NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 51 Number 3, December 2020 Pg. 208-212
Available online at: http://www.ajol.info/index.php/naj

EVALUATION OF COLCHICINE-INDUCED MUTANTS OF CASSAVA FOR ENHANCED MICRO-NUTRIENT AND PHYTO-CHEMICALS

[1]Amanze, N., [1]Eke-Okoro, O. N., [2]Akinbo, O., [1]Njoku, D.N. and [1]Amadi, C.O.

[1]National Root Crops Research Institute, Umudike,PMB 7006 Umuahia Abia State
[2]University of Ouagadougou,Burkina Faso.
Corresponding Authors' email: amanzengozi@gmail.com

Abstract
A field experiment was conducted at National Root Crops Research Institute (NRCRI) Umudike (in the rainforest belt of Nigeria), to develop cassava genotypes with higher yield and increased micronutrient content to meet the current micro-nutrient need of consumers. Four cassava varieties: TMS98/0505, TMS94/4779, TMS94/1632, and TMS98/0581 treated with three levels of colchicines- 0ppm, 2 ppm, and 4ppm were planted on ridges in a 4 x 5m plot size, at a spacing of 1m x1m apart in a randomized complete block design replicated three times. The trial was harvested at 12 months after planting and the proximate analyses of the roots carried out showed that treatment with colchicine at 4ppm concentration significantly ($P<0.05$) increased the zinc and iron content compared to other concentrations. Magnesium content of the roots was however highest at concentration level of 2ppm (0.58 mg/100g). Also, the food components such as crude fibre and starch increased with colchicine concentration, showing that colchicine could be effectively used to improve the nutritional qualities of cassava roots.

Keywords: Colchicine, Polyploidy, Micro-nutrients, Cassava and Food Components

Introduction

Cassava is a prominent root crop, which plays important role in the food security of low income earners. Every part of the plant is important, but of most economic importance is the root. It is efficient in carbohydrate production and provides cheap source of calories for millions of people in Sub-Sahara Africa. According to Tonukari (2004), the starch content of fresh cassava root is about 30%, and gives the highest yield of starch per unit area of any crop known, but highly deficient in essential vitamins and extremely low in protein content which range between 1-3% (Adepoju, and Nwangwu, 2010). The roots however contain trace amount of iron, phosphorus and calcium but relatively rich in vitamin C and minerals (Enidiok, *et al.,* 2008; Salcedo *et al.,* 2010 and Akimbo *et al*., 2011), though not in appreciable amounts. These micronutrient levels are not sufficient enough to take care of the micronutrient requirement of the low income group of people who depend on cassava as their staple food, hence exposing them to endemic malnutrition with regard to essential micro nutrients such as vitamin A, zinc and iron, making malnutrition an immediate health challenge that requires urgent attention. According to Udeh (2011), the use of nutrient-enriched food rather than sourcing essential micro nutrients through supplements and drugs would be a sustainable solution to this critical health problem. In order to use the micro-nutrient rich food approach, it is imperative to develop cassava genotypes with enhanced essential micro nutrients. Different tools have being used in the improvement of nutritional values of cassava. The common breeding approach is through hybridization among appropriate parents. Seedlings with desirable attributes are selected and subsequently propagated clonally. However, selection of parents based on their direct performance may not always be dependable due to the poor knowledge of the gene action involved in the control of the traits, and the diverse genetic structure of the parent, hence the need to explore other breeding tools (Ye *et al*., 2010). Mutation breeding on the other hand has been used in the development of new traits including the increase in food value of root and tuber crops (Jain, 2006 and Mbanaso *et al*., 2006). Increase in protein content and early maturity in rice and rye are some of the traits that had been improved through mutation breeding. This study, therefore, reports the use of mutation breeding through colchicine application in the improvement of the nutritional qualities of four cassava varieties.

Materials and Methods

The experiment was conducted at the National Root Crops Research Institute (NRCRI), Umudike Research farm, which lies within the humid rainforest agroecology of Nigeria. Stakes of four cassava varieties (TMS98/0505, TMS94/4479, TMS98/1632, and TMS98/0581) were treated with mutation-inducing colchicine hormone at 0ppm, 2ppm and 4ppm. The stakes were soaked in the solution for 30 minutes, air dried for 24 hours in the screen house, pre-sprouted in nursery bags and transplanted to the field at 3-leaf stage. At 7 months after planting, twenty five pieces of 25cm stake cuttings of each of the cassava varieties were cut from the mature plants raised from colchicine treated materials and planted on a well harrowed and ridged field in a randomized block design, using 4m x 5m plots and plant spacing of 1m x1m intra and inter-row. Each treatment combination was replicated three times. The field was sprayed with Primextra and Roundup fort, pre and post-emergence herbicides each, using recommended rates. 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 till harvest. At 12 months, the roots were harvested for proximate analyses using the AOAC (1990) methods. Data obtained were statistically analyzed using SAS (1999) statistical package. Mean separation was carried out using Fisher's Least Significant difference (F-LSD).

