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Comparative Analysis of M_1 Progenies against Two Cassava Parents for Improved Food Qualities

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Abstract
Polyploidy cassava seeds produced by two varieties raised from colchicine-treated materials (TMS98/0505 and TMS98/0581) at three levels of concentration: *0, 2 and 4ppm* with their parents were established in the field at the National Root Crops Research Institute Umudike, South Eastern Nigeria. The study aimed to evaluate the level of genetic variability developed in the progenies compared with their parents. In the study, 25cm Stakes of each variety were cut and planted on ridges in a 4 x 5m plot, at a spacing of 1m x 1 m apart in a randomized complete block design replicated three times. The biological seeds derived from the crosses between two varieties were planted in a single-row design at a spacing of 1m intra and 0.3m inter-row. The plots were kept weed-free and NPK fertilizer was applied at the rate of 400kg/ha. The proximate analyses of the roots were carried out using the Association of Official Analytical Chemists (AOAC) method and analyzed using the SPSS statistical package and means were separated using the Duncan multiple range test. The result of the proximate analysis of physicochemical characteristics from the parents and their progenies revealed higher variations in their nutrient quality than the progenies. The higher average range of the starch content of the progenies shows that there is the possibility of developing cassava varieties that are high in starch content from induced mutation. The result also showed that there is zero phenol level in the progenies against the range (0.11 – 0.50%) in the parents, indicating that these developed materials meet the industrial requirement for baby formulae.
Keywords: Polyploidy, progenies, correlations, colchicine and phenol

Introduction

Cassava is a universal crop in the world agricultural system, the second carbohydrate and starch source globally (Adepoju and Nwangwu, 2010; Shore, 2002). It has a high potential to meet the food and raw material needs of the tropical and sub-tropical world. It is a staple food for over 800 million people, used in preparation of divers relishes, (Okogbenin *et al*., 2007), and serves as raw materials to many agro-industries in the production of both human and animal feeds. In recent years, the increased demand for cassava as a raw material has risen beyond the annual production of 52 million tonnes and increased in availability as a staple Food (Okogbenin *et al*. (2012). Hence there is a need to develop varieties with industrial values such as high starch content, high fiber and high nutritional values. Unfortunately, genes needed to develop these traits that will keep pace with population growth and increased industrial requirements are not always available in nature, so there is a need to develop these traits using the available breeding gene pools. However, botanical seed production of cassava is hampered by irregular and erratic flowering (Ibrahim et al 1964. To overcome these challenges, several breeding methods such as recurrent crosses/selection and tools such as genetic markers to locate specific traits, are in use and advancing, but these methods/tools can only be used where the genes needed are available in nature and not in a predominant sterile crop or when novel traits are required (Leitao, 2012). Mutation, which is a natural phenomenon that brings about variation is low and slow, and cannot be relied upon (Manyong, 2000). Hence, the need for a faster means to achieve the needed goal. Induced mutation is one of the techniques very effective in gene manipulation for specific traits (Kasky, 2012). Induced mutation can be physical or chemical and common among the chemical mutagens is colchicine. It has been successfully used in developing variations in some plant species; overcoming inter-specific incompatibilities in cereals specie, (Mehetre *et al*., 2003) and restoring fertility in inter-specific hybrids of *Manihot* species (Nassar, 2002) and *Xanthosoma* species, (Burn and Emirogli, 2008). It has become the ultimate source of all heritable variations in breeding, passing important traits through cell division to the progenies but concentration is important when colchicine is used to induce polyploidy (Shu 2009). Therefore, this study presents a comparative analysis of M1-induced cassava progenies with their parents for improved food quality.

