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Evaluation of cassava (*Manihot esculenta* Crantz) genotypes for total cyanide content, storage tuber and starch yield in South Western Ethiopia

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Abstract

Cassava (*Manihot esculenta*) is one of the main food crops that significantly contribute to food security and poverty alleviation in South and Southwest Ethiopia. Cyanide present in cassava roots poses a health challenge in the use of cassava for food. It is therefore important to identify the sweet and bitter types for food and industry. In this study, 11 farmers preferred genotypes were assessed for storage tuber yield, cyanide content and starch contents at Jimma, Metu and Tepi during 2015-2017 cropping seasons. From the genotypes analyzed, AAGT-108 (128.35 mg/kg) and AAGT-189 (118.5 mg/kg) are identified for bitter type (high HCN content). Based on storage tuber yield, genotype AAGT-108, AAGT-189 and AAGT 192 produced the highest tuber yield in all tested locations. The starch content varies from 5.11 (Qulle at Metu) to 18.40 (AAGT-108 at Tepi). Similarly, genotypes AAGT-108, AAGT-189 and AAGT-192 are the best materials at Jimma as compared to others and gave the mean starch content of 15.22, 16.98 and 16.61 %, respectively. Based on the overall result of hydrogen cyanide and starch contents, genotype AAGT-108 and AAGT-189 are recommended for bitter type (high HCN content). On contrary, the rest of genotypes have sweet type due to low hydrogen cyanide content below 100 mg/kg and are recommended for human consumption.

Keywords: **Cassava, Cyanide, Starch, Storage tuber, Yield**

Introduction

Cassava (*Manihot esculenta* Crantz,) is the sixth most important food crop globally, in terms of annual production (FAOSTAT, 2010). The crop is mainly grown in the tropics, including sub-Saharan Africa, Asia, the Pacific Islands, and Central and South America (Lebot, 2009) for staple food of an estimated 800 million people worldwide (FASTAT, 2013). The crop have ability to survive adverse conditions such as infertile soil, drought, pests and diseases (El-Sharkawy, 1993; Bokanga, 1999) and plays several important roles in Africa serving as a rural staple food, famine-reserve crop, cash crop for households and as a raw material for feed and industrial manufacturing (Nweke *et. al*., 2002).It has been identified as a potentially valuable source of food for addressing food security in developing countries (Montagnac *et al*., 2009) and a major source of food calories for about two of every five Africans (Serge *et al.,* 2013). Although reliable statistical information on the distribution and production of cassava in Ethiopia is lacking, the crop has been cultivated, particularly, in the South, South West, and Western parts to overcome hunger and make a significant contribution in the diets of the people (Tewodros and Zelalem., 2015).

The starchy tuberous roots are the main food source, which are high amount of carbohydrates, fibers, and low level fats and protein, a good proportion of essential amino acids which make them a good dietary source (Montagnac *et. al*., 2009). Conversely, the wider utilization of cassava in Ethiopia is inadequate; due to information on the biochemical composition of cassava is meager. Further, limited exposure to high doses of hydrogen cyanide is a health risk in major growing regions where cassava is a staple food source (Getachew *et. al.,* 2012). Besides, cassava in itself is not a balanced food and malnutrition occurs when cassava is consumed alone as staple food. Studies of nutritional composition on cassava as a food are considerable significance since it may help to identify long forgotten food resource (Tewodros and Biruk, 2012). In this regards, few attempt was made to understand the proximate composition and anti-nutritional factors of the underutilized tubers of cassava to make edible tubers as the safe food sources for mass consumption (Tesfaye *et. al*., 2017). In contrast to cultivated tubers, little is known about

the proximate composition and reasons to expect that some of the species differ in composition from common varieties. Furthermore, several species of cassava also have two cyanogenic glycosides, linamarin and a small amount of lotaustralin, which are catalytically hydrolyzed to release toxic hydrogen cyanide (HCN) toxic to human beings.