Proximate composition determination-The harvested roots of each cassava variety (two large roots) were randomly selected per variety and prepared for proximate analysis using the AOAC's methods (1990).

Moisture/Dry Matter-Tubers were peeled, wiped and chopped into small pieces. Triplicate 2g samples were accurately weighed into pre-labeled, pre-weighed dishes and dried at 130°C to constant weight. Dried samples/dishes were weighed, moisture content (%) was calculated, and dry matter (%) was calculated by 100 – moisture content (%). Other Analyses- Samples were peeled, wiped, chopped and dried in oven. The dried samples were subsequently turned into powder in a high-speed blender and used for the remaining tests.

Carbohydrate content- was obtained by subtracting the total ash content, crude lipid, crude protein and crude fiber from the total dry matter (Oyeleke 1984). The caloric value of each sample was calculated using Atwater factor method [(4 x crude protein) + (9 x crude lipid) + (4 x carbohydrate)] as described by Ihekeronye and Ngoddy (1985). A mixture of concentrated H_2SO_4, a catalyst (selenium), salicylic acid and hydrogen peroxide were used for sample digestion, and subsequently, total nitrogen, phosphorus, calcium, magnesium, sodium, potassium, zinc, iron and manganese in the digests were determined.

Mineral element composition- was determined using the Unicam Solaar Atomic Absorption Spectrophotometer (model 969 Mk II, Unicam Ltd., Cambridge, UK). Potassium, sodium, magnesium and calcium contents were determined by reading their absorbance at 766.5, 589.0, 285.2 and 422.7 nm wavelengths, respectively, whereas the copper, manganese, zinc and iron contents were measured at 324.8, 279.5, 213.9 and 248.3 nm wavelengths, respectively. Total phosphorus was obtained using the ascorbic acid blue color procedure of Okalebo *et al*. (2002) by reading the absorbance at a wavelength of 880 nm on a Helios Gamma spectrophotometer (Helios Gamma UV-Vis Spectrophotometer, Thermo Spectronic, Cambridge, U.K.).

Statistical analysis

Data from the experiment were analyzed using SAS (1999) statistical package. Mean separation was carried out using Fisher's Least Significant difference (F-LSD).

Results and Discussion

Table 1 presents the effect of colchicine concentration on the physico-chemical composition of the raw pulp taken from freshly harvested roots of the treated materials evaluated. There were no significant differences among the three levels of concentration in the moisture content of the four cassava varieties evaluated, although the cassava varieties without treatments had higher water content. With an average dry matter content of 35.5%, there were no significant differences ($p<0.05$) in the dry matter content of the cassava varieties treated with the different level of colchicine with 0, 2 and 4ppm concentrations giving 36.6, 35.9 and 33% mean dry matter, but there were in the same range with the clones. Abraham (1964) reported and recommend for starch industries. The total ash did not show any significant difference across the concentrations, although concentration level at 4ppm gave highest ash content followed by 2ppm and 0ppm (2.437%, 2.50%.and 2.63%) respectively. Crude protein was not significantly affected by the treatment also. On the other hand, crude fiber was significantly affected by concentrations of colchicine as seen in the result (2.25, 2.46 and 2.65) and increased with increasing level of colchicine from 0ppm to 2ppm to 4ppm level as reported by Mbanaso *et al.* (2006), Amanze *et al*. (2010). The cassava varieties evaluated were very rich in carbohydrate content, although there were no significant differences among the different levels (91.81, 91.22 and 90.98) col/kg for 0ppm, 2ppm, and 4ppm respectively. The starch content of the varieties evaluated according to the levels (0ppm,2ppm and 4ppm) were significantly different ($p<0.05$) with colchicine level of 4ppm (34.03%), higher than 32.00% and 29.44% of 0ppm and 2ppm respectively, with an average of 31.70%.

Table 1: Effects of colchicine concentration on mean proximate composition of roots of mutant cassava varieties

Proximate composition	Colchicine concentrations		
	0ppm	2ppm	4ppm
Dry matter content	33.5[a]	35.9[a]	36.6[a]
Moisture content	63.39[a]	64.13[a]	66.42[a]
Ash content	2.47[a]	2.50[a]	2.63[a]
Crude fibre	2.25[b]	2.46[ab]	2.65[a*]
Crude protein	2.64[a]	3.30[a]	2.82[a]
Carbohydrate	91.81[a]	91.22[a]	90.98[a]
Starch content	32.00[a]	29.44[b]	34.03[a]

Means with same letter are not significantly different

Table 2 shows the mineral composition of the cassava varieties evaluated. Colchicine concentration levels seem to significantly affect the major minerals of interest: zinc, iron and magnesium. Both zinc and iron were significantly affected by colchicine concentration level of 4ppm, while magnesium content at concentration level of 2ppm (0.58mg/100g) significantly ($p<0.05$) differed from Mg of concentration level of 0ppm or control (0.41mg/100). These results imply that these essential nutrients can be increased using induced mutation through colchicine treatment, and that the concentration levels have not been attained. The other mineral composition of the cassava materials evaluated: nitrogen, calcium, potassium, sodium and phosphorous were not significantly affected.

