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Agronomic Biofortification of Cassava (*Manihot esculenta* Crantz) as influenced by Rate and Time of Iodine Fertilization in Southeastern Nigeria

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ABSTRACT:

Cassava (*Manihot esculenta* Crantz) being a staple food crop for millions of people particularly in sub Sahara Africa is considered to have a high potential for biofortification with micronutrients, but research information on how fertilization with iodine influences its growth an yield, as well as accumulation of iodine in the edible parts of the crop is scanty. Field trials were therefore carried out for two seasons (2012 and 2013), in Calabar (latitude $05^{o}3^{'}$ and $04^{o}27^{'}$ North and longitude $07^{o}15^{'}$ and $09^{o}28^{'}$ East), Southeastern Nigeria, to investigate the growth, yield, and iodine content of fresh cassava tubers, and gari (fried/roasted dehydrated cassava flour) as affected by rate and time of soil-applied potassium iodide. 20 cm-long stem cuttings of the cassava (varieties TME 419 and TMS 30555) were planted horizontally on 4.0 × 4.0 m, manually prepared, flat-tilled plots, at 1.0 ×

1.0 m spacing, and at one cutting per stand. The experiment was a split-split-plot laid out in randomized complete block design replicated thrice, with cassava variety, rate, and time of fertilization constituting the main-, sub-, and sub-sub- plots, respectively. Treatments were factorial combinations of the two cassava varieties, five rates of potassium iodide (0, 1.5, 2.0, 2.5, 3.0 kg/ha) and three application times (8, 10, 12 weeks after planting). The growth and yield of cassava was not significantly influenced by soil-applied iodine ($P > 0.05$), but all cases of applied iodine resulted in a significantly higher concentration of the element in the tubers, and in gari, relative to the unfertilized control, irrespective of variety planted. Incremental rates of fertilization induce progressively higher levels of iodine accumulation, the highest being at the rate of 3.0 kg KI/ha. However, the iodide concentration in the fresh root tubers, or in gari at this concentration did not differ significantly from the lower rates of 2.0 or 2.5 kg KI/ha. Most of the iodine accumulated when fertilization was at 10 WAP, with the combination of 2.0 kg/ha at 10 WAP, followed closely by 1.5 kg/ha at 10 WAP, giving the best results. It would appear that the accumulation began just before the onset of tuberization and peaked at the early stages of bulking. The concentration of iodine in gari was high and similar to that in the fresh root tubers, indicating that biochemical changes arising from fermentation as well as exposure to high temperatures during frying did not reduce the iodine content of gari. The high iodine content in gari suggests that it is potentially bioavailable to consumers.

Keywords: Cassava variety, Iodine, Agronomic biofortification, Time of application, Rate of application.

INTRODUCTION

Mineral malnutrition is a global challenge to mankind which can be addressed through dietary diversification, mineral supplementation, food fortification and/or increasing mineral concentration in edible crops (i.e. biofortification) (Welch and Graham, 2000; Graham, 2003; White and Broadley, 2009). Given that strategies to increase dietary diversification, mineral supplementation and food fortification have not always been successful, it has been proposed that biofortification of crops through the application of mineral fertilizers, combined with breeding varieties with an increased ability to acquire mineral elements could be an immediate strategy to increase mineral concentration in edible crops (Graham *et al*., 2001; Bouis *et al.*, 2003; Genc *et al*., 2005; White and Broadley, 2005). Of the five most prevalent micronutrient deficiencies, zinc, selenium, and iodine are mobile in the soil and suitable as fertilizers, while iron and vitamin A can only be meaningfully improved by plant breeding/biotechnology (Graham *et al*. 2001).

Iodine is a trace element essential to the health of humans and livestock, as an important constituent of the thyroid hormones, thyroxine and triiodothyronine, both of which play biological roles in growth and development (Hetzel and Maberly, 1986). When the thyroid gland malfunctions or when it receives inadequate iodine, it results in a wide range of physiological abnormalities collectively referred to as Iodine Deficiency Disorders (IDD) (Fernando *et al*., 1987). This results in developmental disability, mental retardation, cognitive impairment, increased infant mortality and hypothyroidism (FAO, 2004). In Nigeria, about 38.8 million of school age children are with insufficient iodine intake (Benoist *et al.,* 2004), and Cross River State falls within the goiter-endemic or 'goiter belt' of the country (NDHS, 2003).

