugu	Excellent (1)	V. Good (4)	Excellent (1)	Excellent (1)	V. Good (4)	Good (6)	Good (6)
Imo	V. Good (2)	V. Good (2)	Good (6)	V. Good (2)	Excellent (1)	V. Good (2)	Good (6)
Nasarawa	Good (3)	Good (3)	Poor (6)	N.T.	Good (3)	V. Good (1)	V. Good (1)
Ogun	Good (3)	V. Good (1)	Good (3)	Good (3)	Good (3)	V. Good (1)	N.T.
Mean*	2.0 (2)	2.42 (4)	3.0 (6)	1.83 (1)	2.28 (3)	2.71 (5)	4.0 (7)

The figure in parenthesis is the rank on a scale of 1–7, where 1 = most preferred and 7 = least preferred. N.T = Not tested. * = mean ranking

Gender responsive cassava breeding seeks to identify and breed products that address the specific needs of men and women alike while recognizing that women are the true custodians of household food security and their opinion and preferences in terms of cassava preferred traits are of uttermost importance. This defines NRCRI's breeding objective as a shift towards increased freedoms, opportunities for both men and women and a more equal and food secure Nigeria.

1.3.3. Genetic variability in NRCRI cassava breeding program

There exists a minimal genetic variability for the different root quality traits in cassava such as dry matter content, starch, root mealiness, etc. Recent efforts have yielded improvements through new introductions of cassava from South America. For example in 2012, Nigeria officially released a variety, CR36-5, originally received as an in vitro seedling from CIAT after national evaluation trials. The variety is currently one of the highest dry matter and starch yielding ones. Accumulation of genes for prioritized root quality trait can be aided with the power of genomics if the most critical traits responsible for the best gari and fufu quality are determined.

Biofortified cassava with enhanced levels of beta carotene are being cultivated by Nigerian farmers since 2012 (Fig. 1) and it has become apparent that scaling out will be more successful if the biofortified

varieties have higher dry matter and starch contents. Genomic dissection of the genes controlling both beta carotene and dry matter indicated that they are co-located on at least chromosome 1 (Rabbi et al., 2017). Genetic methods to manipulate this in a pleitropic manner might be helpful.

NRCRI will work from 2019 to ensure that validated high throughput phenotyping methods for root quality traits such as for dry matter, starch, carotenoids are used routinely. We shall seek to further collaborate with the RTBFoods and NextGen Cassava projects to develop more for mealiness and high cyanogenic potentials. These would be subsequently be predictable using the portable NIRS. The NextGen Cassava will further used these to develop models for genomic predictions for the most relevant traits and to enlist them in the genomic selection index being developed for cassava.

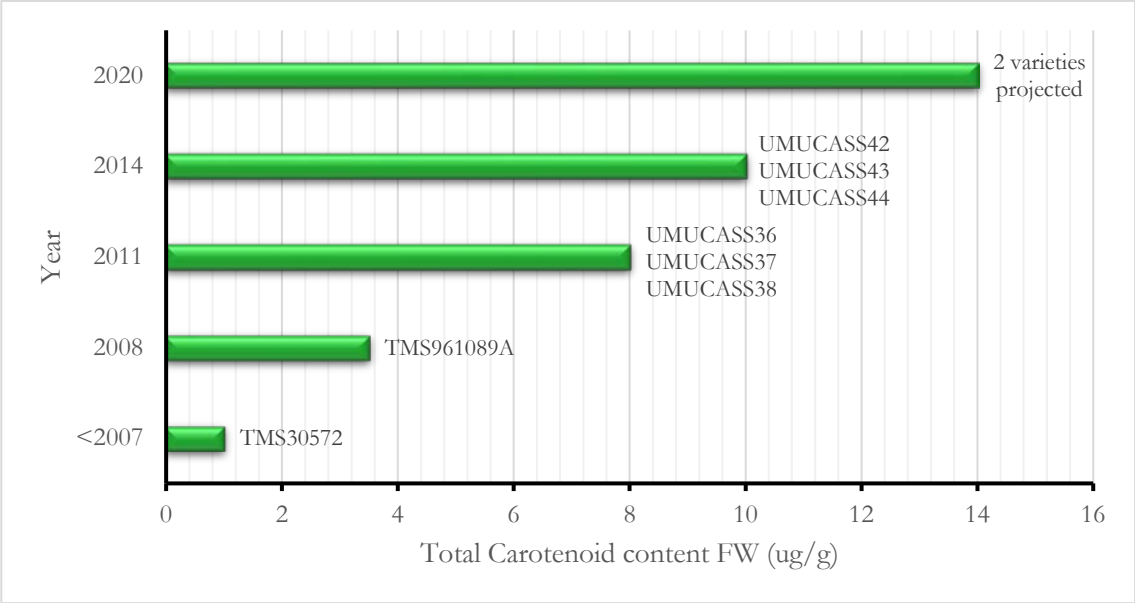

Figure 15 : Pro-vitamin A in Nigeria showing the six biofortified varieties released and those in the pipeline.

1.4. Cassava Gap Analysis

At CIAT, the biochemical basis for softening in response to boiling or fermentation have not yet been established. The degree of association between these two response variables has not been determined either. What is clear is that boiling results in a gradual and consistent reduction of starch and cyanogenic glucosides in the root (Ezeigbo et al., 2015).

In Nigeria, determination of most critical traits responsible for the best gari and fufu quality, is the major focus of the RTBFoods project. For example, studies by Teeken et al. (2018) identified the trait dynamics differentiated by gender and during focus group discussions indicated that preferences were governed by the stretch-ability of the gari and fufu. Would it be sufficient that the pectin or amylose contents would be key determinant of preferred gari and fufu and if yes, what would be the thresholds? Would the size of starch granules play any role in root mealiness?

In Uganda, by the end of this project, data on five traits are expected to be generated: 1) root dry matter content; 2) beta carotene content; 3) softness; 4) cyanogenic potential; and 5) starch content. This presents opportunities to address both knowledge and/or methodological gaps notable of which will include:

- empirical assessment of both narrow-sense and broad-sense heritabilities for the target traits;
- assessment of genetic gain associated with breeding for the five target traits;
- development of less-drudgery and efficient root trait phenotyping methods for the five target traits;
- documentation of protocols to be used for routine assessment of the five target traits.

2. BREEDING YAM FOR QUALITY TRAITS

Introduction

Yam (*Dioscorea spp.*) is a versatile staple crop which posses a huge potential to address sustainable food supply and predictable source of income in tropics and sub-tropics: (1) it produces more agricultural value per unit area of land compared to all major arable crops, (2) it is largely traded locally and nationally, not internationally hence far less susceptible to large-scale market shocks and price speculation experienced by more widely traded staples, such as grains. The crop is most important in West Africa where 93.5% of its global production (ca. 62 million tonnes in 2016) occurs. It supports the livelihood of over 300 million people as a source of staple food and cash income. Yam ranks as the most important source of dietary calories in West Africa. It also makes a substantial contribution to protein in the diet, ranking as the third most important source of supply, much greater than the more widely grown cassava, and even above animal protein sources. Yam is a crop with the highest gross value of production which is about $14 billion US dollar. The yam value exceeds all other African staple crops and is equivalent to the summed value for the top three cereal crops (maize, rice and sorghum) (FAO, 2000-2016). Yam-based cropping systems occupied 20% of the lowland humid and sub-humid zones of nine countries in West Africa (Manyong et al. (1996) where population is increasing most quickly (Alabi et al., 2019). Yam is a strategic crop for sustainable food production and wealth creation in West Africa and beyond to efficiently address the ever-expanding food, nutrition and income demand. The yam farming, however, is complex and presents many challenges than any other crops in the region and characterized with very low productivity where farmers achieving only 20% of its potential yield (FAO, 2016). Yam farming has relied heavily on area expansion instead of progressive productivity gain. Its cultivation, traditionally practiced in a shifting-rotation 'bush-fallow' system, is now evolving towards permanent plot farming with reduced fallow periods due to limitations of new land availability. The crop requires fertile land rich in organic matter for optimum productivity but the current sedentary type of yam farming being practiced with low or no externally added nutrients and underdeveloped agronomic and post-harvest practices is facing chronic yam productivity and quality challenges. The co-occurrence of soil nutrient decline with multiple stresses from diseases, pests, and weeds that limits productivity and reduces product quality in many traditional yam belt areas, which are further exacerbated by the complexity of global climate change modifying the growing environments and/or adding new stress factors, are becoming major challenges. Yam production systems of West Africa must cope with these challenges that can result in annual yield losses of over 50% (Amusa 2001, Adeniji et al., 2012). The vast majority of yam production occurs in smallholdings which require integrated low-tech solutions to these problems, and small-scale growers are particularly vulnerable to variable incomes. Processing and culinary innovation that uses an optimal mix of techniques can improve yam product quality and deliver social benefits. However, integrated food system technologies that adequately improve yam food product quality while reducing dead-ends from yam genetic improvement are currently lacking. Opportunities exist for investments in food quality to improve the yam sector in West Africa and beyond. West Africa is known for very long-standing practices and engagements with yam cultivation, trading, processing, and consumption. The economic value of the yam industry has grown quite rapidly in recent years. Yam as a non-traditional export commodity is shooting upwards in Ghana and starting with great boom in Nigeria. There is an increasing demand for yams for consumption as food in both the domestic and foreign markets, as well as a growing interest in developing starch extracts from yams for industrial use. Yam sector is very strategic for West Africa as a source of food, income generation, and social relevance. Real and potential opportunities exist for research intensifying the role of yam crop for food security and economic development in the region. Studies on efficiency-equity trade-offs (Alene et al., 2009) and poverty-based priority setting (Alene et al., 2007) have together demonstrated the possibility of directing greater benefits to the poor through increased yam improvement research. Over a 20-year period, such research generates an economic surplus of over US$7.5 billion in Nigeria, with over US$677 million accruing to poor households. The internal rate of return to investments in yam research is estimated at 131%. Increased yam research has the potential to reduce poverty by >4.5%. If improved varieties were used and a 30% yield increase realized, the production value of yam will increase to $17.9 billion. However, the current lack of improved yam-based food technologies that further support the yam productivity gains from genetic improvement are the main challenges in current yam production practices. Product quality and culinary innovations are

essential to change the current yam production practices and to thereby satisfy the food and nutritional needs of an ever-increasing African population. Investment in yam-based food product quality research will improve yam crop production and strengthen value chain through novel yam product processing and quality solutions that are intrinsically linked to the sustainable yam production.