The level of total cyanide contents of different varieties and plant parts of cassava is 1–1550 mg HCN equivalents / kg fresh material = ppm, in the root parenchyma and 900–2000 ppm in the root cortex (peel) (Cardoso *et.al.,* 2005). Cassava leaves contain 20–1860 ppm of total cyanide (Bradbury and Denton, 2011). The World Health Organization (WHO) set a safe limit of 10 ppm total cyanide for cassava flour (FAO/WHO, 1995). This maximum limit of has been adopted in different countries for example, 10 ppm for cassava chips by Food Standards Australia and New Zealand (Fsanz, 2009), while 40 ppm is the limit in Indonesia (Djazuli and Bradbury, 1999). Internationally, the Codex Standard for 'sweet cassava' (those varieties with low levels of cyanogens) is 50 ppm (fresh weight basis, FAO/WHO, 2005), but many countries have yet to formally adopt recommended limits (Kolind-Hansen and Brimer, 2009).

In spite of its food security and industrial importance, there are no efforts so far done in the nutritional composition; industrial value and information on the biochemical composition of cassava genotypes are scarce. Furthermore, the glycoside attributes of the existing genotypes have never been assessed and the level of anti- nutritional factors on cassava at country level is still unknown; which hinders the wider utilization and researchers to access the biochemical composition of cassava in the country. Cognizant of these facts, the present study was designed to evaluate the yield, hydrogen cyanide and starch contents of cassava genotypes collected from Southwest Ethiopia for use and conservation.

Materials and Methods

Study areas: A field studies were conducted during the 2015/16 and 2016/17 growing seasons at Jimma, Tepi and Metu agricultural research centers. The detail descriptions of tested sites are presented (Table 1).

Table 1. The geographical description of the study sites

Location	Altitude (m.a.s.l.)	Latitude	Longitude	Rainfall (mm)	Temperature (^{0}C)	
					Maximum	Minimum
Jimma	1753	7° 40.00' N	36° 47'.00' E	1521.1	26.2	12.1
Metu	1550	8°18' .00' N	35°35' .00' E	1520	28.0	12.2
Tepi	1200	7° 3' .00' N	35° 18' .00'E	1685.9	29.9	15.4

Source: JARC, 2010

Plant materials, experimental design and management: A total of 11 cassava genotypes were collected from major growing areas of Southwest Ethiopia. The experiment was laid out in randomized complete block design (RCBD) with three replications. The gross plot size for each treatment was 4m x 4m, using inter-row spacing of 1.5m and intra-rows spacing of 1m. Cuttings of the same size and age were used as planting material. One month after planting, seedlings were earthed up followed by frequent weeding. All other agronomic practices were followed according to the recommendations.

Data collection: Data were collected from six plants from each plot and the average values were used for data analysis. The characters that are used for data collection were: tuber length (cm), tuber fresh weight (t/ha), hydrogen cyanide content (mg/kg fresh storage sample) and starch content (% fresh storage sample) and tuber dry weight (t/ha).

Determination of starch content: The starch content was determined by a modified method of Asaoka *et al.* (1992). A cassava storage tuber was collected from tested materials from each location.500 g of fresh storage tuber was taken from the distal, middle and apical sections of washed tubers from each of the middle four plants randomly. After peeling, the tuber cut into small pieces with stainless steel knife and then milled using an electric grinder, to obtain fine powder cassava flour. The starch particle was isolated using excess pure water. The slurry was filtered through 1mm sieve mesh. The residue was washed three times with 500 ml of water each time to remove remnants of starch. The filtered was allowed to settle for 2 hours for each wash before decanting the liquid. Hence, starch was suspended and non-starch materials removed by decanting the supernatant. Subsequently, the starch was dried in oven at 30-33ºC until constant weight attained and dried. The amount of dried starch

obtained from one kg of fresh cassava tuberous roots was weighted and expressed as a percent of the fresh storage tuberous.

Determination of hydrogen cyanide Content: Determination of hydrogen cyanide contents (HCN mg/Kg). The duplicate samples were harvested from the fields and transported immediately to the laboratory (Food and nutritional laboratory Addis Ababa). The collected samples were cleaned, peeled and washed with potable water. Samples from the distal, middle and apical sections of peeled tubers were cut into cube. The acid titration method (AOAC, 2005) for the determination of hydrocyanic acid in beans was used. One hundred (100) ml of H_2O was added to 25 g of the sample in a 500 ml Kjedahl flask for steam distillation. The distillate was collected in 20 ml 0.02N. $AgNO_3$ acidified with 1 ml HNO_3. The apparatus was adjusted so that the tip of the condenser dipped below the surface of the liquid in the receiver. After 150 ml had passed over, excess $AgNO_3$ was titrated with 0.02 KSCN using Fe alum an indicator. The results were calculated as fresh matter basis as follows:

100/W ×0.27)] × [(V$AgNO_3$ = sample HCN/100g mg

1 ml of 0.01 N $AgNO_3$ = 0.27 mg of HCN

Where: VAgNO3 Volume of silver nitrate = [(20-(2×V KSCN))]

V KSCN Volume of potassium thiocyanate consumed in and W: weight of sample

Data analysis: The collected data were analyzed by using SAS statistical Software package (version 9.0 of SAS Institute Inc, 2000). Both quantitative and quality data were subjected to analysis of variance (ANOVA) using the RCBD procedure as suggested by Gomez and Gomez (1984). Means were separated using the Least Significant Difference (LSD) procedure at the 5% and 1% level of significance.

Results and Discussion

Mean performance of Storage tuber and length: The mean storage tuber yield (t/ha), storage tuber length (cm) hydrogen cyanide (mg/kg fresh tuber weight) and starch contents (%) of tested cassava genotypes and locations are presented in Table 1. The result of the study revealed that there were significant ($p<0.05$) differences among cassava genotypes collected from southwest Ethiopia. Based on combined mean performance of Jimma, genotype AAGT-108 (70.54 t/ha), AAGT-189 (61.49 t/ha) and AAGT 192 (61.45 t/ha) were produced the highest storage tuber yield. Similarly, genotype AAGT-108, AAGT-192, AAGT-028 and AAGT-189 gave the highest performance at Metu and Tepi with storage tuber yield of 77.8 t/ha, 76.4 t/ha, 74.15 t/ha and 58.6 t/ha, and 78.55, 66.78, 72.99, and 77.59 t/ha, respectively. Likewise, storage tuber length of tested genotypes and locations showed similar trends as storage tuber yield. Genotypes AAGT-189, AAGT-108 and AAGT-191 produced the longest storage tuber with 56.75, 52.40 and 52.90 cm, respectively. However, released varieties Qulle and Kello provided the longest storage tube at Metu and Tepi. Based on overall performance genotypes AAGT-028, AAGT-189 and AAGT-192 are the best performed genotypes in all tested locations.

Mean performance of hydrogen cyanide and starch content: The mean hydrogen cyanide and starch contents are important quality parameter in cassava breeding. In this study, genotypes AAGT-108, AAGT-189 and AAGT-192 produced the highest mean hydrogen cyanide contents with value of 105.35, 118.5 and 11.7 mg/kg of fresh tuber for Jimma, 71.05, 75.50 and 76.05 mg/kg fresh sample for Metu and 128.35, 88.35 and 76.25 mg/kg for Tepi, respectively. The starch contents of established genotypes with tested locations are presented in Table 2. At Jimma, genotypes AAGT-108, AAGT-189 and AAGT-192 are the best materials as compared to others and gave the mean starch content of 15.22, 16.98 and 16.61 % per one kg. of fresh storage tuber cassava sample. Similarly, the genotype performed well at Metu and Tepi with the value of 10.18, 10.83 and 10.91 % at Metu and 18.4, 12.7 and 15.65% per kilogram of fresh storage tuber cassava sample, respectively.

In the present study, the mean storage tuber yield showed high significant differences ($p<0.05$) among cassava genotypes from southwest Ethiopia, this suggested, the presence of high degree of genetic variability in the materials evaluated and the existence of considerable genetic diversity among cassava genotypes for selection. The result of this study is similar with the report of Tewodros and Getachew, (2013) who reported cassava genotypes collected from southwest Ethiopia had significant difference storage tuber yield and related traits. Similarly, Tesfaye *et.al*, (2017) also reported high significant ($p<0.01$) difference among cassava genotypes tested at Hawassa, Amaro, Jima and Sekota areas of Ethiopia. The storage tuber length is also vary significantly ($p<0.05$) among tested cassava genotypes. The longest tuber length is obtained from genotypes AAGT 189, Qulle, AAGT 108 at Jimma, Metu and Tepi with values of 56.75, 53.10 and 40.40 cm, respectively.