Except for sea foods, iodine does not occur naturally in staples, its concentration in staples depending on that of the soils in which the crops are grown (Hetzel, 1986). This implies that food grown on iodine-deficient soils may not provide adequate amounts of the element to those who consume it. At present, dietary supplementation based on the addition of iodized salt is the strategy for correcting iodine deficiency in the diet of Nigerians, but studies in Cross River State, have shown that over 70 % of school-age children are deficient in iodine, and more than 25 % of households consume salts with inadequate iodine concentration (Abua *et al*., 2008).

Iodine is present in soils as iodide, iodate and organic iodine compounds. Little is present in the soil solution and most soil iodine is associated with organic matter, clays and oxides of iron and aluminum (Fuge and Johnson, 1986). The prevalent form of iodine in the soil solution is iodide (I^-), but iodate may also occur depending upon pH and redox conditions, and it is thought that plant root cells take up iodine as the iodide anion (Umaly an Poel, 1971; Mackowiak and Grossl, 1999; Zhu *et al*. 2003; Blasco *et al*. 2008). The iodine follow the chloride (Cl^-) transport pathway with H^+/anion symporters catalyzing I^- uptake and anion releasing I^- into the xylem (White and Broadley, 2001).

Although iodine is not a plant nutrient (Ohira *et* al., 1973; Udoh and Ndon, 2016), and quite often regarded as being harmful to plants, studies on its effect on plant growth and yield have shown mixed results. It is however, generally agreed that the form (iodide or iodate), mode of application (foliar or soil) and concentration of the element determines whether it will have a harmful or a beneficial effect (Borst Pauwels, 1961; Mackowiak and Grossl, 1999; Dai *et al.* 2004; Lawson *et al.*, 2015). Lehr *et al.*, (1958) found that in soil culture, iodine applied as iodide improved fruit yield of tomatoes by increasing the number and weight of fruits, while Borst Pauwels (1961) showed that various crops react to minute applications of iodide or iodate. The vegetative growth of spinach, white clover, fodderbeet tomatoes, perennial ryegrass, turnips (aerial parts), barley, flax, wheat and mustard was positively influenced by iodine, but development of oats and to a lesser extent of the roots of turnip was hindered by all the rates of application used. This worker concluded that in general, iodate had a more favorable effect on growth than iodide, particularly in the initial stages of development, due in part to the fact that plants absorb iodate more slowly than iodine, since during early life plants are highly sensitive to an over dosage of iodine. Portianko and Kudria (1966) observed stimulation of pollen germination by iodine while Pais and Jones (1997) reported "stimulatory effects" on plants with the application of iodine at a concentration of 0.1 mg L^{-1}. However, Barker and Mapson (1966) observed negative influence of iodine acetate on respiration and carbohydrate metabolism of plant tissue, while Mackowiak and Grossl (1999) reported detrimental effect of iodine on biomass yields. Fertilizer iodine readily accumulates in roots and leaves (Mackowiak and Grossl, 1999; Zhu *et al*., 2003), but little is distributed via the phloem to other parts of the plant (Muramatsu *et al*., 1995).

It has been reported that the iodine concentrations in root crops and leafy vegetables can be increased greatly by the application of iodine fertilizers, and although iodine is not readily mobile in the phloem, its concentration in tubers, fruits and seeds can also be increased by fertilization to nutritionally significant concentrations (Jiang *et al.,* 1997; Rengel *et al.*, 1999; Dai *et al.*, 2004). Because cassava (*Manihot esculentus* Crantz) is an important component in the diets of more than 800 million people in tropical and sub-tropical Africa, Asia and Latin America (FAO, 2007), it is considered to have a high potential for biofortification. Cassava is regarded as a food security crop due to its inherent tolerance to stressful environments (Barrat *et al.* 2006), and is cultivated mainly by resource-limited farmers for its starchy roots which can be processed into several different products including gari, flour, bread and starch (James *et al.,* 2012). Estimates of the Food and Agriculture Organization of the United Nations (FAOSTAT, 2011) put world production of cassava at more than 230 million metric tonnes annually, with Nigeria, Brazil and Thailand being the largest producers.