2.1. Yam Breeding for Quality Traits at IITA : Nigeria

The International Institute of Tropical Agriculture (IITA) in collaboration with its national yam research programme partners in West Africa have made significant progress in yam breeding. The primary focus of this collaborative breeding effort is development and deployment of robust varieties with unique combinations of preferred traits required for production and consumption majorly on two extensively cultivated species, *Dioscorea rotundata* and *Diocorea alata*. The breeding programs generally targeted traits related with yield limiting and quality reducing factors, however, the specific breeding targets varied according to the region and species involved (Mignouna et al., 2007). From the inception of formal yam breeding in 1970's to date, the breeding targets have evolved over the years to meet the changing needs and preferences of farmers and other end-users. The breeding targets have since then been gradually included new traits along with basic focus on high and stable tuber yield, higher dry matter, resistance to economically important diseases (e.g. anthracnose, viruses, tuber rots) and pests (e.g. nematodes), tuber characteristics cherished by consumers (e.g. size, shape, and culinary quality) in the region; and plant architecture (e.g. dwarf genes) that reduces the need for staking (Asiedu et al., 1998; IITA, 1999, Mignouna et al., 2007). These efforts over the last five decades have resulted in identification of trait progenitors and commercial release of improved cultivars, however, farmers continued to grow landrace cultivars with low or minimal rate of variety turnover or replacement in the production system. High level resistance genes to virus, anthracnose, and nematodes, shrub-like or dwarf plant architecture with stiff or stout vine base and early branching, tubers less susceptibility to deformation in the soil, tolerance to low soil fertility, drought and heat, and high level of sensory and processing attributes suit consumer and market needs for fresh and processed yams are some of the missing traits in advanced breeding lines and released cultivars. The local farmer varieties are still the leading and dominate cultivars in the yam cultivation and consumption systems of Africa. In order to address the apparent less successful product innovation with yams breeding, the goal setting or specifications of the varieties have been refocused and restructure in recent years. As a result, the breeding targets have been transformed from undifferentiated product portfolio to a differentiated product concept where client needs are clearly profiled and translated to product specification. The yam breeding program currently focusing on what clients really want as centre of its variety development plan. Accordingly, precise description of what product to breed for and traits or features that constitute the preferred product or varieties are the current targets. The yam teams are implementing a product concept in yam breeding by identification and prioritization of right features or traits for the varieties the clients (growers and consumers) require or demand with clear roadmap to achieve the target product in a specified timeframe. The general framework for new variety design in yams is whom and where the new variety is targeted to serve. The new product concept attempt to ensure tangible benefits to the clients (growers and consumers) with clear product differentiation: key attributes of the product for the target client and benchmark for improvement. The yam breeding program therefore envision implementing best product concept that can drive rapid and successful uptake of new variety in the production and consumption system. With the current framework, in addition to the traditional traits, the yam breeding activities are focusing on tuber quality, nutritional value and metabolites that define yam cultivar acceptability for consumption. Traits such as colour of tuber flesh, tuber oxidation, tuber shape, starch property, dry matter content and other functional properties are now routinely measured in breeding programmes as they influence the acceptability of newly developed yam varieties.

2.1.1. Phenotyping for quality traits

The yam quality phenotyping routinely used in yam breeding programs includes fresh tuber physical quality assessment, and physico-chemical and functional properties of fresh tuber for predicting boiled yam and pounded yam food quality. In addition, breeding lines and trait progenitors are being phenotyped for yam flour characteristics.

Long list of nutritional and anti-nutritional quality traits related with yam flour characteristics, pounded yam characteristics and boiled yam characteristics are now being routinely measured in breeding programs. These included traits such as colour of tuber flesh, tuber oxidation, tuber shape, dry matter, peel loss, starch yield, pasting property of starch, flour yield, and other functional properties such as ash content, total protein, fat, amylose, sugar, vitamin, phytate and tannin are now routinely measured in breeding programmes as they influence the acceptability of newly developed yam varieties.

Table 24. Quality traits being assessed on yam products

Product profile	Traits
Pounded yam	Pasting profile of starch using RVA, color, consistency, stretchability, texture
Boiled yam	Sensory attributes: taste, color, texture, aroma, over all acceptability of boiled yam slices
Yam flour	Peel loss, flour yield, flour color

Methods

Table 25. Methods being used for assessing yam food quality traits

Method	Traits
Consumer preference	Color, taste, aroma, texture, overall acceptability of boiled or pounded yam
Proximate composition	Dry matter, ash, protein, fat, amylose, starch and sugar, vitamin, phytate and tannin
NIRS	Prediction of moisture content, ash, protein, crude fibre, tannin, sugar, phytate, amylose content on fresh tuber and processed food products
Gas Chromatography-Mass Spectrometry (GC-MS) Profiling	Metabolites
RVA	Pasting properties of yam starch

2.1.2. Genetic variability in IITA breeding program

Currently the active yam breeding programs in West Africa are composed of IITA and its national partner programs from Benin, Côte d'Ivoire, Ghana and Nigeria. Through collaborative effort, the IITA-derived breeding lines, released varieties, popular farmer cultivars and landrace accessions are being extensively characterized for fresh tuber and processed food quality traits in West Africa. The programs are currently studying physicochemical and functional properties for predicting food quality and pasting characteristics of fresh yam as indicators of textural quality in major food products. In addition, tuber micronutrient density, specifically for iron, zinc, total carotenoids, ascorbic acid (vitamin C), phytate, and tannin content have been assessed in different yam germplasms. A diverse panel of accessions from four *Dioscorea* species routinely used in yam breeding programmes are also being studied for metabolomic profiles in collaboration with advanced research institutes to augment the breeding of new yam varieties with improved consumer and agronomic traits. Physico-chemical and functional properties of diversity panels, mapping populations, trait progenitors and breeding lines were assessed at IITA with support from Africayam, EDITYAM and other projects.

Table 26. Type of genetic materials being assessed for quality traits under different studies at IITA. This table summarized very recent and on-going studies for quality breeding in yams at IITA

Genetic material	Different studies
Breeding lines	A total of 232 genotypes (61 *D. rotundata* and 171 *D. alata*) grown at three sites in Nigeria (Abuja, Ibadan and Ubiaja) were evaluated for nutritional and anti-nutritional quality traits: Dry matter, ash, protein, fat, amylose, starch, sugar, vitamin, phytate and tannin.
Elite clones under multi-location trials for release	20 *D. rotundata* and 12 *D. alata* elite clones in multi-environment trails were assessed for consumer preference traits (boiled yam) and RVA properties
Diversity panels	119 *D. rotundata* diversity panels being assessed for tuber oxidation, tuber flesh color and pasting properties of starch using RVA
Trait progenitors	36 trait progenitors routinely used in yam crossing block were evaluated for tuber oxidation, tuber flesh color, yam flour characteristics and pasting properties of starch using RVA
Advanced breeding lines	49 advanced breeding lines grown at two sites in Nigeria were assessed for yam flour characteristics, dry matter, peel loss and pasting profile of starch
Early and advanced breeding lines	Routinely assessed for tuber flesh color, tuber oxidation and dry matter content

2.2. Yam Breeding for Quality Traits at NRCRI: Nigeria

The goal of NRCRI's yam research (National Root Crops Research Institute**)** has been to enhance the adoption of improved technologies by farmers, and thus contribute to a sustainable increase in productivity of yam-based systems. In contributing to achievement of this goal, the objective of the genetic improvement component has been to develop and disseminate improved yam genotypes with high and stable yield of tubers with good storage and food qualities suited to the relevant cropping systems. Yam breeding has resulted to 21 varietal releases and more recently official registration of five landraces (See Annex1). The breeding pipeline adopted within these process include seedling evaluation, clonal evaluations, preliminary yield trials, advanced yield trials, uniform yield trials, two years multilocational trials and one year on farm verification.

2.2.1. Phenotyping for quality traits

At the early stage of breeding cycles, we characterize and advance clones based on yield, response to diseases and pest. Food quality traits often considered alongside the agronomic traits include tuber flesh colour, physico-chemical factors in fresh yam tubers (granule morphology; starch granule size, histological structure of the cells), physico-chemical composition of yam starch (amylose/ amylopectin ratio, swelling, water binding capacity), pasting characteristics of fresh yam tubers, as well as calcium, phosphorus and cellulose contents of yam tubers that are indicators of textural quality in 'pounded yam. These quality attributes are significant in determining the quality of yam end use products (Otegbayo *et al.,* 2010). Starch accounts for 80% (on dry weight basis) of the yam tuber. It has been reported as one of the dominant factors which affects the physicochemical, rheological and textural characteristics of food products from different yam species (Amani *et al*., 2004). Otegbayo *et al*., (2011), reported that granule size, swelling power, amylose and water binding capacity of yam starch can be indicators of textural quality in 'pounded yam'. Investigating the physico chemical properties of yam flour, starch and also non-starchy polysaccharides (lignin, pectin, cellulose, hemicelluloses) in yam could also give insight to quality factors which can predict the quality of food products such as boiled yam, thus serving as screening tools to breed for these specific traits in order to produce tubers with qualities that will be acceptable by the end users (farmers, consumers, processors). In boiled yam quality attributes such as colour and taste (sweet, bland, sour) as well as textural attributes such as hardness, mealiness/sogginess/waxiness is very important.

At the advanced stages of evaluation we consider food qualities using participatory varietal selection. Breeders, farmers and processors involved in the improvement of yam for food quality have to cook the yam into various food forms to assess the suitability of clones for specific products. Carrying out such assessment is very cumbersome; extremely time consuming, expensive and the result could be subjective. Optimum cooking time is assessed by placing a sample each in pre-heated (boiled) water

and checking at intervals to detect changes in texture. An approximate of ten chunks of each sample is placed in boiling water and first checked after 10 min of boiling and thereafter at 5 min interval to determine optimum cooking time of each genotype. The optimum cooking time is taken to be the approximate time within which samples were cooked right through the middle. This is determined by piercing through with a fork. Consumers of the two most important food products (boiled yam and pounded yam) describe their subjective impressions either in qualitative tests e.g. focus group discussing or in quantitative tests. A hedonic scale of 1 - 5, where 1 = like very much and 5 = dislike very much, is always used for descriptive purposes, to assess colour attractiveness, texture, aroma, taste description and perception, after-taste and general acceptance. The intensity of taste and after-taste, are measured on a scale of 1 - 5 where 1 = very sweet and 5 = bitter. Texture is scored on a scale of 1 - 4 where 1 = very mealy and 4 = waxy; aroma on a scale of 1 - 5 where, 1 = very high and 5 = none; colour on a scale of 1 - 5 where 1 = white and 5 = purple and general acceptability on a scale of 1 - 4, where 1 = very good and 5 = very bad. Data is collected on enzymatic oxidation (time for browning of cut surface) 1 - 3; 1 = < 1 min, 2 = 1 - 2 min and 3 > 2 min. The genotypes are thus ranked for individual attributes and general acceptability. The mean rank for each genotype is normally calculated. The percentage of farmers, processors and consumers giving a particular ranking to an attribute of a genotype is calculated as well. It is important to note that due to the limited capacity of sensory analysis; only a few lines can be objectively evaluated within a short time. Secondly, owing to the quantity of material required in each sample to be evaluated, such screening can only be done close to the end of the selection cycle when there are sufficient numbers of tubers per genotype. However by this stage the numbers of genotypes have been significantly reduced on the basis of other selection criteria and several lines with potentially superior tuber quality would have been lost. Hence it has been difficult to make progress in the breeding of yams for food quality because of lack of appropriate screening techniques.

2.2.2. Status of NRCRI breeding populations in respect to quality

Based on the protocols mentioned above the underlisted trials (starting with the most advanced) are ongoing

On farm verification of D. rotundata genotypes

One of the pre-requisite for varietal release is a nationally coordinated multi locational testing and evaluation. The potential candidate(s) for release need to be tried across diverse agro-ecological zones to ascertain their area of specific and wide range agro ecological adaptation /stability and consumer acceptability. The two years data set (2016 and 2017) resulted to a reliable estimation of the genotype mean yield across locations, response to abiotic/ biotic stress and post-harvest traits.

The twenty *D. rotundata* genotypes that were evaluated at multisite include TDr8902157, TDr1100585, TDr1100034, TDr0900002, TDr1100873, TDr8902665, TDr1100835, Amula, TDr1100163, TDr1100497, TDr1100396, TDr1100421, TDr0900058, TDr1100101, TDr0500491, TDr1100278, TDr1100492, Meccakusa (control), TDr0900082, and TDr1100582.

Post-harvest data collection encompassed participatory varietal selection (PVS) cooking qualities using a set of well-structured questionnaire, sensory evaluation and farmer perceptions (See Figures 1-8). This provided reliable guidance for selecting the best genotypes that were advanced to on- farm verification in 2018.