Table 2: Effect of colchicine concentration on mean mineral composition of roots of mutant cassava varieties

Element (mg/100g)	Concentration of colchicine (ppm)		
	0ppm	2ppm	4ppm
N	0.42[a]	0.52[a]	0.47[a]
Ca	0.89[a]	1.00[a]	0.95[a]
Mg	0.41[b]	0.58[a]	0.48[ab]
K	0.56[a]	0.44[a]	0.46[a]
Na	0.23[a]	0.29[a]	14.56[a]
P	0.47[a]	0.47[a]	0.51[a]
Fe	5.07[b]	4.78[b]	6.20[a]
Zn	0.36[b]	0.31[b]	1.61[a]

Mg=milligram per 100cm^2. Means with same letter are not significantly different
N=Nitrogen, Ca=Calcium, k=Potassium, Na=Sodium, P= Phosphorous, Zn= zinc, Fe=iron and Mg=Magnesium

Vitamins composition of treated and untreated cassava varieties: Some vitamins were significantly different among the three levels of colchicine concentrations evaluated. Colchicine treatments, especially at 4ppm (3.51 mg/100g), seem to enhance the accumulation of hydrogen cyanide compared to the control (2.74 mg/100g). Same trend was also observed in phenols where the roots of colchicine-treated cassava stems exhibited higher phenol contents (0.26mg/100 for 2ppm and 0.17 mg/100 for 4ppm) compared to the control (0.13mg/100). For vitamin C (range: 22.51 – 22.52 mg/100g), thiamin (0.16 – 0.19 mg/100g), nicotinic acid (0.51 mg/100g) and riboflavin (0.43 – 0.53 mg/100g), there were no significant ($p<0.05$) difference, but colchicine treatment effects where marginal difference in content exists. The non-existence of notable effect of colchicine treatment on these phytochemicals made their values similar to that of Li *et al.* (2012).

Table 3: Effects of colchicine concentration on mean phyto-chemical and vitamin composition of roots of mutant cassava varieties

Element (mg/100g)	Concentration of colchicine (ppm)		
	0 ppm	2 ppm	4 ppm
HCN	2.74[b]	3.16[ab]	3.51[a]
Phenol	0.13[b]	0.26[a]	0.17[b]
Alkaloid	0.31[b]	0.34[b]	0.34[b]
Vitamin C	22.51[a]	22.51[a]	22.52[a]
thiamin	0.16[a]	0.16[a]	0.19[a]
nicotinic	0.51[a]	0.51[a]	0.51[a]
riboflavin	0.53[a]	0.53[a]	0.43[a]

HCN=Hydro cyanide. Means with same letters are not significantly different

The improvement of micronutrient composition of cassava is very important due to endemic micro-nutrient in many cassava consuming communities in Sub-Sahara Africa. The results obtained in this study indicated that colchicine treatment effected changes and improvement in the genetic materials of the treated cassava varieties for some key nutritional traits. The results showed increase in some micronutrient levels of the treated materials. The micro nutrients increased include crude fiber and starch, hence can be used to develop cassava varieties with high fibers which will meet the food requirements of the aged, while varieties with high starch (34.03, 32.00 and 29.44mg/100) content can be developed using this same medium to meet the high demand on starch for domestic and foreign industries.

The increase in the major minerals zinc, iron and Magnesium, is a simple indication that these essential nutrients can be improved using induced mutation, while the progressive increase with increase in consecration level shows that the maximum level has not been attained. The other mineral compositions: Nitrogen, Calcium, Potassium, Sodium and Phosphorous which were not significantly affected might be as a result of level of concentration or genetic makeup. The increasing effect of colchicine on magnesium and HCN showed that it can be used directly to develop resistance or tolerance in cassava for drought and easy adaption in the northern or arid regions of the county. Moreover, Hydro cyanide (HCN) is no longer a challenge in cassava utilization, since higher percentage of cassava produced is used industrially. Hence, to increase the magnesium content of cassava using colchicine, concentration level 2 gives a better result.