When cassava is propagated by stem cuttings, adventitious roots arise from the basal cut surface of the stake and occasionally from the buds under the soil. These roots develop into fibrous roots and only a few subsequently bulk to become storage roots, while the others remain thin and continue to function in water and nutrient absorption (Alves *et al*., 2002). Once a fibrous root becomes a storage root, its ability to absorb water and nutrients decrease considerably. The development of storage roots begins with secondary growth in several fibrous roots, and starch deposition has been observed to occur at about 25-40 days after planting in many cultivars (Cock, 1984), but visible root bulking is noticeable only when root thickness is about 5 mm or more, normally reached 2-4 months after planting (El-Sharkawy, 2004).

On the basis of pot experiments, Ansa *et al*. (2016) reported that cassava varieties differ in their response to soil-applied iodine and the application of potassium iodide at the rate of 2.5 kg/ha was safe for agronomic fortification of the crop in Southeastern Nigeria. However, given that plants grown in a confined soil body (pots) often respond to higher rates of fertilization, this field study sought to determine the response of rainfed cassava to various iodine rates, applied at different stages of plant growth.

Materials and Methods

A field study was conducted in two seasons (August, 2012 to May, 2014) at the Teaching and Research Farms of the University of Calabar, Calabar, (latitude $05^{o}3^{'}$ and $04^{o}27^{'}$ North and longitude $07^{o}15^{'}$ and $09^{o}28^{'}$ East), Southeastern Nigeria. Calabar falls within the tropical rainforest climatic zone with typical primary vegetation and mostly secondary forest regrowth vegetation. Rainfall in the area is bi-modal with peaks in June and September and with a short dry spell in August, and averages 1900 mm – 3500 mm. The relative humidity is 80 – 91%, average annual temperature of 25 – 32.5^{o} C, and a daily sunshine of 4-6 hours of sunshine (Afangide *et al.* 2010; Nwajiuba and Oyenike 2010).

20 cm-long stem cuttings of the cassava varieties TME 419 and TMS 30555 were planted horizontally on 4.0 × 4.0 m, manually prepared, flat-tilled plots, at 1.0 × 1.0 m spacing, and at one cutting per stand, in the first week of August, 2012, and repeated in 2013. Treatments were factorial combinations of the two cassava varieties (TME 419, TMS 30555), five rates of fertilization with potassium iodide (0, 1.5, 2.0, 2.5, 3.0 kg/ha) and three application times (8, 10, 12 weeks after

planting}. The experiment was a split-split-plot laid out in randomized complete block design replicated thrice, with cassava variety, rate, and time of fertilization constituting the main-, sub-, and sub-sub- plots, respectively. Potassium iodide was thoroughly mixed with 200kg/ha NPK 15-15-15 and applied in bands as per treatment. . Weeds were controlled by hoeing and hand pulling, twice at 4 – and 8 – WAP, while 'earthing up' to cover exposed tubers was routinely done.

Random soil samples taken with auger at a depth of 15 -30 cm at the start and conclusion of the experiment were composited, sieved with 2 mm mesh and analyzed for physico-chemical properties. Soil pH was determined by the glass electrode meter in 1:1 soil: water suspension; organic carbon by the dichromate wet oxidation method; total nitrogen by Kjeldhal digestion and distillation method (Udo and Ogunwale, 1978); available P by the Bray P1 method (Bray and Kurtz, 1945); exchangeable bases by the flame photometer method, and the particle size distribution by Bouyoucos (1951) method. Soil, cassava tuber, and processed cassava (Gari) samples were analyzed for iodine content using the method of Yamada *et al.,* (1996).

Data Collection

Harvesting of cassava tubers was at 12 months after planting (i.e. 48 WAP). Plant height was measured as the vertical height from the ground to the top of the canopy; number and weight of root tubers was taken as the roots with length greater than 20 cm (Fukuda *et al.,* 2010), weighed with an electronic weighing scale, while harvest index was computed as weight of roots divided by weight of roots plus weight of aboveground biomass (Kawano, 1990).

The harvested tubers were processed into 'Gari' (James *et al*., 2012), and the iodine content was then determined. Data were analyzed using the statistical software GenStat 10.3 DE Release (2011), and comparison of means was by Least Significant Difference (LSD) at 5 % level of probability.