Table 27. Candidate clone selection for on-farm trial

Based on tuber yield advantage over the best check variety in the trial		Based on consumer acceptability for boiled yams (taste, color, aroma, appearance		Mean yield and preference by farmers at harvest/vegetative trait		Best candidate for on-farm verification
TDr0500491 **TDr0900002** **TDr1100101** **TDr1100582** **TDr1100585** **TDr1100835** **TDr1100492**	45% of the test location performed better than best check variety	TDr11000497 TDr1100396 TDr1100163 TDr0500491 TDr1100421 TDr1100492	Rated good for sensory traits in majority of the test location as per logistic regression analysis	TDr1100163 TDr1100497 TDr0900058 TDr0900002 TDr1100492	Based on over all tuber yield and farmer preference at harvest and vegetative stage	TDr1100163 TDr1100497 TDr1100492 These three clones merit for yield advantage and preference of farmers for harvest and sensory traits

The three selected clones have been established in eight locations this year for farmers verification. Candidate(s) that pass will be nominated for varietal release late this year.

Multi locational evaluation of 16 D. rotundata genotypes

Sixteen *D. rotundata* genotypes that passed uniform yield trial evaluation in 2016 were advanced to multi location testing in 2018. Selections will be participatory with farmers and consumers at vegetative, harvest and post-harvest stages for two years. The clones include TDr0002405, TDr0900061, TDr0900067, TDr1000003, TDr1000006, TDr1000016, TDr1000021, TDr1000048, TDr1000078, TDr1000179, TDr1000344, TDr1000360, TDr1000459, TDr1400633, TDr8902665, and local checks.

(c) Multiplication and screening of two D. rotundata breeding populations for agronomic and post-harvest qualities

Surviving one hundred and forty seven and one hundred and twenty individuals generated from 2015 crosses will be multiplied in 2018 under field conditions. These individuals from two independent populations have been previously maintained in screenhouse. Individuals from these populations will be phenotyped for multiple traits including post-harvest traits like tuber flesh colour, oxidation, pasting characteritiscs, starch quality and dry matter.

Evaluation of four early breeding populations

Evaluation of *D. rotundata* clonal materials generated from seeding advancement in 2017 will be undertaken. The four independent populations is already established under screen house condition. Individuals will be screened for tuber flesh colour and oxidative qualities.