Methodology

This experiment was conducted at the National Root Crops Research Institute, Umudike Research farms, which lies within the humid rainforest agroecology of Nigeria, to assess the effect of induction mutation on the cassava progenies produced from colchicine-treated cassava stem. Five cassava varieties namely: TMS98/0505, TMS94/4479, TMS94/1632, TMS92/0057, and TMS98/0581 were soaked in colchicine solution at two levels: 2%,(400mg of colchicine powder dissolved into one litre of distilled water), 4% (4100mg of colchicine powder dissolved into 50cl of distilled water), while 0% the control were soaked into ordinary water and left to dry under shade for 24 hours. Seven months later, twenty pieces of stakes of about 25cm each of the five cassava varieties were cut from the plants raised from the treated materials and planted in a well-harrowed and ridged field in a 4x5 randomized complete block design, at a spacing of 1mx m intra and inter-row replicated three times. At the end of the year, seeds harvested from the experiment were sorted and stored. In 2014, 6,117 botanical cassava seeds from two cassava parents (TMS98/0505 and TMS98/0581) harvested, sorted and stored were planted directly on ridges in the field at a spacing of 0.3cm intra–row using a single-row design after viability test. The field was kept weed-free by applying pre-emergence herbicides as recommended one week before planting, followed by two hand weeding. Fertilizer (NPK 15: 15: 15) was applied at the rate of 400kg per hectare, while supplemental manual weed control was used to maintain the field until harvest. The parents were also planted to provide means for comparison. 12 months after planting, storage root samples were randomly selected from both parents and progenies, which were used for proximate analysis using AOAC's methods (1990). Data obtained were statistically analyzed using Statistical Package for the Social Science (SPSS) software. The means were separated using the Duncan multiple range test.

Results and Discussion

The proximate compositions of the parent materials and the progenies were analyzed to compare their food qualities. Table 1 shows the proximate analysis of the parent materials and their progenies. Their mean values show that some progeny's compositions were higher than the parents. The dry matter content, ash content, crude fibre content, starch content and crude protein of the progenies (41.83, 3.63, 3.53 and 36.24)mg/100 were higher than their parents (38.45, 2.74, 2.38 and 27.7)mg/100 These values were in line with Boonbongkarn *et al.* (2013) and Rodríguez *et al.* (2008), and higher than the values reported by Ephraim *et. al.* (2010) and Enidiok and Otuechere (2008) who reported lower values of (0.28 - 0.52) mg/100 among the parents and (0.28 - 0.35) mg/100 among the progenies. The mean value of moisture content and carbohydrate of the parents (61.55, 2.92, and 91.34) mg/100 were higher than their progenies (58.17. 2.49 and 89.46) mg/100g respectively, Ephraim *et al.* (2010), had a similar result. This result indicated promising genotypes with better proximate composition levels that could meet the demand for high-quality cassava values.

Moisture contents

The average moisture content of the parents ranged from (30.0 - 76.20) mg/100 while the range of moisture content of the progenies was (43.70 - 79.52)mg/100. This high range is the same as reported by Kenneth (2012) who reported high moisture content in six cassava varieties he evaluated.

Starch Content

The average starch content of the parent varieties ranged from (20.48-40.80) mg/100, while the starch content of the progenies ranged from 20.90 to 67.40%. These cassava starch content values are similar to the range of values reported by Nuwamanya *et.al*. (2011) who reported an average range of 70.36 to 89.90% for parents and an average range of (73.48 to 93.85) mg/100 for the progenies.

Dry matter content

The dry matter content of the parent material had an average range of 23.80 to 48.70%, while it was 20.48 to 56.30% among the progenies. These values are similar to the range of values for cassava varieties as reported by several researchers (Chávez *et al*., 2008; Ramanandam *et al*., 2008; Teye *et al*., 2011) who recorded values ranging from (29.8 to 40.7)mg/100, with an average of (34.50)mg/100. This shows that there is the possibility of developing cassava varieties that are high in dry matter content from induced mutations.