Results and Discussion

Physical and Chemical Composition of Soil at the Study Site

The soil was an acid, sandy loam, low in total nitrogen content, and available phosphorus. It contained moderate amounts of organic matter, exchangeable potassium and magnesium, and exchangeable cations (Table 1). The native soil iodide concentration was high (119.6 mg/kg) and applied fertilizer iodine increased it to 135.2 mg/kg the following year. Although the fertility of the soil was low, it was adequate for cultivation of cassava which does best in deep, fertile, well-drained light-to-medium-textured soils, but is tolerant of poor soils, and can give economically adequate yields on soils that are too poor for other crops (Udoh and Ndon, 2016).

Under acid conditions, exchangeable Fe and Al^{3+} come into solution and may become phytotoxic and/or cause the deficiency of such nutrients as phosphorus (Opara-Nadi, 1988), as well as form complexes with iodine thereby rendering it unavailable to plants (Fuge and Johnson, 1986).

Table 1: Physico-chemical properties of the soil at the site of study

Property	Year	
Physical (%)	2012	2013
Sand	78.4	79.6
Silt	19.4	19.6
Clay	0.81	0.80
Textural class	Sandy loam	Sandy loam
Chemical		
pH (H_20)	5.4	5.4
Organic matter (%)	1.81	1.74
Total nitrogen (%)	0.14	0.16
Available P (mg/kg)	6.0	5.8
Extractable Fe (mg/kg)	108.6	137.0
Soluble I (mg/kg)	119.6 ±4.17	135.2 ±8.65
Exchangeable bases (meq/100 g soil)		
Calcium	1.30	1.25
Potassium	0.25	0.22
Magnesium	1.61	1.66
Sodium	0.05	0.10
ECEC	9.82	9.85

Effect of Iodine fertilization on growth and yield of Cassava

Soil-applied Iodine, at the various times and concentrations evaluated, did not significantly affect the growth and yield of the two cassava varieties studied, as differences in plant height, harvest index, and number and weight of tubers per plant were not statistically significant (Table 2). Similarly, the interaction effect of cassava genotype with time of fertilization applying iodine was similarly not statistically significant (Table 3). Although trace amounts of iodine could be beneficial to plants, high soil concentrations inevitably results to toxicity, irrespective of growth stage, but especially at the early plant stages of growth. Mackowiak and Grossl (1999) reported no effect on rice biomass yields with application of 0.1 and 1.0 $mg/kg^{-1}L$ iodate, but a small negative effect was recorded when the iodate concentration was increased to 10.0 $mg/kg^{-1}L$. The iodide at 1.0 and 10.0 $mg/kg^{-1}L$ was detrimental to biomass yields. Similarly, Zhu *et* al. (2003) found that a high concentration of iodide (≥ 10 µg M) had detrimental effects on plant growth, while iodate had little effect on the biomass production of spinach plants. In lettuce (*Lactuca sativa* L.), Voogt *et al.* (2010) found that different forms and various rates of soil-applied iodine did not affect plant biomass while Lawson *et al.* (2015) working with lettuce obtained or exceeded the desired iodine content {50-100 µg I $(100 \text{ g FM})^{-1}$} at a fertilizer rate of 7.5 kg IO_3^- without significant yield reduction or impairment of marketable quality. In contrast, supplying KI at the same rate resulted in a much lower iodine enrichment and clearly visible growth impairment.

Table 2: growth and yield of cassava as influenced by fertilization with potassium iodide

Treatment	Plant height (cm)	Tubers/plant (no)	Tuber weight (kg/plant)	Harvest index
Variety				
TME419	183.4	5.8	6.1	0.67
TMS30555	175.9	5.4	5.3	0.58
LSD(0.05)	Ns	Ns	Ns	Ns
Rate (kg/ha)				
0	177.7	6.1	5.6	0.61
1.5	188.2	5.1	5.3	0.54
2.0	183.9	5.3	5.8	0.57
2.5	176.2	5.7	5.6	0.60
3.0	183.0	5.7	5.9	0.59
LSD(0.05)	Ns	Ns	Ns	Ns
Time(WAP)				
8	190.4	5.9	5.6	0.55
10	188.5	5.8	5.9	0.63
12	186.0	5.8	6.0	0.59
LSD(0.05)	Ns	Ns	Ns	Ns
Interaction	Ns	Ns	Ns	Ns

Effect of iodine fertilization on accumulation in cassava root tubers and in Gari