Table 1. Mean fresh storage tuber yield (t/ha) and tuber length (cm) of cassava genotypes of in tested locations and years

Genotypes	Storage tuber yield (t/ha)									Storage tuber length (cm)								
	Jimma			Metu			Tepi			Jimma			Metu			Tepi		
	2015	2017	Mean	2015	2017	Mean	2015	2017	Mean	2015	2017	Mean	2015	2017	Mean	2015	2017	Mean
Qulle	68.47	62.8	65.64	62.9	34.6	48.75	71.88	26.06	48.97	41.7	56.1	48.90	55.0	51.2	**53.10**	35.1	38.7	36.90
Kello	48.01	44.4	46.21	75.7	34.4	55.05	77.71	31.18	54.45	44.0	53.4	48.70	47.3	43.4	45.35	30.9	38.8	34.85
45/72NR	62.01	54.9	58.46	53.3	41.3	47.30	37.92	77.60	57.76	45.0	45.7	45.35	46.0	34.8	40.40	31.8	34.0	32.90
45/72NW	51.41	54.5	52.96	54.1	58.5	56.30	77.46	75.75	76.61	43.3	53.5	48.40	39.7	35.8	37.75	32.5	40.5	36.50
AAGT 028	38.50	39.9	39.20	80.1	68.2	**74.15**	68.42	77.56	72.99	47.3	46.3	46.80	39.3	36.5	37.90	27.6	41.3	34.45
AAGT 108	73.38	67.7	**70.54**	78.4	77.2	**77.80**	79.59	77.51	78.55	51.0	53.8	52.40	41.7	44.2	42.95	28.9	51.9	**40.40**
AAGT 189	58.48	64.5	**61.49**	45.1	72.1	58.60	76.71	78.46	77.59	58.0	55.5	**56.75**	35.0	52.2	43.60	29.7	37.8	**33.75**
AAGT 191	47.38	52.2	49.79	65.4	78.9	72.15	64.58	53.99	59.29	56.0	49.8	52.90	40.7	38.5	39.60	27.1	31.9	29.50
AAGT 192	74.40	48.5	**61.45**	80.6	72.2	**76.40**	51.88	81.67	66.78	47.0	49.4	48.20	44.7	46.0	45.35	31.1	37.9	**34.50**
AAGT 200	52.50	67.3	59.90	74.2	62.1	68.15	64.84	50.48	57.66	61.0	45.4	53.20	35.0	41.9	38.45	30.9	34.5	32.70
Local	40.88	64.1	52.49	64.3	52.0	58.15	70.46	74.77	72.62	53.3	45.1	49.20	43.0	43.1	43.05	28.1	33.4	30.75
Mean	55.95	56.44	56.20	66.7	59.2	62.95	67.4	64.1	65.75	49.8	50.3	50.05	42.5	42.5	42.50	30.3	38.2	34.25
LSD (5%)	7.17	19.1	12.71	37.2	21.1	11.87	17.18	25.67	17.93	19.5	13.7	8.71	16.2	11.8	9.14	6.2	8.6	7.41

Table 2. The mean hydrogen cyanide content (HCN mg/kg) and starch content (%) from one kg. fresh tuber sample of cassava genotypes over locations and years