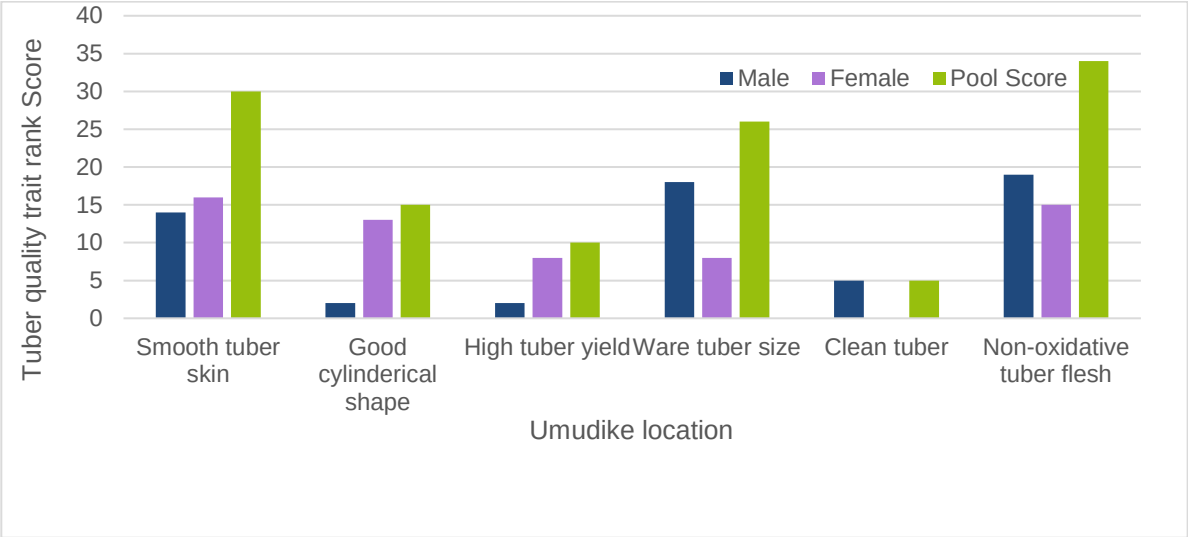

Figure 16. Farmers preferred tuber quality traits in Umudike location

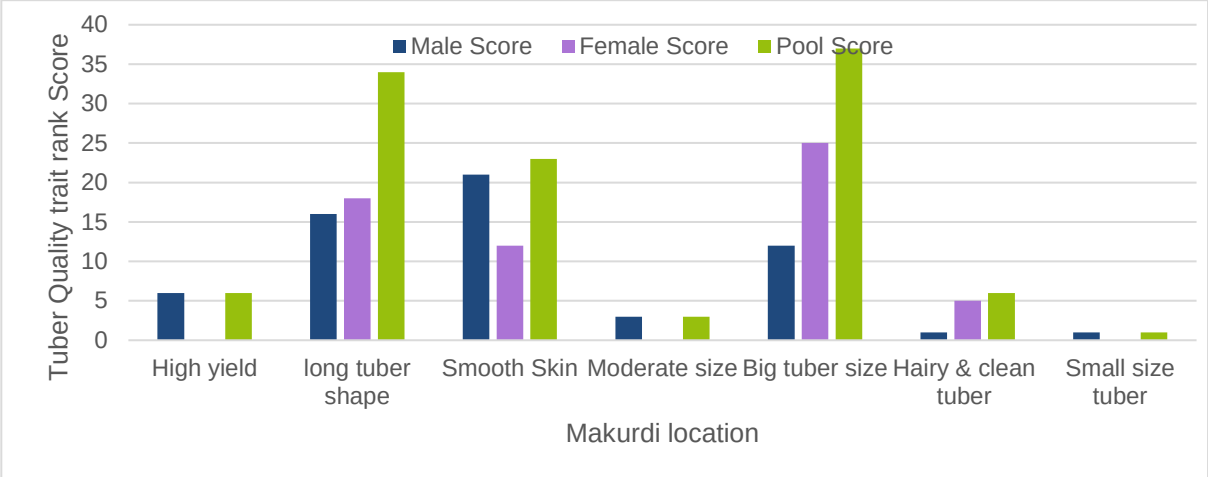

Figure 17. Farmers preferred tuber quality traits in Makurdi location

Figure 18. Farmers preferred tuber quality traits in Uyo location

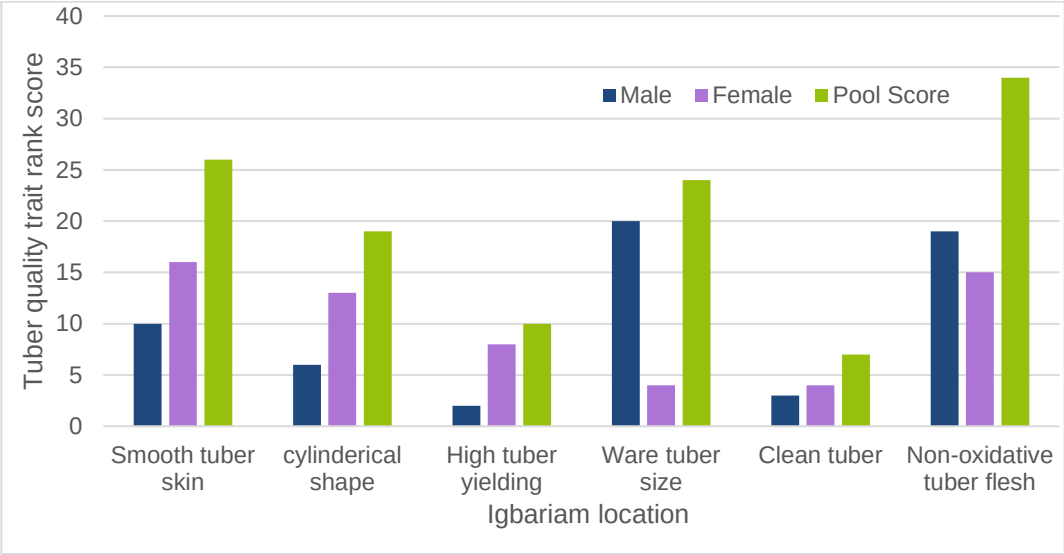

Figure 19. Farmers preferred tuber quality traits in Igbariam location

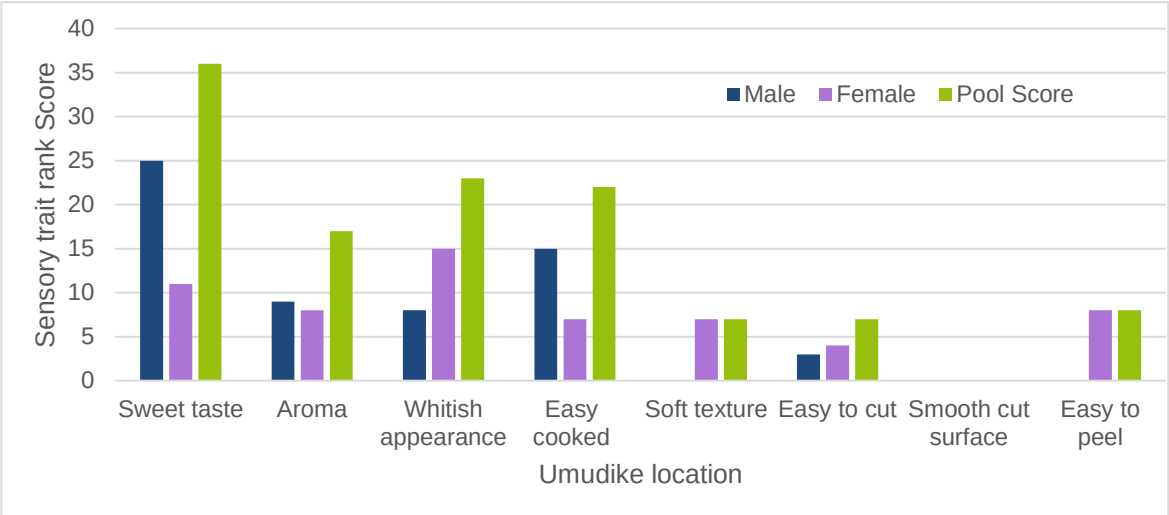

Figure 20. Farmers preferred sensory traits in Umudike

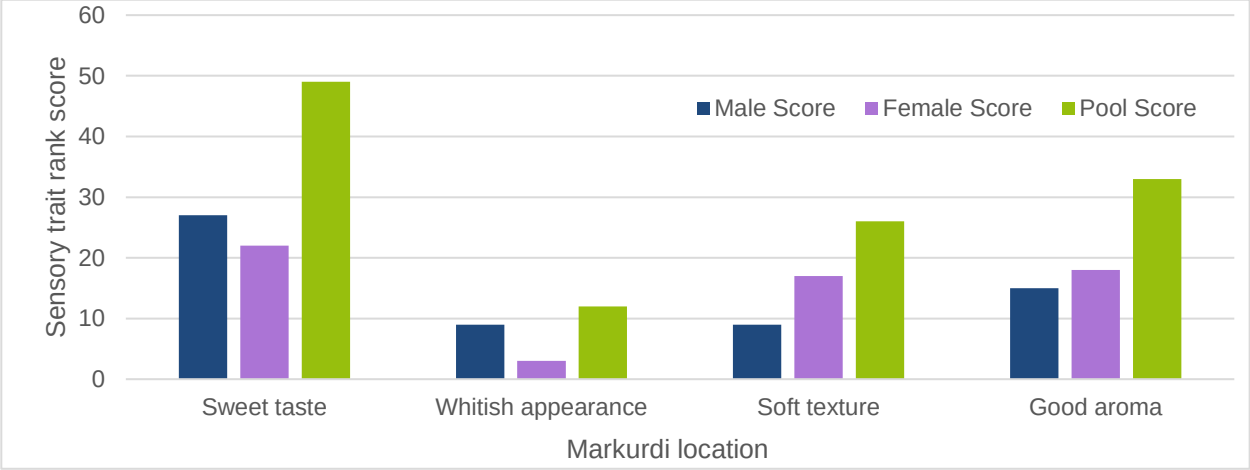

Figure 21. Farmers preferred sensory traits in Makurdi

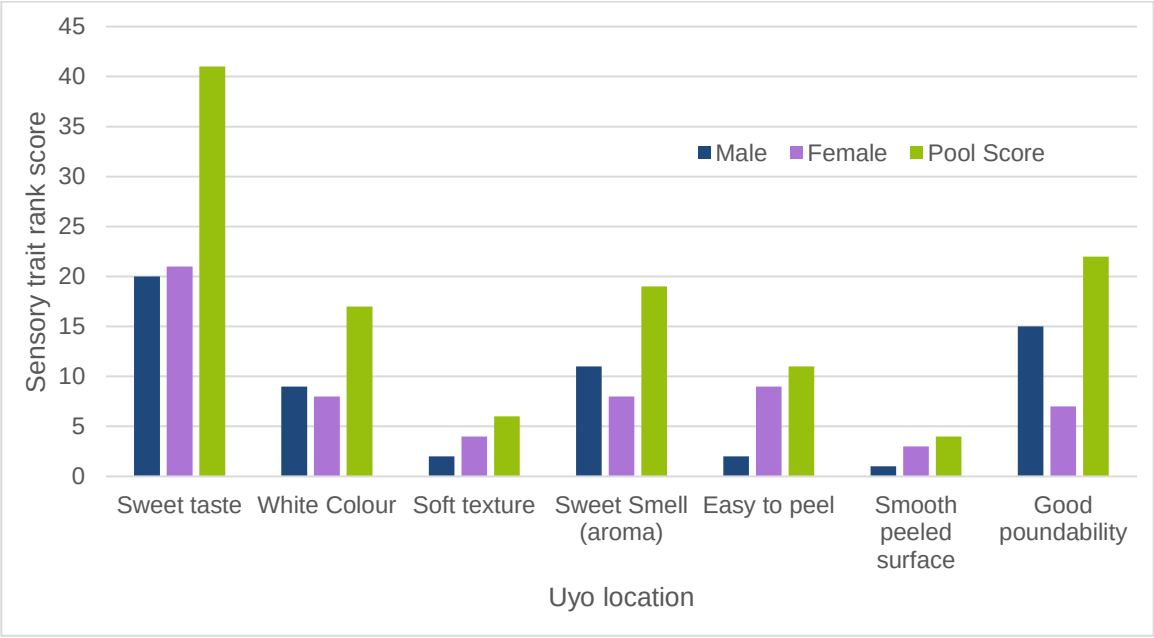

Figure 22. Farmers preferred sensory traits in Uyo

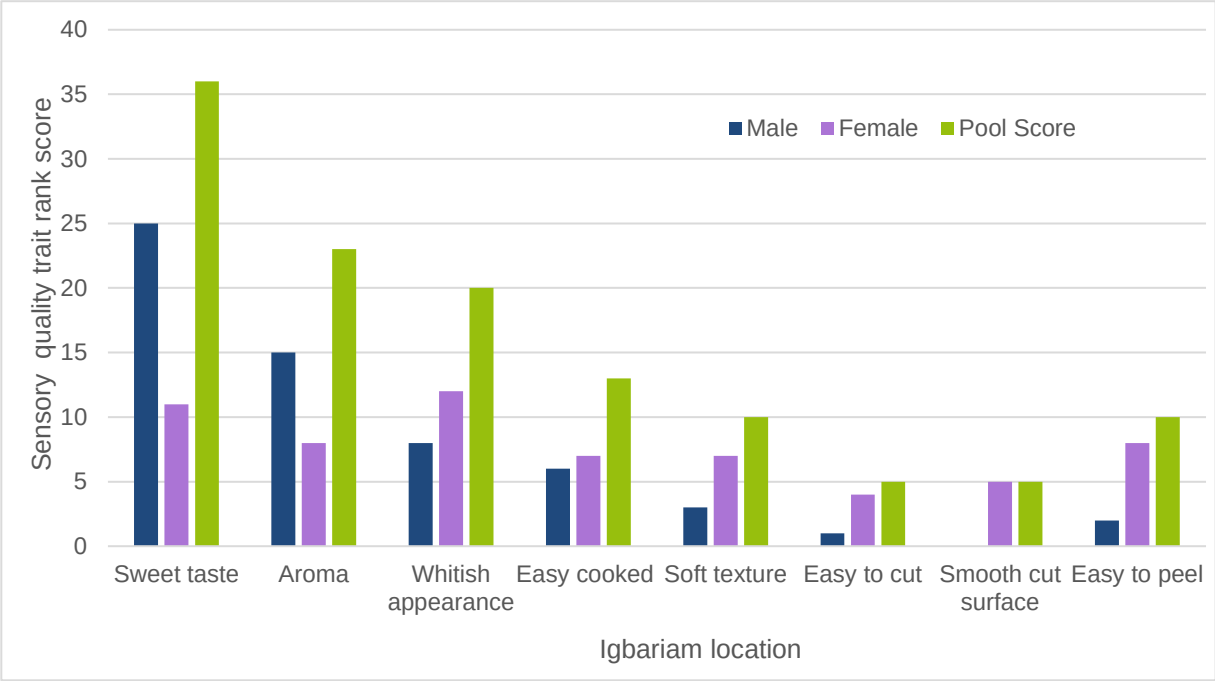

Figure 23. Farmers preferred sensory traits in Igbariam

2.3. Yam Breeding for Quality Traits at CNRA IITA: Côte d'Ivoire

The main objective of yam breeding program at CNRA is the selection of varieties for food quality, particularly for boiled and pounded yam. *Dioscorea alata* and *Discorea rotundata* are the two species considered. To reach this objective, breeding starts by selecting clones which are resistant to pests and diseases and have high tuber qualities. The main traits observed are: the shape and the smoothness of the tubers (that means absence of roots or hair on the tubers), the colour of the tuber flesh, the dry matter content, the oxidation, the absence of brown spot, the appreciation of the boiled and pounded yam by consumers. The last attribute is appreciated by participatory evaluation by farmers and consumers. They appreciate the cooking time, the aroma, the acceptability, the flavor, the lumpiness,

the springiness, the looseness, the sweetness, the steakiness, the smoothness, the firmness, the elasticity. The other traits are appreciated by visual observation and weighing for the dry matter.

2.3.1. Phentotyping for quality traits

In 2010, a study was conducted on 91 accessions of *D. alata* yam germplasm of CNRA. This study showed that most of the accessions had desired traits for the external aspect of the tuber.

The presence of anchor roots on the tuber, the level of ramification of the tuber and the tuber shape gave the best description of the yam tuber. Most accessions (70.33 %) do not have anchor roots on the tuber. Eighty-five percent of the accessions had tubers not ramified and 44 % of the germplasm had round shape of tuber. For the flesh, 91 % did not have oxidation whereas 72 % showed smooth aspect of the parenchyma;

Figure 24. Smooth tuber of the variety Amadouo

Figure 25. White and purple flesh tubers of Dioscorea alata

2.3.2. Participatory quality evaluation by farmers and consumers

In 2006, participatory evaluation was conducted on four landraces of slicing, pre-cooking and drying in the yam chips process. The survey conducted on 122 farmers revealed four ways of yam processing: roasted, boiled, stewed and pounded yams. In one region, flour was made for couscous.

In 2018, a participatory evaluation was done on 59 breeding lines of *D. alata* and *D. rotundata*.The sensory parameters testes were : the color, the perception of the cooked tuber, the presence of brown spot, the presence of fiber, the cooking time, the friability, the firmness, the elasticity, the aroma, the savor and the global acceptance. For the color, the values varied from 2,00 ± 0,71 (TDa0700154) to 4,75 ± 0,45 (Krenglè). The range went fom 1 (bad) to 5 (very good). This work is on-going. Preliminary results showed a variability among the varieties selected for food qualiy according to farmers and consumers. Farmers and consumers indicated also their preferences. These observations have to be

measured by precise tools like NIRs or physico-chemical methods to establish links for the breeding scheme.

Figure 26. Pounded yam and a woman participating to the sensory evaluation at Bouaké.

2.3.3. Genetic variability in CNRA breeding program

The genetic material is tested at the earlier stage for diseases and the row tuber is phenotyped (shape, absence of roots, oxidation). After the advanced yield level, the food quality traits are accessed. In 2018, 10 clones (5 *D. alata* and 5 *D. rotundata*) were tested at Bouaké and Gagnoa. Two were selected from the yam germplasm and 8 were from the breeding clones introduced from IITA. Five of them are submitted for releasing. Many other clones are in the pipeline for quality testing during the next years. Twenty parents having contrasted quality traits (10 *D. alata* and 10 *D. rotundata*) are used for crosses. More than 2000 hybrids have been created and are at different stages of breeding.

2.4. Yam Breeding for Quality Traits at CIRAD-INRA: Guadeloupe

CIRAD and INRA breeding programs have been oriented to the creation of hybrids (diploid for INRA and polyploid for CIRAD) combining a good quality as regard to Caribbean preferences, anthracnose resistance and enhanced tuber yield.

Several improved hybrids have already been released through the process of participatory evaluation in the framework of RITA (https://coatis.rita-dom.fr/guadeloupe/?HomePage). Since 2012, in this multi-sites selection platform hosted by farmers, a total of 19 hybrids pre-selected by CIRAD and INRA were evaluated in 10 sites. Quality (i.e. taste, flesh browning, and colour) was evaluated during groups tasting. These tastings were conducted during farmers meetings and were a qualitative evaluation of the cooked hybrids. A website have been design that compiles all results from these evaluation plateform to help farmers to choose among varieties (https://ziyanmannou.cirad.fr).

To now, the main criteria linked to quality applied during the selection process are related to the tuber form, the flesh browning and colour and are visually assessed. More complex traits such as taste or cooking ability are used during the final selection stages on few promising hybrids and on hedonic scales.

Phenotyping related to quality is mainly conducted on diversity panel to identify possible genitors. These work is also the support of “high-throughput” phenotyping methods development. To now, most of the work has been done on physico-chemical characterization. Lebot et al., (2006) studied 48 accessions from Vanuatu and linked physico-chemical analysis to boiled quality assessed as “good”, “average” or “poor”. Good quality accessions were characterized by a higher starch content and amylose/starch ratio which is related to firmness and elasticity (Bourrieau, 2000). A broader diversity was also characterised

for these parameters, without comparing it to cooked quality (Lebot and Malapa, 2013), in order to develop NIRS calibration for *D. alata* breeding programs.

At CIRAD_Guadeloupe, during two growing seasons, physico-chemical analysis were conducted on 23-27 accessions producing flower in order to identify genitors (Arnau G., Cavalbio project, 2014-2017, European Union and Guadeloupe regional grant). In parallel, several “high-throughput” phenotyping methods have been develop to measure other traits related to quality (Cornet D., Cavalbio project, 2014-2017, European Union and Guadeloupe regional grant). These methods are mainly based on picture analysis and will allow the phenotyping of large populations. For example, tuber flesh browning and colour are now automatically assessed using repeated pictures of sliced tubers.

Figure 27. Example of the browning phenotyping method (Denis Cornet, Cavalbio project).

In the framework of AfricaYam, two biparental populations have been created to develop genetic resources/tools and select improved hybrids. Parents have been chosen to study quality traits. The tuber shape of the common female (74F) is cylindrical and the flesh is yellowish, while males shapes are oval and flesh colour is white for Kabusa and white-cream for 14M. Males are also characterized by a higher starch content and a lower sugar content than the female.

Figure 28. The biparental populations in field (Gemma Arnau, AfricaYam project).

These populations were the support of the development of the first *D. alata* high-dense genetic map and will be the support of the studies of genetic determinism of quality traits.