Carbohydrate contents

The carbohydrate content of parents ranged from (91.7 to 90.99) mg/100 with a mean value of (91.34)mg/100, which was a little higher compared to the progenies with a range of 87.39 to (93.47))mg/100 with a mean of (89 46)mg/100. These values are higher than those of Adepoju and Nwangwu (2010), who reported a mean value of carbohydrate content at (32.6) mg/100 and Richardson, (2011), who reported carbohydrate content of (42.7, 40.6) mg/100 and 37.6 mg/100.

Crude protein content

The protein content of parent materials ranged from (1.31 to 5.69) mg/100 which was a little higher than the progenies ranging from (1.09 to 4.38) mg/100. The low protein contents of these materials corroborate the long-held view that the protein content of cassava is very low (Nassar and Dorea, (1982). This finding is also similar to the findings by Charles *et al*., *(*2005); Ceballos *et al*., (2006) and Li *et al*., 2012). Ceballos *et al*. (2006) further suggested that the wide range of protein values found in their study (0.95- 6.42) mg/100 were genetic and provided excellent opportunities for the improvement of protein levels through conventional breeding methods**.**

CF=Crude fiber

The mean crude fibre content of the progenies ranged from (1.98 to 5.20) mg/100 while the fibre content of parents higher ranged from (1.98 to 2.59) mg/100. This result simply shows a wide variability found in the botanical seed and provides an opportunity for the selection of cassava for different purposes.

Vitamins and Amino Composition of the parents and their progenies

The ash Composition which gives the total mineral and vitamin content of a crop, varied from (1.78 - 3.22) mg/100 in the parents compared to (2.01 to 5.2) mg/100 in the progenies, which is comparable and even higher than the values by Odebunmi *et al.* (2007) and Offor *et al*. (2012) in recent reports that ash contents for cassava averaging (2.65) mg/100. The mean values of the thiamine, niacin and riboflavin content of the progenies were lower than their parents as shown in Table 2. Several researchers (Charles *et al*., 2005; Ceballos *et al*., 2006; Sankaran *et al*., 2008; Li *et al*., 2012; Manary and Hirschi, 2012) had similar results and proved that they were genetic and provided excellent opportunities for improvement through the conventional breeding method. The zero-phenol value of the progenies is very low compared to the range of the parents' level (0.11-0.50mg/100g) making them good and safe raw materials for industrial baby food formulae. The other values of vitamins for the parents such as alkaloid, vitamin C, thiamine, niacin and riboflavin were almost within the same range of the progenies which required additional improvement to increase the vitamin in the leaves as they are important elements in human nutrition. The hydrogen cyanide (HCN) of both parents (2.92 – 3.34mg/100g) and progenies (1.88 - 5.60mg/100g) had almost the same mean values. This is similar to the report of Wilson and DuFour, **(**2002), who also reported the same range of HCN in bitter cassava varieties. This element serves as a cassava antioxidant as a deterrent to animal attack. Moreover, it is no longer a problem in cassava utilization as processing removes it and a higher percentage is been used industrially (Okogbenin *et al*., 2005). Table 3 shows the mean mineral composition of the two parents; TMS 98/0505 and TMS92/0581 compared with the mean mineral values of the progenies. The result showed that the values of magnesium, potassium, phosphorous and sodium, (8.18, 0.58, 0.23 and 0.58) mg/100g, of the progenies were higher than the mean values of the parents (0.49, 0.43, 0.43 and 0.21) mg/100g respectively. This result shows that the possibility of developing cassava varieties that will supply these essential micronutrients is high. Although the mineral contents were relatively low when compared to the contents of sodium and potassium found in the literature (1.67 to 29 7) mg/100g, the potassium (4.5-9.7) mg/kg values of these materials were higher. The mean calcium content of the parents was 0.98 mg/kg as against the mean calcium content of the progenies (0.97 mg/100g), although there was no significant difference the result showed that there were individuals with higher calcium values as seen in the maximum mean (1.91 mg/kg) indicating a favourable disposition.