Fertilizing cassava with potassium iodide induced significant levels of accumulation of iodine in the root tubers, as well as in gari processed from them, this being significantly influence by the rate an time of fertilization, but not by the variety of cassava cultivated (Table 3). All cases of applied iodine resulted in a significantly higher concentration of the element in the tubers, relative to the unfertilized control, irrespective of variety planted, with incremental rates of fertilization inducing progressively higher levels of iodine accumulation, the highest being at the rate of 3.0 kg KI/ha. However, the iodide concentration in the fresh root tubers, or in gari at this concentration did not differ significantly from the lower rates of 2.0 or 2.5 kg KI/ha (Table 3). Similarly, significantly higher levels of iodine accumulation in the edible parts of the plant occurred when fertilization was at 10 WAP, with the combination of 2.0 kg/ha at 10 WAP, followed closely by 1.5 kg/ha at 10 WAP, giving the best results (Table 4). It would appear that iodine accumulation in tubers occurred shortly after the onset of tuberization, but peaked during the early stages of bulking. Given that very little of the fertilizer iodine absorbed by roots is translocate to other parts of the plant (Marumatsu *et al*., 1995), root tubers may themselves have been involve in iodine absorption.

The gari iodine content was similar to that in cassava root tubers from whence it was obtained (Fig. 1). This indicates that biochemical changes due to fermentation and exposure to high temperatures during frying/roasting of grated fresh cassava tubers (James *et al*., 2012), did not significantly reduce the iodine concentration in gari. The high iodine concentration in gari might suggest that it is potentially bioavailable to consumers, which is in agreement with the conclusion of Jiang *et* al. (1997), Camak (2004), and Rayman (2008), that the application of mineral fertilizers containing iodine can have a significant impact on the nutritional status of vulnerable populations. However, it would be necessary to conduct feeing trials in order to confirm this assertion, and determine the cost effectiveness of this strategy, before recommendations can be made. Nevertheless, the concentration of iodine found in cassava in this study was similar to that in beans and maize samples sourced from markets in the North Central Nigerian States of Nasarawa and Plateau (Etonihu *et al*. (2011).

Reports by Zhu *et al.* (2003), found that increases in iodine concentration in the growth medium significantly enhanced iodine concentration in plant tissues of spinach, while Lawson *et al*. (2015) obtained increased iodine accumulation in the edible parts of field-grown lettuce and kohlrabi with soil or foliar iodine fertilization. Similarly, Ujowundu *et al.* (2010) reported increased iodine uptake by fluted pumpkin (*Telfairia occidentalis*) and water leaf (*Talinum triangulare*) with soil-applied iodine, and with iodination of irrigation water, while Landini, *et al*. (2011) found in tomato that iodine was taken up better when supplied to the roots using hydroponically grown plants.

Table 3: iodine concentration of two cassava varieties as influenced by time and rate of fertilization with potassium iodide

Treatment	Root tuber	Gari
Cassava variety		
TME419	83.6	76.9
TMS30555	77.4	74.2
LSD(0.05)	Ns	Ns
Rate of fertilization (kg/ha)		
0	22.6	22.6
1.5	68.4	68.0
2.0	75.0	74.8
2.5	82.7	82.3
3.0	85.0	85.0
LSD(0.05)	4.1	4.1
Time of fertilization (WAP)		
8	78.6	77.5
10	86.2	87.0
12	80.9	79.4
LSD(0.05)	3.5	3.3

Table 4: interactive effect of time and rate of iodine fertilization on fresh tuber iodine content (mg/kg)

Rate (kg/ha)	Time (WAP)			
	8	**10**	**12**	**Mean**
0	**12.6**	**12.6**	**22.9**	**22.9**
1.5	**88.6**	**94.1**	**92.0**	**91.6**
2.0	**90.2**	**95.7**	**91.4**	**92.3**
2.5	**90.8**	**93.7**	**90.2**	**91.6**
3.0	**90.5**	**93.9**	**91.2**	**91.9**
Mean	**76.6**	**80.1**	**77.5**	
LSD (0.05)	**1.3**			

Figure 1. Iodine content of two cassava varieties as influence by time and rate of fertilizing with potassium iodide.
Key: 419 = TME 419, 30555 = TMS 30555, K = Kg/ha, W = Weeks after planting

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