2.5. Yam Breeding for Quality Gap Analysis

Development of yam varieties highly cherished by consumers for food quality is the main target of yam breeding programs. Large number of breeding materials have been profiled for quality traits using different methods. The quality trait evaluation is laborious and often challenging when it comes to screening larger number of genotypes and samples. In this sense, most of the traits related to quality had been evaluated in few combination of genotypes × environment. Moreover, yam breeding is based on the creation of new hybrids (heterozygous). The first gap to fill to improve selection process is thus to estimate the part of variance related to genotype and the part of variance related to additive genetic variance that can be combined in progenies. Indeed, the heritability (narrow/broad-sense) of the most important traits have to be addressed before going further.

Efficiency in breeding for quality breeding would be improved by adopting high-throughput and low cost early screening to eliminate “bad” genotypes. Indeed, it will allow to reduce the cost of maintenance of large populations or increase breeding population size at a constant cost. This goal is aimed by the RTBfoods project using NIRS technology or genetic/genomic information. Whether, these methods are concerning early-phenotyping or marker assisted selection. However, to be sure they can be adopted, our project needs to go further that methods development and should address the question of their day-today uses, that is to say:

- when to apply them? Indeed, during the first generation only few materials are available to test and multiply progenies (few small tubers).
- what is their efficiency in breeding populations? Indeed, if methods are developed on diversity panel, their application in breeding populations (reduced and oriented diversity) have to be discussed.
- And more generally, at which cost (human work and price) for which efficiency? This question may be program specific.

3. BREEDING POTATO FOR QUALITY TRAITS

Introduction

As one of the most versatile food crops, which produces more dry matter and protein per hectare than the major cereal crops, potato (*Solanum tuberosum*) has been planted worldwide (Carputo et al., 2005, Storey, 2007). The most important aspects of potato production are tuber quality, that includes biological, sensorial, and industrial traits. Since most quality traits are genetically controlled, breeding work can successfully meet the needs of a changing and demanding world. The potato needs a continued improvement of quality that are most likely influenced by consumer's choice (Carputo et al., 2005).

Develop an efficient selection method for quality traits can increase the adoption by fulfilling the end-user expectations. Once genetic variability has been produced, it is necessary to identify selection procedures that reduce time and costs to develop a new variety. Breeding potato for quality traits at International Potato Center is a continuous effort, where the primary objective is to obtain information about the potential or aptitude of intermediate and advanced clones for diverse end-users, ranging from fresh consumption to processed products. This provides important information to guide potato breeding and selection programs, as well as for the recommendation of varieties for specific uses and determination of parental value for quality traits.

3.1. Potato Breeding for Quality Traits at CIP: Uganda

Three hundred "trait observation network" (TON) panel clones are set for phenotyping assessment in Uganda in 2019. A corresponding single nucleotide polymorphism (SNP) dataset will be available in an open access database at International Potato Center (CIP) for further genotyping. Within RTBFOODS, at least 150 genotypes (panel clones) and locally-preferred varieties will be phenotyped within diverse environments that influence quality traits (e.g., cool highlands and warm mid-elevation or lowland agro-ecology) in Uganda. Phenotypic data and GWAS for productivity and resilience traits can be contributed from the BMZ/GTZ funded project "Accelerating the Development of Early-Maturing-Agile Potato for Food Security through a Trait Observation and Discovery Network", established in Ethiopia, Peru and China.

This panel is mostly comprised of CIP's advanced tetraploid populations B3, B1 and LTVR and is a dynamic collection of bred clones previously subject to analysis of structure and successfully used for genome-wide association studies (GWAS). Population B is under improvement for high levels of horizontal resistance to late blight along with economically important traits such as tuber yield, quality for table and industry, adaptation to wide environments and tolerance to other biotic/abiotic stresses. The LTVR population is characterized mainly for its resistance to the most important virus diseases [potato virus V (PVY), potato virus Y (PVX) and potato leafroll virus (PLRV)) of potato, early tuberization in short day-length conditions, mid-maturity under long days and adaptation to warm, arid environments.

3.1.1. Quality traits assessment at CIP

The quality assessment on potato at CIP has mainly been applied for advanced clonal selection and genetics studies for parental value prediction. CIP has adopted standard procedures[2] for determining: i) specific gravity and dry matter content, ii) texture and flavor components of cooking quality, iii) storage behavior, iv) chipping and French-frying performance, v) oil content, and vi) contents of undesirable secondary products such as glycoalkaloids. However, quality traits have not been a target on CIP's breeding population.

[2] https://research.cip.cgiar.org/confluence/display/SET/Processing+Protocol

Gastelo et al. 2014 have explained that the potato breeding program of CIP aims to generate improved populations and clones with resistance or tolerance to biotic (virus and late blight) and abiotic stresses (drought and heat). For that CIP has used wide genetic resources (including wild, landrace and improved germplasm) to develop improved populations adapted to stressful conditions of the tropics on highland and lowlands. CIP has also increased the level of micronutrient densities (Fe and Zn) on a diploid population by recurrent selection and introgressed those traits to tetraploid population, combining high level of micronutrients with tolerance to biotic and abiotic stresses.

Advanced potato clones combining different traits for a diverse potato agroecologies are available for international distribution. CIP has developed catalogue[3] to provide relevant information about advanced potato clones that combine several traits, such as resistance to late blight, viruses, high productivity, quality traits among others. Annex 2 shows list of morphological, agronomical and post-harvest traits for potato assessed at CIP's breeding program.

3.1.2. Breeding potato for quality traits

Several publications have discussed breeding strategies for several quality traits on potato (Bradshaw et al., 2003, D'hoop et al., 2008, Bradshaw et al., 2009, Slater et al., 2016, Gastelo et al., 2017). However, it's expected greater genetic gain combining new tools (HTTP, GWAS, GWS) accessing in earlier breeding stages, characteristics such as sugar profiles, texture profile (dry matter, cooking time, cell wall, cooking time), nutritional and antinutritional (glycoalkaloid) and sensorial (aroma, taste).

Approaches as mapping association for quality traits and genomic wide selection in potato has been successfully applied (D'hoop et al., 2008, Werij, 2011, Slater et al., 2016). Slater et al. 2016 comparing the expected genetic gain from genomic selection with the expected gain from phenotypic/pedigree selection, found that genetic gain can be substantially improved by using genomic selection approach. Understanding the genetics of potato quality traits associate with marker assisted breeding, can also improve tuber quality.

Pedigree database and variety catalogue are valuable information for breeding. In potato there are some open data information available online (exp. Annex 3), as potato pedigree database of Wageningen UR, (http://www.plantbreeding.wur.nl/potatopedigree/), the European cultivated potato database (http://www.europotato.org/menu.php) and Catalogue of CIP's advanced clones (https://research.cip.cgiar.org/redlatinpapa/pages/home.php).

The factors affecting tuber quality in potato include the genetic (cultivar), crop maturity, agronomic practices, environmental conditions, storage temperatures, the presence of pests and diseases. Traits that are genetically controlled can be grouped as follows: 1) biological traits (proteins, carbohydrates, vitamins, minerals, reduced amounts of toxic glycoalkaloids; 2) sensorial traits (flavour, texture, colour); and 3) industrial traits (tuber shape and size, dry matter content, cold sweetening, oil absorption, starch quality) (Bradshaw et al., 1994, Carputo et al., 2002, D'hoop et al., 2008,). Color, size and shape are also crucial quality aspects for consumers. Breeders must take all the demands into account from farmers, processors, distributors and consumers while at the same time realize that "quality" is a continuously changing concept for each of the parties involved in the process.

The big challenge remains on the integration of all the knowledge in a genomics-assisted breeding strategy and high-throughput phenotyping platforms aiming genetic gain increment for yield/selection of varieties with outstanding quality.

3.2. Potato Breeding for Quality Gap Analysis

Particularly in early stages, the breeding process for quality traits in potato is a laborious task. It starts with thousands of seedling and after several steps of clonal selection a small number of elite clones combining desirable traits, will be promoted as a potential variety. This process can be divided in two

[3] https://research.cip.cgiar.org/redlatinpapa/pages/home.php

major phases - hybridization and clonal selection. “Good by Good” is the old adage been used by many breeders to emphasize the importance of choosing the best parents for the hybridization. With the right genetic variability on hands, the next steps will be the adoption of the most efficient selection scheme to identify the elite lines.

The lack of more efficiente and effective tools to access target quality traits on potato in early selection stages, such as sugar/texture profile, nutritional/antinutritional and sensory analysis, have restricted the use of the genetic resources available to development varieties that meet end-user’s preferences. It is expected an efficient high-throughput phenotyping tool will help to develop new improved varieties and breeding parents with high genetic merit for quality traits for boiled and fried potato.

4. BREEDING SWEETPOTATO FOR QUALITY TRAITS

Introduction

Sweetpotato, Ipomoea batatas (L.) Lam. (2n = 6x = 90), is an important food crop, ranking seventh globally with 106.6 million tons production (FAOSTAT 2014). Sweetpotato provides a rich source of carbohydrates, dietary fiber, vitamins, and micronutrients, is low in fat and cholesterol, and due to its resilience and adaptability, it serves an important role in food security for subsistence farmers in sub-Saharan Africa (SSA) and many developing countries on other continents. Provitamin A-rich orange-fleshed sweetpotato (OFSP) cultivars are important in combating vitamin A deficiency, the leading cause of blindness and premature death in sub-Saharan Africa (SSA).

In SSA, sweetpotato roots are consumed mainly in homes although they may be eaten in restaurants as part of a meal on the street as a snack. The basic methods of cooking roots used in almost all areas are boiling, steaming, baking or roasting and frying. Variations on these basic methods may be used to produce a variety of dishes with characteristics to suit the tastes of local consumers. Most methods of preparation are simple, but consisting of boiling or baking the sweetpotato in its skin, after which the root is peeled or the flesh is scooped out and eaten. Alternatively, roots may be peeled and cut into pieces before boiling. Boiling is the commonest method in rural areas in most SSA countries (Woolfe, 1992; Suguri et al., 2012).

Consumer preference and sensory evaluation plays an important role in research projects and has been applied in rural village settings. Various sensory and consumer approaches have been developed and applied and various statistical tools used to analyze and interpret the results. For example, in cultivar selection, sensory panels and consumer preference studies have been used to investigate regional and seasonal variations in the preference and sensory characteristics of sweetpotato cultivars (Tomlins et al., 2007). There are models relating sensory attributes used by a trained panel to consumer preference. There are simple techniques for use by farmers to evaluate sweetpotato under field conditions.

Consumer testing requires evaluation of suitable methods for rapidly assessing preference in rural and urban locations. Tomlins et al. (2007; 2003) used sweetpotato cultivars and a sensory panel to develop models for selecting local sweetpotato cultivars for quality and understanding sensory changes during shelf-life.

Consumer testing is time consuming and expensive because it requires interviewing at least 100 consumers. In East Africa, consumers in rural locations may be remote and vary in literacy and education. Tomlins et al. (2007; 2003) developed simplified methods for rapidly measuring consumer acceptability in both rural and urban locations, such as the ranking method (ISO reference) and a simpler approach based on asking the consumer to say which of the sweetpotato cultivars they prefer the most.

Most consumer preference studies are undertaken in developed nations and are directed towards high income consumers. However, appropriate consumer preference and sensory evaluation approaches can have an important role to play in sweetpotato research, production and marketing for low-income consumers in developing countries, leading to increased uptake by these groups. In consumer preference, by interviewing sufficient consumers (100 or more) it is possible to assess the views of the low-income consumers and how they make a choice. The use of simplified consumer testing methods (only to choose the most preferred cultivars out of those offered) can facilitate consumer preference studies where people have minimal education such as in rural areas and the urban poor. The application of appropriate statistical tools (e.g. principal component analysis, discriminant analysis) allows models to be developed that enable consumer preference to be combined with sensory panels so that sensory results can be used as a low-cost approach for predicting consumer preference of sweetpotato culitvars. Consumer preference of some sweetpotato culitvars varies from season to season implying that new cultivars should be evaluated over more than one season (Tomlins et al., 2007).