Genotypes	Hydrogen cyanide contents									Starch content (%)								
	Jimma			Metu			Tepi			Jimma			Metu			Tepi		
	2015	**2017**	**Mean**	**2015**	**2017**	**Mean**	**2015**	**2017**	**Mean**	**2015**	**2017**	**Mean**	**2015**	**2017**	**Mean**	**2015**	**2017**	**Mean**
Qulle	51.10	52.73	51.9	28.9	42.30	35.6	59.2	56.70	57.95	7.33	7.57	7.45	4.15	6.07	5.11	8.49	8.14	8.32
Kello	55.00	42.87	48.9	56.7	54.80	55.75	67.3	47.80	57.55	7.89	6.15	7.02	8.14	7.86	8.00	9.66	6.86	8.26
45/72NR	77.90	56.70	67.3	39.1	46.70	42.9	80.8	76.40	78.6	11.18	8.14	9.66	5.61	6.70	6.16	11.6	11.0	11.30
45/72NW	59.30	60.30	59.8	47.2	50.60	48.9	72.9	80.30	76.6	8.51	8.65	8.58	6.77	7.26	7.02	10.4	11.5	10.95
AAGT 028	87.50	67.50	77.5	75.6	67.30	71.45	62.0	67.10	64.55	12.55	9.68	11.12	10.8	9.66	10.23	8.90	9.63	9.27
AAGT 108	113.1	98.75	105.9	52.6	89.50	71.05	148.4	108.3	128.35	16.23	14.2	15.22	7.55	12.8	10.18	21.3	15.5	18.40
AAGT 189	128.6	108.4	**118.5**	52.6	98.40	75.5	77.90	98.80	88.35	18.45	15.5	16.98	7.55	14.1	10.83	11.2	14.2	12.70
AAGT 191	66.00	59.20	62.6	98.6	79.20	88.9	59.40	52.70	56.05	9.47	8.49	8.98	14.1	11.3	12.70	8.52	7.56	8.04
AAGT 192	118.6	112.8	115.7	59.3	92.80	76.05	115.8	102.4	109.1	17.02	16.2	16.61	8.51	13.3	10.91	16.6	14.7	15.65
AAGT 200	66.90	44.70	55.8	64.8	44.00	54.4	62.00	52.10	57.05	9.60	6.41	8.01	9.30	6.31	7.81	8.90	7.48	8.19
Local	47.90	51.10	49.5	55.3	40.50	47.9	52.40	52.40	52.4	6.87	7.33	7.10	7.93	5.81	6.87	7.52	7.52	7.52
Mean	79.26	68.65	74.0	57.3	64.19	60.76	78.01	72.27	75.14	11.37	9.85	10.61	8.23	9.21	8.72	11.2	10.3	10.75
LSD (5%)	2.3	1.1	1.58	1.9	1.50	0.45	0.10	0.23	2.10	0.27	0.14	0.27	0.11	0.15	0.57	0.14	0.12	0.42

The length of tuber is highly affected by the soil texture of cassava grown. This result is supported by the report of Tewodros and Yared, (2015), who reported the storage tuber length of cassava grown in clay soil and high moisture stress areas of southern Ethiopia are reduced significantly. Besides, Tesfaye *et al*, (2017) reported variety Kello produced longest mean storage tuber of 40.67 cm grown in major growing areas of Ethiopia. However, in this study the storage tuber length obtained from Jimma (48.90) and Metu (53.10 cm) is higher than the report of Tesfaye *et al,* (2017). The hydrogen cyanide and starch content are the important quality parameter in cassava breeding. According to Montagnac *et al*., (2009) and André *et al*., (2016), cassava genotypes are classified in two groups, according to the difference on toxicity and palatability of the storage tubers: sweet (slightly sweet; that is, plants that have less than 100 mg/kg cyanuric acid) or bitter (those have a perceptible bitter taste due to the high concentrations of HCN, that is, values over 100 mg/kg fresh sample). There is no morphological characteristic that may tell these two groups apart (Dufour *et. al*., 1988). In the present study, cassava genotypes AAGT 189, AAGT 191 and AAGT 108 produced the highest cyanogenic glycosidecyanide content with value of 118.5, 88.9 and 128.35 mg/kg fresh sample, respectively. The result obtained from this study was similar with the report of André *et al.,* (2016), who reported, the cyanide contents ranged from 15-225 mg/kg of cassava collected from Brazil. On contrarily, the result obtained from this study was higher than the report of Ezeigbo *et al.,* (2015) reported the cyanide contents of cassava genotypes ranged from 36.65-62.57 grown in Abia state of Nigeria. Similarly, the mean starch contents of cassava genotypes ranged from 5.11-18.4%. The lowest starch content obtained from genotype Qulle at Metu and the highest mean starch content was collected from genotype AAGT 108 at Tepi. The result obtained from this study was lower than the study of Muleta and Mohammed, (2017) who reported the starch contents ranged from 62-91%. Similarly, the starch content obtained from in this study almost similar with the study of Ezeigbo *et al, (*2015) reported the starch contents of five cassava genotypes from Nigeria ranged from 17.48-20.62%.

Conclusion and Recommendation

The yields of cassava are highly affected by genotype and location (environment) which indicated the yields and quality performance of genotypes varies from one location to another. Similarly, the hydrogen cyanide content also varied from one location to other within and among genotypes. Based on the overall mean result of hydrogen cyanide and starch contents, genotype AAGT-108 (128.35mg/kg) and AAGT-189 (118.5 mg/kg) are recommended for bitter type (high HCN content). As a result, the National Variety Release Committee officially released genotype AAGT-108 (Melko-108) as bitter type for production. On contrary, the rest of genotypes have sweet type due to its cyanide content below 100 mg/kg and are recommended for human consumption after full processing

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