Potassium and sodium

The potassium content of the parent materials had an average range of (0.36 and 0.68)9mg/100g, while the progenies had an average range of (0.35 and 0.87) mg/100g. These values are similar to the values obtained by Adepoju *et al*. (2010). The mean values of sodium ranged from (22.30 to 34.9) mg/100g for the parents, while the values of potassium ranged from (817.30 to 1301.20)mg/100g for the progenies. These values were compared favourably to those of Rojas *et al*. (2007), whose mineral contents of six cassava varieties studied had an average of (623)mg/100g for potassium and 30mg/100g for sodium. The value of iron in the parents ranged from (5. 12 to 5.89) mg/100g, while it was (1.00 to 7.24) mg/100g in the progenies. This result compares favourably with the iron level of Chavez et al (2000) who obtained a level of (2.6 to 4.9) mg/100g, and the higher iron level in the progenies shows the possibility of increasing the quantity of iron in cassava through induced mutation. For zinc, the range obtained was lower than the range in the literature by Chavez *et al*. (2000) (3.4 to30.9) mg/100g, higher in the parents showing probably that it is not transferable.

Conclusion

The proximate analysis carried out showed that there is a wide range of variations in cassava botanical seeds which could be exploited for variable uses and that more variations can be developed in cassava using colchicine mutation breeding.

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Table 1: Comparison of the Physicochemical components of the parents and their progenies

Parameter(mg/100g)	TMS0581	TMS05050	Mean of Progenies	STD	Max	Min
DM	39.63	37.27	41.83	7.25	56.30	20.48
MC	60.37	62.73	58.17	7.25	79.52	43.70
ASH	2.54	2.94	3.63	0.89	5.40	2.04
CF	2.17	2.59	3.53	0.80	5.20	1.98
CP	3.21	2.63	2.49	0.85	4.38	1.09
CHO	91.70	90.99	89.46	1.38	92.23	85.90
STARCH	31.83	27.76	36.24	8.89	51.40	20.90

***Key**: DM =Dry matter content, Ash = Ash content of parent, CF = Crude fibre of parent, CP = Crude protein of parent, CHO= Carbohydrate content of parent*

Table 2: Vitamins and Amino acid composition of parents and their progenies

Parameter(mg/100g)	TMS98/0505	TMS	Mean of parents	Mean of Progenies	Max	Min
HCN	1.84	5.20	3.13	3.44	5.6	1.88
Caloric	386.70	382.22	384.46	375.18	386.56	363.18
Alkaloid	0.16	0.53	0.35		1.11	0.14
Vit.C	21.08	42.41	20.25	17.56	28.58	6.91
Thiamine	0.23	0.18	0.21	0.14	0.23	0.00
Niacin	0.57	0.95	0.53	0.46	0.80	0.25
Riboflavin	0.012	0.12	0.04	0.04	0.02	0.10
Phenol	0.11	0.50	0.80	0.00	0.00	0.00

Key: HCN – Hydrogen cyanide, Niacin, Riboflavin

Table 3: Mineral composition of two parents compared with the M1 progenies

Mineral (mg/100g)	TMS05050	TMS0581	Mean of parents	Mean of Progenies	STD	Max	Min
N	0.51	0.41	0.46	0.40	0.14	0.70	0.18
Ca	0.80	1.17	0.98	0.97	0.29	1.91	0.41
Mg	0.45	0.54	0.45	8.18	50.87	3.34	0.24
K	0.46	0.41	0.43	0.65	0.14	1.03	0.35
Na	0.21	0.22	0.21	0.23	0.19	1.44	0.11
P	0.41	0.46	0.43	0.58	0.13	0.87	0.33
Fe	5.12	5.89	5.50	4.20	1.59	7.24	1.00
Zn	0.21	11.61	0.32	0.22	0.09	5.00	0.00

Key: N = Nitrogen, Ca = Calcium, Ma = Manganese, K = Potassium, Na = Sodium, P = Phosphorus, Fe = Iron, Zn = Zinc