In Uganda, the traits in preferred sweetpotato are high yielding, resistance to common pests and diseases, early or medium maturity with good in-ground storability, suitable for piecemeal harvest with no fibers, and of good marketability, medium sweetness, and powdery texture (Bashaasha et al., 1995).

The food product profile selected for the study under WP4 is: a) boiled sweetpotato – which is the commonest form in which sweetpotato is consumed in most countries in SSA b) puree (mashed sweetpotato) – for producing bakery and other products is increasing in importance. C) and fried sweetpotato.

4.1. Sweetpotato Breeding for Quality Traits at CIP: Uganda

Uganda has released to-date 27 sweetpotato cultivars; 5 released in 2017 and 22 between 1995 and 2013 (Mwanga et al., 2016). Most of the cultivars were evaluated on-farm for acceptability. Most of the parents are in two separate breeding populations at Namulonge, Uganda, separated on the basis of simple sequence repeat markers (David et al., 2018).

Mapping populations developed under the Genomic Tools for Sweetpotato Improvement (GT4SP) project, including the bi-parental Beauregard x Tanzania (BxT) population (317 genotypes) phenotyped for SPVD resistance at Namulonge, and an 8 x 8 (population Uganda B x population Uganda A) panel of roughly 1900 genotypes is being phenotyped in Uganda in three locations (Namulonge, Kachwekano and Serere) representing different agroecologies. The 16 sweetpotato cultivars (parents) are part of germplasm sourced from different agroecologies and a few selected introductions. The parents have been extensively used as parents in African sweetpotato breeding programs. Previous work using 31 simple sequence repeat (SSR) markers separated the 16 accessions into two genetic groups, Population Uganda A and Population Uganda B. To revisit their phylogeny and genetic diversity, and to reveal genes and alleles associated with agronomic traits, the parents were re-sequenced and aligned to the reads of the diploid sweetpotato wild relatives Ipomoea trifida and I. trilobal reference assemblies (http://sweetpotato.plantbiology.msu.edu/). These populations are likely to have the diversity of user preference traits of interest for the targeted product profiles. The BxT population has been phenotyped for SPVD resistance in Uganda and phenotyping in Ghana is in progress. Consideration of quality attributes is already anticipated in Ghana under GT4SP, and this project will allow for application of the specific HTPP methods. Genomic data of both populations and analytical methods for QTL/GWAS will be available from GT4SP.
Heterosis increment studies in sweetpotato (All use NIRS for quality traits – beta carotene, minerals and sugars**):**

1) Mega-clones (important clones across regions) – 4 x 12 crosses (48 families) – without separation of genepools, without selection of recombining ability, without inbreeding (Peru).
2) PJ1 x PZ1 population (two populations at CIP developed independently since 2004) - 231 families clones (49 PJ parents and 31 PZ parents) - with separation of genepools, without selection of recombining ability, without inbreeding (Peru).
3) A x B population with 8 x 8 parents (64 families) from Namulonge tested at Namulonge - with separation of genepools, without selection of recombining ability, without inbreeding (Namulonge, Uganda).
4) Population Uganda A x Population Uganda B with 8 x 8 parents (64 families) from Namulonge tested at Umbelusi / Mozambique) - with separation of genepools, without selection of recombining ability, without inbreeding (Mozambique)
5) PJ and PZ populations (tracing back to 49 PJ parents and 31 PZ parents - with separation of genepools, without selection of recombining ability, with inbreeding, ready to cross PJ" x PZ" to determine the gain of one complete reciprocal recurrent selection cycle (Peru)
6) New parents (42 PJ' and 42 PZ' parents for wide adaptation & earliness (WAE) (Peru)
7) 25 PJ'' and 28 PZ'' parents for non-sweet sweetpotato (NSSP) (Peru and Ghana)
8) 23 PJ''' and 23PZ''' parents for high iron (HIFE) in experiments to determine genetic gains due to heterosis increments and recurrent selection (Peru and Mozambique)
9) SPVD resistance cross (80 x50) (Uganda)

10) Phenotyping SPVD resistance (8 x6) (Uganda)
11) Beuregard x Tanzania mapping population (Peru, Uganda)

4.1.1. Phenotyping for quality traits

NIRS is used for quality traits – beta carotene, minerals and sugars at all the sweetpotato support platforms in SSA (Ghana, Mozambique and Uganda). In Uganda, the traits in preferred in sweetpotato are high yielding, resistance to common pests and diseases, early or medium maturity with good in-ground storability, suitable for piecemeal harvesting with no fibers, and of good marketability, medium sweetness, and powdery texture (Bashaasha et al., 1995). National sweetpotato programs in sub-Saharan Africa evaluate acceptability of storage based on a common procedure developed by the programs and consider the following traits (Grüneberg, 2010):

1. Fresh weight of storage roots
2. Dry weight of storage root samples
3. Dry matter content
4. Appearance of cooked samples assessed using a 1 to 9 scale where 1 = very appealing, 3 = appealing, 5 = somewhat appealing, 7 = unappealing, 9 = very unappealing, with numbers in between representing intermediate ratings.
5. Fibers in cooked storage roots, assessed by inspection and tasting (scale, 1 to 9 scale where 1 = non-fibrous, 3 = slightly fibrous, 5 = moderately fibrous, 7 = fibrous and 9 = very fibrous, with numbers in between representing intermediate ratings).
6. Storage root sweetness in cooked samples, determined by taste test (scale, 1 to 9 scale where 1 = non-sweet, 3 = slightly sweet, 5 = moderately sweet, 7 = sweet and 9 = very sweet, with numbers in between representing intermediate ratings).
7. Storage root texture in cooked samples, determined by taste test (scale, 1 to 9 scale where 1 = very moist, 3 = moist, 5 = moderately dry, 7 = dry and 9 = very dry, with numbers in between representing intermediate ratings)
8. Overall taste of cooked samples assessed using a scale of 1 to 9 scale where 1 = excellent, 3 = good, 5 = fair, 7 = poor and 9 = horrible, with numbers in between representing intermediate ratings.
9. Appearance of cooked samples assessed using a 1 to 9 scale where 1 = very appealing, 3 = appealing, 5 = somewhat appealing, 7 = unappealing, 9 = very unappealing, with numbers in between representing intermediate ratings.

In participatory plant breeding in central Uganda, farmers used 52 traits for ranking sweetpotato consumer preference (Table 28). What is in the farming system is driven by a combination of taste preference and field performance.

Table 28. Important root traits used to rank preference of sweetpotato cultivars in a participatory plant breeding trial in Central Uganda (there were no orange-fleshed cultivars)

1	Good root yield		27	Good root yield on poor soils
2	Roots sweet when cooked		28	Easy/quick to cook
3	Big roots		29	Ample planting material
4	Drought resistance		30	Resistant to Alternaria
5	Roots mealy when cooked		31	Few exposed roots
6	Early root maturity		32	Long-lived plants
7	Weevil resistance		33	Crop resistant to weeds
8	Attractive colour of roots		34	Less 'kigave'‡ of roots
9	Non-fibrous roots when cooked		35	Easy peeling roots
10	Continuous root yield for piecemeal harvesting		36	Does not require big ridges/mounds
11	Marketability		37	Roots close to surface for easy harvesting
12	Straight roots		38	Many roots
13	Resistant to caterpillars (Acrae acereta)		39	Crop resistant to rain
14	Long storage of roots in soil		40	Crop resistant to diverse weather conditions
15	Soft texture of roots when cooked		41	Roots resistant to millipedes
16	Long roots		42	Smooth skin on roots
17	Resistant to rats and other vertebrates		43	Thin peel on roots
18	Resistant to SPVD		44	Few black spots on skin of roots
19	Extensive foliage		45	Hard (solid) storage roots
20	Non-sappy roots		46	Good root shape
21	No loss of taste as the crop gets older		47	Roots do not break during harvesting
22	Nice looking at table		48	Attractive flesh
23	Nice flavour when cooked		49	Roots not too sweet when cooked
24	Few cracks in roots		50	Roots not watery when cooked
25	Yields satisfactorily in poorly tilled soil		51	Lots of foliage for animal feed

The food product profile selected for the study under WP4 in Uganda is: a) boiled sweetpotato – which the commonest form in which sweetpotato is consumed in most countries in SSA b) puree (mashed sweetpotato) C) and fried sweetpotato. The key traits are: resistance to sweetpotaot weevil and sweetpotato virus disease (SPVD), drought and heat tolerance, storage life, and culinary quality including beta-carotene.

Traits data to record: Establishment, number of plants, vigor, vine weight, virus, Alternaria blight, number of roots, root shape, weight of market and non-marketable roots, root weight, skin color, flesh color, weevil damage. Quality traits: dry matter, beta-carotene, iron, zinc, calcium, magnesium. NIRS will be calibrated for quality traits of cooked storage roots.

4.1.2. Genetic variability in CIP breeding program

Specifically, Population Uganda B x Population Uganda A (64 families, about 1,880 genotypes) at Namulonge, Uganda, will be used to provide information required for WP4. Other populations will be a source of information to complement results from Population Uganda A x Population Uganda B.

Table 29. Description of the parents from population Uganda A & B. ***In bold and italic*** are the landrace cultivars.

Accession	Name	Country of origin	Ancestral data	Other identification	Flesh Color
Parents from Population Uganda A (small crossing block)					
CIP443750	***Ejumula***	Uganda	Farmer variety of unknown parentage	TC--/SP/UG 417	Orange
CIP191133.1	NASPOT 1	Uganda	Selection from bulked seed from a 1991 polycross block with 24 parents	TC--/SP/UG 440; NIS/91/52	Cream
CIP443752	***Dimbuka-Bukulula***	Uganda	Farmer variety of unknown parentage	Farmer variety described in breeding reports	Cream
NASPOT5/58	NASPOT 5/58	Uganda	Selection from bulked seed from a 1991 polycross block with 24 parents	Breeding line (progeny of NASPOT 5), not released	Orange
CIP100200.1	NASPOT 7	Uganda	Progeny of Kakamega (SPK004) from seed produced in a 2000 polycross block with 24 parents	NIS/2002/SPK004/1	Orange
CIP441768	***Kakamega (SPK004)***	Kenya	Farmer variety of unknown parentage	SPK004	Orange
CIP100200.4	Kabode (NASPOT 10 O)	Uganda	Progeny of Kakamega (SPK004) from seed produced in a 2000 polycross block with 24 parents	TC08/SP/UG 055; NIS/2002/SPK004/6/; NASPOT 10 O	Orange
NK259L	NK259L	Uganda	Breeding line (progeny) from New Kawogo selected at same time as NASPOT 11 (also from same parent)	Breeding line selected during participatory plant breeding but not released	White
Parents from Population Uganda B (big crossing block)					
CIP440001	Resisto	USA	Obtained from CIP/Kenya, KEPHIS	Used in crossing block as parental source of beta-carotene	Orange
Magabali	***Magabali***	Uganda	Farmer variety of unknown parentage	Farmer variety described in survey and germplasm characterization reports	Cream
CIP191133.5	NASPOT 5	Uganda	Selection from bulked seed from a 1991 polycross block with 24 parents	TC--/SP/UG 446; NIS/91/316	Orange
CIP440167	***Wagabolige***	Uganda	Farmer variety of unknown parentage	TC08/SP/PE 152; TC--/SP/UG 355;TC08/SP/UG 108;TC08/SP/UG 188	Cream
Mugande	***Mugande***	Uganda	Farmer variety of unknown parentage	Farmer variety described in survey and germplasm characterization reports	White
CIP100201	NASPOT 11 (Tomulabula)	Uganda	Progeny of New Kawogo from seed produced in a 2000 polycross block with 24 parents	TC08/SP/UG 027; NIS/2003/NKA1081L; NASPOT 11	Cream
CIP441745	***New Kawogo***	Uganda	Farmer variety of unknown parentage	Farmer variety described in survey and germplasm characterization reports,	Cream
CIP420020	***Huarmeyano***	CIP/Peru	Breeding landrace from Peru	Farmer variety from Peru used in crossing block in Uganda; source for SPFMV resistance,	Orange

4.2. Sweetpotato Breeding for Quality Gap Analysis

Food product profiles are high priority following major constraints such as diseases e.g. SPVD and pests such as weevils have been controlled e.g. using cultivars with disease field resistance. Equipment ideal for field screening for quality traits (e.g. beta-carotene, starch and sugars, dry matter) would increase accuracy and precision and would be more rapid enabling faster release of cultivars.