Conclusion

The evaluation of the effects of colchicine treatment of cassava has shown that its use can be effective in enhanced accumulation of crude fibre, an important food trait, and increase in some key minerals (Mg, Fe and Zn) and phyto-chemicals (alkaloids and phenols) (Akimbo, 2008). The tendency of colchicine treatment of cassava leading to increased content of hydrogen cyanide content (HCN) is a negative occurrence since the reduction of HCN in cassava roots is an important breeding objective in most cassava improvement programs. Understanding the genetic correlation of some of these food and nutritional traits with HCN may help in deploying an appropriate breeding strategy to reduce HCN while increasing the alleles of the favorable traits.

References

Abraham, A., Picnicker, P.K.S. and Matthew, P.M. (1964). Polyploidy Relation to Breeding in Tuber Corps. *Ind. Bot. Soc. 43: 278-83. city of America*. Oct.12-19 2011, San Antonio TX: USA. absorption relative to casein. *J. Bioequiv. Availab,* 4: 040-043.

Adepoju, O.T. and Nwangwu, J.O. (2010), Nutrient composition and contribution of noodles (*abacha*) and local salad from cassava *(Manihot spp)* to nutrient intake of Nigerian consumers. *Afr. J. Food Sci.,* 4: 422-426.

Akimbo, O. A. (2008). Introgression of High protein and Pest Resistance Genes from Inter-specific Hybrids of maihot esculenta SPP flabellifolia into cassava (manihotes culenta crants):Thesis plant science Natural and Agricultural Science Unit of the Free State abloemfontein. P. 263.

Amanze, N.J., Eke-Okoro O. N., Ikeorgo, J.G. and Ohaneje, T. (2010). Influence of Variety, Micro–nutrient and Growth Hormone on some Reproductive and Yield parameters of Yam, proc. Agricultural Society of Nigeria: October 16th-20th 2006. Pp. 374-3.

AOAC (1990). Official Methods of Analysis, 15th Ed., Association of Official Analytical Chemists, Washington, DC.

Charles, A.L., Sriroth, K. and Huang, T.C. (2005). Proximate Composition, Mineral Contents, Hydrogen cyanide and Phytic acid of 5 cassava genotypes. *Food Chemistry,* 92: 615–20.

Enidiok, S. E., Attah, L. E. and Otuechere, C. A. (2008). Evaluation of Moisture, Total Cyanide and Fiber Contents of Garri Produced from Cassava (*Manihot utilissima*) Varieties Obtained from Awassa in Southern Ethiopia. *Pakistan Journal of Nutrition*, **7**(5): 625-629.

Ihekoronye, A.I. and Ngoddy, P.O. (1985). Integrated Food Science and Technology for the Topics. Macmillan Publishers ltd, London. Pp. 65-193,283-294

Jain, S.M. (2010). Mutagenesis in crop improvement under the climate change. *Rom Biotechnol Lett.,* 15(2):88–106.

Li, X., Yang, J., Manary, M. and Hirschi, K.D. (2012). Biotechnologically-modified cassava: Protein absorption relative to casein. *J. Bioequiv. Availab.,* 4: 040-043.

Mbanaso, E.N.A., Nwanchukwu, E.C., Egesi C.N., Chukwu L. I., Nwoko S.U. and Ogbonye P. (2006). Induction of Flowering and Fruiting in Cocoyam using Various Concentration of Gibberellic Acid(GA3). NRCRI Annual Report, (2005. Pp. 153-156.

Okalebo, J. R., Gathua, K. W. and Woomer, P. L. (2002). *Laboratory Methods of Soil and Plant Analysis: AWorking Manual.* (2nd ed.). TSBF-CIAT and Sacred Africa, Nairobi.

Oyeleke, O.A. (1984). Outlines of Food Analysis, Department of Biochemistry Ahmadu Bello University, Zaria, Nigeria.

Salcedo, A., Valle A.D., Sanchez B., Ocasio V., Ortiz A., Marquez P. and Siritunga D. (2010). Comparative evaluation of physiological post-harvest root deterioration of 25 cassava (*Manihot esculenta*) accessions: visual vs. hydroxycoumarins fluorescent accumulation analysis. *African Journal of Agricultural Research,* 5: 3138-3144.

SAS (1999). Statistical Analytical Systems/ STAT User's Guide Version 8(2) carry NC: SAS Institute inc., 1999. *Statistics.* Rome, Italy.

Udeh, S. (2011). A multivitamin may not be the best choice, unless you choose the right one: Issue No 46 of Healthy Eating Magazine, pp11 published by Healthy media communication.

Tonukari, N.J. (2004). Cassava and the future of starch**.** *Electronic Journal of Biotechnology*, **7**(1): 5-8

Ye, Y. M., Tong, J., Shi, X.P., Yuan,W. and Li. G.R. (2010). Morphological and cytological studies of diploid and colchicine-induced tetraploid lines of crape myrtle (*Lagerstroemia indica* L.). *Scientia Horticulturae***,** 124: 95–101.