5. BREEDING MATOOKE FOR QUALITY TRAITS

Introduction

The East African Highland bananas (Musa AAA group) also referred to as EAHB, is an endemic group of bananas found in the Great Lakes region (Uganda National Council for Science and Technology 2007). They are grown at altitudes between 900 and 2000 m above sea level, and are mainly found in Burundi, Kenya, Rwanda, Tanzania, and Uganda, plus in some areas of Cameroon and the Democratic Republic of Congo. As described by Batte et al., (2018), the cultivars within the group require an average of 2000–2500 mm of rain evenly distributed throughout the year (Uganda National Council for Science and Technology 2007) as they are very drought susceptible (Kissel et al., 2015, 2016).

In Uganda, cooking banana cultivars are locally known as 'matooke' and serve as staple food to a large part of the population. Uganda produces over 8 million tons of 'matooke' bananas annually, which makes it the second largest banana producer in the world (Batte et al., 2018). The banana breeding programme at the International Institute of Tropical Agriculture (IITA) works on improving three types of bananas: "matooke", "mchare" bananas and plantains. "Matooke" improvement is carried out in Uganda where IITA collaborate with the Banana Research Programme of the Ugandan National Research Organization (NARO). "Mchare" breeding is done in Arusha in Tanzania, while plantain breeding takes place in Ibadan and Onne in Nigeria. This document in particular concerns "matooke" breeding, which is the focus of WP4 under the project "RTBFoods".

5.1. Matooke Breeding for Quality Traits at IITA: Uganda

5.1.1. Breeding programs

"Matooke" breeding started in Uganda in 1992, as a collaboration between IITA and Ugandan National Agricultural Research Organization (NARO). The key breeding objective was to improve yield through the incorporation of resistance to black Sigatoka. "Matooke" quality was a priority from the start. The aim was not to improve the quality or "matookeness", but to maintain or stay as close as possible to the quality of the landraces. This was incorporated in the product profile for "matooke" breeding, where the trait "table quality" was, and still is, given the highest market priority, in the same range as yield. Selection objective is set to an acceptable threshold of a score of 4 and above on a hedonic scale of 1 to 6 (Table 30).

Table 30. Banana PRODUCT PROFILE: Matooke (Source: IITA and NARO Banana Breeding Programmes)

Banana PRODUCT PROFILE: Matooke				
Region/Market segment	**Trait (economic, sustainability, livelihood) and value**	**Target trait level**	**Market Priority**	**Selection Objective**
Highlands of East and Central Africa				
Fresh market and processing	Yield	25% greater than Mbwazirume variety across a range of soil and management conditions	*1*	Maximize
	Table quality	A general acceptability score of at least 4 (on a hedonic scale of 1 to 6), using Mbwazirume as a check (acceptability is tested after cooking as taste, aroma, colour, texture/mouth-feel)	1	Reach threshold
	Earliness: planting to harvest	300 to 390 days	2	Minimize
	Plant stature (girth at 1m/height ratio)	A ratio of at least 0.15	2	Maximize
	Plant height	Less than 350 cm	2	Minimize
	Suckering behavior	75% follower sucker growth at harvest	2	Maximize
	Resistance to black Sigatoka	INSL at flowering of 70% and above	2	Reach threshold
	Resistance to weevils	Resistance higher than that of the susceptible check (Kibuzi)	2	Maximize
	Resistance to *Radopholus similis*	Resistance higher than that of the susceptible check (Valery)	2	Maximize
	Resistance to BXW	Sources of resistance to be identified	2	Opportunistic
	Bunch orientation	Pendulous score of 1 or 2	1	Opportunistic
	Drought tolerance (water productivity)	Tools to be developed	3	Reach threshold
	High ProVitA content	Average -Carotene(µg/100 g) higher than 150	1	Opportunistic

5.1.2. Phenotyping for quality traits

Astringency (or lack of), yellow pulp colour, aroma, taste, and mouthfeel have been identified as the best indicators of “matookeness” (Ssemwanga, 1995; Ssemwanga and Thompson, 1994). However, these have not yet been conclusively associated with physio-chemical compounds. Evaluation for “matooke” quality in the hybrids is done qualitatively at different stages of trials, starting with Early Evaluation Trials (EET) up to variety release. At EET level, testing is done in a simple way, where each triploid hybrid carrying a bunch with the right fruit-filling attributes, and resistant to black Sigatoka is checked by the breeding team for:

- Colour of the pulp: creamish/yellow desired in matooke
- Browning of the pulp and sap upon oxidation: poor matookeness
- Astringency of the pulp and sap: astringent hybrids indicate poor “matookeness”

At the Preliminary Yield Trial (PYT) level, the advanced hybrids from EET are tasted systematically in a sensory preference test. Landrace “Mbwazirume” is used as a control in a blind test, where all the lines are coded. Each evaluation seating comprises at most five hybrids. The bunches are cooked following the traditional method of cooking “matooke”, using banana leaves to wrap the peeled fingers,

adding little water to the pot and steaming. To avoid differences in cooking, each line is individually wrapped and labelled, and all the small packages of wrapped lines are wrapped together in more banana leaves in one same cooking pot. Steaming is done on wooden fire until steam comes through the wrapped genotypes (muwumbo), a general indication that the bananas are well cooked. After steaming, the individually wrapped lines are mashed. A team of 15 to 20 people, Ugandans, evaluates the lines and gives them a score on a hedonic scale of 1 to 6, where 6 is the best quality. Scoring is done on pulp colour, aroma, taste, mouthfeel and general acceptability, with the first four traits being indicators of the last one. A genotype with an average score of 4 or above for general acceptability and meeting the agronomic traits requirements qualifies for advancement to further evaluation, namely Advanced Yield Trial (AYT), and Multilocational/On-Farm Trial. At AYT level, the sensory preference test is repeated. At the on-farm trial level, the last evaluation for “matooke” hybrids, the sensory preference test is carried out with farmers as panel members. At this level, the hybrids are prepared in all possible ways that banana is cooked in the specific area where the trial is conducted. Data collected at on-farm trials level are included in the variety release process (Ssali et al., 2010).

To ensure that the hybrids produced have “matooke” quality, and to cater for sterility and poor seed set observed in triploids, banana breeding is carried out in 3 phases as summarized in **Erreur ! Source du renvoi introuvable.** (Nyine et al., 2018). Briefly, breeding starts with EAHB 3*x* x 2*x* crosses, 3*x*, the pest and disease susceptible landrace, but with the right “matooke” quality is used as a female parent. The 2*x* used at this level carries the required resistance to meet the needs of the product profile for yield and other agronomic traits (Table 30). At this level, the number of seeds is very low. This cross produces 2*x*, 3*x* and 4 *x* hybrids. After evaluation, depending on the requirements for each ploidy, the 2*x* can be incorporated in the 2*x* x 2*x* breeding scheme, where they can later be used to improve 4*x*. The 3*x* hybrids can be advanced for further testing. The 4*x* hybrids start another stage of pollination, where they are crossed with improved 2*x* to generate 3*x* to be evaluated in EET.

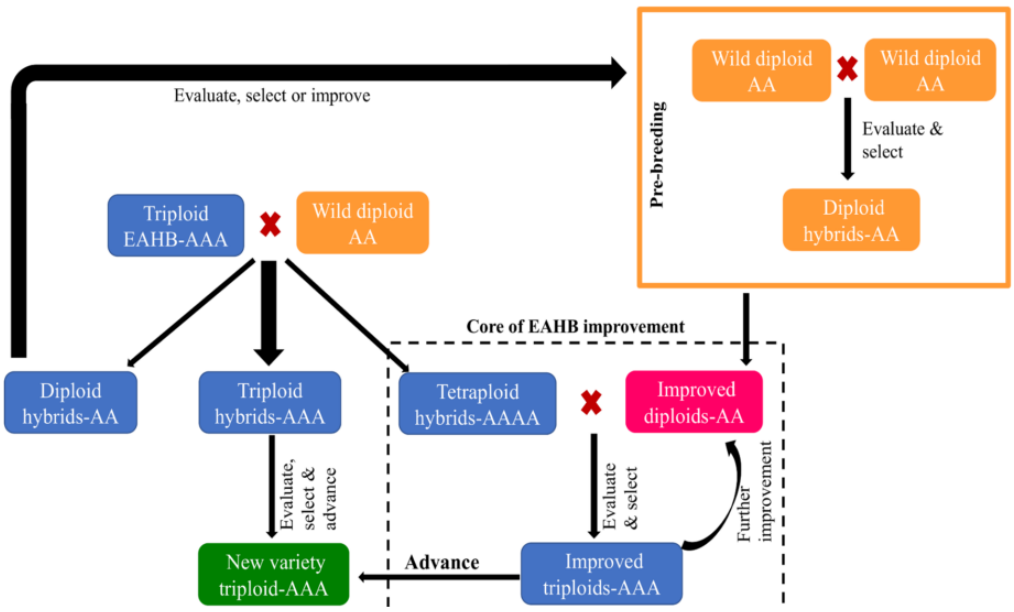

Figure 29. EAHB improvement scheme: breeding starts with landrace 3x to ensure the preservation of “matooke” quality Source: (Nyine et al., 2018)

5.2. Matooke Breeding for Quality Gap Analysis

Evaluation for “matooke” quality in the developed banana hybrids is a tedious task, involving a lot of logistics and people. Moreover, we rely on “matooke” consumers for evaluation, making the whole method biased. There is a need to develop easily measurable, field-based, precise method to quantify “matookeness” at an early stage of breeding. Those traits could be genetically/genomically mapped, and molecular tools would be further developed for selection at nursery level, even before EET.

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7. ANNEXES

7.1. Annex 1: List of yams officially released in Nigeria

Variety name	Outstanding characteristics	Agro-ecological zones	Year of release
TDR 89/02677	Stable yield, very good cooking and pounding qualities, cream tuber parenchyma, 25% tuber dry matter content	Forest and Southern Guinea Savanna	2001
TDR 89/02565	Stable yield, very good cooking and pounding qualities, cream non oxidizing parenchyma, 35% tuber dry matter.	Forest and Southern Guinea Savanna	2001
TDR 89/02461	Stable yield, very good as cooking and pounding qualities, cream parenchyma, 26.7% tuber dry matter.	Forest and Southern Guinea Savanna	2001
TDR 89/02665	Stable yield, very good cooking and pounding qualities, cream non oxidizing parenchyma, 35.3% tuber dry matter	Forest and Southern Guinea Savanna	2003
TDR 89/01213	Stable yield, very good cooking and pounding qualities, white non-oxidizing parenchyma, tuber dry matter = 29.8%	Forest and Southern Guinea Savanna	2003
TDR 89/01438	Stable yield, very good cooking and pounding qualities, white non-oxidizing parenchyma, tuber dry matter = 29.3%	Forest and Southern Guinea Savanna	2003
TDR 95/01924	Stable yield, very good cooking and pounding qualities, white non-oxidizing parenchyma, tuber dry matter = 32.8%	Forest and Southern Guinea Savanna	2003
DRN 200/4/2	High yielding, pests and diseases tolerant, very good for fufu, frying and boiling (35t/ha)	Yam Zones in Nigeria	2008
TDa 98/01176	High yielding, pests and diseases tolerant, very good for pounded yam, frying and boiling, suitable for both rainy and dry seasons yam production (26-30t/ha)	Yam Zones in Nigeria	2008
TDa 98/01168	High yielding, pests and diseases tolerant,good for pounded yam, frying and boiling. (24-28t/ha)	Yam Zones in Nigeria	2008
TDa 98/01166	High yielding, pests and diseases tolerant, very good for pounded yam, frying and boiling, suitable for both rainy and dry seasons yam production (26-30t/ha	Yam Zones in Nigeria	2008
TDr 95/19158	High yielding, pests and diseases tolerant,good for pounded yam, frying and boiling. (29.4t/ha)	Yam Zones in Nigeria	2008
TDr 89/02602	High yielding, pests and diseases tolerant, very good for yam, fufu, frying and boiling. (31.5t/ha)	Yam Zones in Nigeria	2009
TDr 89/02660	High yielding, pests and diseases tolerant, very good for yam, fufu, frying and boiling. (31t/ha)	Yam Zones in Nigeria	2009
TDa 00/00194	High yielding, pests and diseases tolerant, very good for yam, fufu, frying and boiling. (37.5t/ha)	Yam Zones in Nigeria	2009
TDa 00/00104	High yielding, pests and diseases tolerant, very good for yam, fufu, frying and boiling. (37.5t/ha)	Yam Zones in Nigeria	2009
UMUDa-4	High Yielding good for Amala, pounded yam, frying and boiling. (33.3t/ha)	Yam Zones in Nigeria	2010

UMUDr - 17	High yielding under dry season yam cropping system. (30t/ha)	Yam Zones in Nigeria	2010
UMUDr - 18	High yielding, pests and diseases tolerant, very good for yam fufu, frying and boiling. (31t/ha)	Yam Zones in Nigeria	2010
UMUDr /020	High yielding (37.2t/ha), adaptable to low soil fertility and low soil moisture	Yam Zones in Nigeria	2016
UMUDr/021	High yielding (33.2t/ha), adapted to low soil fertility.	Yam Zones in Nigeria	2016
UMUDr/022 (Obiaturugo)	High yield (28.6t/ha), tolerant to major pests and diseases	South west and South east	2016
UMUDr/023 Amola)	Tolerant to viruses	South west and South east and North central	2016
UMUDr/024 (Hembakwasi)	Tolerant to viruses, moderately yielding	Yam Zones in Nigeria	2016
UMUDr/025 (Ekpe)	Tolerant to viruses, moderately yielding	Forest zones	2016
UMUDr/026 (Aloshi)	Tolerant to viruses	Foests zone	2016

7.2. Annex 2: List of Morphological traits, Agronomical traits and Characteristics post-harvest for potato at CIP.

#	Name of Trait	Description of Trait	Trait Class	Describe how measured (method)	Bibliographic Reference	Scale
1	Predominant tuber skin color	Freshly tubers harvested washed and dried before the evaluation of tubers. Skin color-register must be the most representative of the tuber and recorded with the help of the color table.	Morphological traits	Visual categorization	Gomez, R. 2004.	1 = White- cream; 2 = Yellow; 3 = Orange; 4 = Brownish; 5 = Pink; 6 = Red; 7 = Purplish-red; 8 = Purple; 9 = Blackish
2	Predominant Tuber flesh color	Observation of the secondary color described cross sections made approximately in the center part of the freshly harvested tubers	Morphological traits	Visual categorization	Gomez, R. 2004.	1 = White; 2 = Cream; 3 = Pale; 4 = Yellow; 5= Intense Yellow; 6 = Red; 7 = Purple; 8 = Violet
3	General tuber shape	Observation of the general shape of the tubers, the ratio between the diameter and the length of the overall shapes delimiting tuber	Morphological traits	Visual categorization	Gomez, R. 2004.	1 = Compressed; 2 = Rounded; 3 = Ovoid; 4 = Obovoid; 5 = Elliptical; 6 = Oblong; 7 = Long-oblong; 8 = Elongated
4	Tuber shape depth of eyes	Observate several tubers and determine the depth of eyes	Morphological traits	Visual categorization	Gomez, R. 2004.	1 = Protruding; 3 = Shallow; 5 = Slightly deep; 7 = Deep; 9 = Very deep
5	Dormancy Period (DLS) Lowland	The dormancy period should be evaluated in Diffuse light storage to Lowland	Agronomical traits	The dormancy period should be counted as number of days from haulm cutting to sprouting of 80% of the tubers (8 to 12 tubers depending on the size of the experimental unit) with at least one sprout longer than 2 mm. Tubers should be checked at 10 day-intervals for monitoring sprouting initiation and growth, and to accurately record the dormancy period	Carli et al. 2010.	
6	Dormancy period (DLS) Highland	The dormancy period should be evaluated in Diffuse light storage to highland	Agronomical traits	The dormancy period should be counted as number of days from haulm cutting to sprouting of 80% of the tubers (8 to 12 tubers depending on the size of the experimental unit) with at least one sprout longer than 2 mm.	Carli et al. 2010.	

				Tubers should be checked at 10 day-intervals for monitoring sprouting initiation and growth, and to accurately record the dormancy period		
7	Flavor	Taste the cooked tuber, immediately determining after cooking	Characteristics post-harvest	Tuber flavor after cooking in a 1-5 scale	International Potato Center (CIP) 2014.	1 = Very poor; 2 = Poor; 3 = Intermediate; 4 = Good; 5 = Very good
8	Texture of tuber after cooking	Test the texture of the tuber, immediately determining after cooking	Characteristics post-harvest	Visual categorization	International Potato Center (CIP) 2014.	1 = Watery; 2 = Less watery; 3 = Waxy; 4 = Less mealy; 5 = Mealy
9	Oil Absorption Rate	The method requires a knife to press, scales, paper towels and samples of chips	Characteristics post-harvest	Oil absorption =100- [(weight final / weight initial) *100]	International Potato Center (CIP). 2006.	
10	Cooking Quality	Cooking test	Characteristics post-harvest	Visual categorization	International Potato Center (CIP) 2014.	1=A = Totally solid and firm; 2=AB = Solid; 3=B = Partially Solid; 4=BC = Partially and Slightly disintegrated; 5=C = Partially disintegrated; 6=CD = Totally disintegrated; 7=D = Totally and strongly disintegrated
11	After cooking darkening	Measure the degree of darkness immediately after cooking	Characteristics post-harvest	Visual categorization	International Potato Center (CIP) 2014.	1 = Light; 2 = Moderately light; 3 = Moderately dark; 4 = Dark
12	Flesh Color after cooking	Observe the color of the pulp after cooking	Characteristics post-harvest	Visual categorization	International Potato Center (CIP) 2014.	1= Cream; 2= Dark Yellow; 3= Light Cream; 4= Light Yellow; 5= Purple; 6= White; 7= Yellow; 8= Dark cream
13	Chipping color	Measure the degree of darkening that occurs during frying	Characteristics post-harvest	Visual categorization	International Potato Center (CIP). 2006.	1 = Light; 2 = Moderately light; 3 = Moderately dark; 4 = Dark; 5 = Very Dark
14	Percentage Dry matter	Percent dry matter content of tubers	Characteristics post-harvest	Percentage Dry matter of the tubers, estimated by multiplying by 100 the ratio between the dry weight (obtained after drying the tubers in the oven until constant weight), and the fresh weight of the sample, using a sample of about 100 grams of fresh samples.	International Potato Center (CIP). 2006.	
15	Cooking Time (minutes)	Choose two or three well washed tubers and cook them,	Characteristics post-harvest	Visual categorization	International Potato Center (CIP) 2014.	1=10-15; 2=16-20; 3=21-25; 4=26-30; 5=>30

		evaluate every 5 minutes				
16	Vitamin C (mg/100 g, dry weight basis)	The vitamin C concentration is quantified through comparison with a standard curve of the L-ascorbic acid.	Biochemical traits	The vitamin C concentration is quantified through comparison with a standard curve of the L-ascorbic acid. Accessed on https://research.cip.cgiar.org/confluence/display/cipqnl/Ascorbic+acid	International Potato Center (CIP) 2014.	
17	Content of iron in dry weight basis	Content of iron in dry weight basis	Biochemical traits	The mineral concentrations (Fe, Zn, Ca, K, Na, Mg and P) are determined by inductively coupled plasma--optical emission spectrophotometry (ICP-OES) using a Radial View.	International Potato Center (CIP) 2014.	
18	Content of zinc in dry weight basis	Content of zinc in dry weight basis	Biochemical traits	The mineral concentrations (Fe, Zn, Ca, K, Na, Mg and P) are determined by inductively coupled plasma--optical emission spectrophotometry (ICP-OES) using a Radial View.	International Potato Center (CIP) 2014.	
19	Anthocyanins Total	Tubers with skin and flesh color red and purple are used for the detection of anthocyanins.	Biochemical traits	TA analysis of freeze dried samples was optimized for freeze dried and milled potato samples from the method proposed by Jansen and Flamme (2006)	Burgos et al. 2013.	
20	Antioxidant Ability Hydrophilic	Antioxidant Hydrophilic Ability	Biochemical traits	AAH evaluation was done by the ABTS and DPPH assays which were optimized for freeze dried potatoes based on the methods reported by Re et al. (1999) and Brand-Williams et al. (1995).	Burgos et al. 2013.	
21	Antioxidant Ability Lipophilic	Antioxidant Lipophilic Ability	Biochemical traits	AAL evaluation was done by the ABTS and DPPH assays which were optimized for freeze dried potatoes based on the methods reported by Re et al. (1999) and Brand-Williams et al. (1995).	Burgos et al. 2013.	

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7.3. Annex 3: Characteristics of CIP392797.22 variety available online at Catalogue of CIP Potato Varieties.

CIP 392797.22 - UNICA

Parentage: 387521.3 X APHRODITE

Country of Selection: Peru

Country where cultivated: Vietnam, Laos, Uzbekistan, Peru

Is a variety resistant to PVY. The plant have medium vegetative period with decumbent plant habit and lilac flowers with white acumen abaxial surface. The variety has a wide adaptation to the tropics under short days. The tubers are oblong with cream flesh are excellent for processing as chips (crisps) and French fry (chips). The variety was realized in Peru in 1998.

Tuber skin predominant color	Red	Tuber flesh predominant color	Cream
Tuber skin secondary color	Absent	Tuber flesh secondary color	Absent
Tuber skin secondary color distribution	Absent	Tuber flesh secondary color distribution	Absent
Tuber shape	Oblong	Tuber shape depth of eyes	Shallow
Tuber shape unusual	Absent		

Reaction traits	
Late blight (LB)	Susceptible
Potato virus X (PVX)	Resistant
Potato virus Y (PVY)	Extreme Resistance
Potato leaf roll virus (PLRV)	Resistant
Bacterial wilt (BW)	Moderately susceptible
Root knot nematode (RKN)	Moderately resistant
Leaf miner fly (LMF)	Highly susceptible

Agronomical performance	
Adaptability	
Tuber yield (kg/plant)	0,96
Dormancy period - DLS highland	109
Sprouting pattern	Apical dominance

Post-harvest performance	
Use	
Dry matter (%)	21
Oil absorption rate (%)	39

Nutrient concentrations in tubers	
Vitamin C (mg/100g, dry weight basis) min	36,38
Vitamin C (mg/100g, dry weight basis) max	164,34
Vitamin C (mg/100g, dry weight basis)	77,78
Fe (mg/kg, dry weight basis) min	13,02
Fe (mg/kg, dry weight basis) max	27,00
Fe (mg/kg, dry weight basis)	19,58
Zn (mg/kg,dry weight basis) min	6,19
Zn (mg/kg,dry weight basis) max	25,86
Zn (mg/kg, dry weight basis)	18,12

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