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Bio-fortification of Quality Protein Maize (Zea *mays* L.) with Beta Carotene

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Authors' contributions

This work was carried out in collaboration among all authors. Author DOI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KB and JEU managed the analyses of the study. Author ASA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Alike other cereals, maize is deficient in certain essential amino acids such as lysine and tryptophan. While lysine is critical in protein synthesis for the growth of tissues and important in the absorption of calcium from the intestinal mucosa, tryptophan is the biological precursor of B-vitamin and niacin. Increasing protein quality alone may have a displacement effect on the efficiency of provitamin A, carotenoid bio-conversion and utilization. The research was designed to improve the quality protein maize with beta carotene in order to further enhance its nutritional content by hybridization, through the conventional method of breeding, using randomize complete block design (RCBD). Materials used were quality protein maize (white endosperm) genotype and yellow (rich in beta carotene) maize. Pollination was manually conducted and controlled, to exclude the possibility of unwanted pollination. Harvested seeds indicated a successful cross of both varieties as seen in the pigmentation of the hybridized maize seeds, which appeared pale yellow. However, the two varieties and their hybrid seeds harvested were subjected to laboratory analysis to compare their nutritional content. All the data collected were subject to statistical analysis using SPSS and mean separated by DMRT at 5% probability level. The result shows that individual

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varieties were higher in crude protein (13.5%) for yellow maize and Quality protein maize (12.9%), than the hybrid QYM (11.9%). Similarly, hybrid maize seed had higher percentage of lysine (1.37%), tryptophan (0.54%) and methionine (0.85%) than the individual varieties. Also the ear diameter correlated positively to 100 seeds weight.

Keywords: Bio-fortification; hybridization; pollination; protein; correlate.

1. INTRODUCTION

Maize is one of the most important crops used as food, feed and industrial applications [1,2]. In most parts of the world, Maize alone contributes over 20% of the total calories in human and livestock diets [2,3,4]. Globally, maize is cultivated in 184 million hectares with the global production of 1,016 million tons [5,6]. Maize taste has been easily accepted by the local population and therefore, it has been rapidly replacing traditional starchy foods like cassava (Pingali and Heisey, 1999). Importance of maize cannot be over-emphasized in the developing world, including its potential to mitigate the present food insecurity and reduce poverty. Maize is a preferred staple food for over 900 million poor consumers, 120-140 million poor farm families and about one third of malnourished children [7]. In sub-Saharan Africa, absence or shortage of maize invariably leads to famine and starvation. It is estimated that by 2025, maize would have become the crop with the greatest production in developing countries and the world, and by 2050, the demand for maize in developing countries will double [7]. Therefore, maize is a definite solution to hunger and it can salvage the famine population.

However, maize, alike other cereals, is deficient in certain essential amino acids, such as lysine and tryptophan [1,6] (Lauderdale, 2000). Unlike local maize varieties which is the major staple food and feed for human and domesticated animals, quality protein maize is not commonly cultivated in the developing countries [8]. According to Onimisi [9] Lysine is critical in protein synthesis for the growth of tissues and found to be important in the absorption of calcium from the intestinal mucosa. On the other hand, Tryptophan being an essential amino acid is the biological precursor of the B-vitamin, niacin. Generally, efforts to improve protein quality in maize began in mid-1960s with the discovery of mutants (opaque 2 genes) that produce enhanced levels of lysine and tryptophan [1,3,4,10,11]. Finally, this mutant genes through conventional breeding work by scientists from International Maize and Wheat Improvement Centre (CIMMYT) developed a derivative of opaque-2 maize called Quality Protein Maize (QPM) which has hard endosperm, maize variety with similar yield to normal maize and nutritionally enhanced [12,13].

Future directions for quality protein maize (QPM) efforts include: developing yellow and orange varieties for dissemination, which contain higher levels of beta-carotene and other carotenoids. QPM varieties are being crossed with high carotenoid varieties and traditionally bred to contain an enhanced overall nutritional profile [13]. Some researchers maintain that increasing protein quality alone may have a displacement effect on the efficiency of provitamin A, carotenoid bioconversion and utilization [14]. Therefore, yellow or orange QPM would have an even greater impact on health and nutrition for target countries such as Africa. Some of the reasons why the importance of maize cannot be over-emphasized in the developing world; are due to the fact that maize production inputs is very high compared to other cereals. Therefore, this project was initiated to review the conventional means of improving QPM with beta-carotene using the yellow maize to cross the QPM. The outputs of the researched work enable conclusions and recommendations to exploit the potential of quality protein maize as food, feed and industrial raw materials mainly for Nigerians and other developing countries farmers who relied mainly on maize for their source of energy and protein.

Malnutrition is a persistent problem in Africa, especially in rural areas where poor people depend on staple foods and have limited access to a diverse diet. Therefore, this research was designed to explore the conventional means of improving QPM with beta-carotene using the yellow, to exploit the potential of quality protein maize as food, feed and industrial raw materials mainly for Nigerians and other developing countries farmers whose livelihood is dependent largely on maize and feed processors. The research is aimed at assessment and comparison of agronomic performance and yield of quality protein maize, yellow maize and the cross of yellow maize (YM) and bio-fortified quality protein maize (QPM).

2. MATERIALS AND METHODS

The research work, conducted at the Teaching and Research Farm of the Federal University Wuakri during the 2018/2019 farming seasons. The farm was situated on latitude 7º52'17.00ºN and longitude 9º46'40.30ºE. It falls within the guinea savannah agro-ecology of Nigeria, with annual rainfall of 1058 mm-1300 mm and the relative humidity dropping to about 15% alongside with the annual temperature of 28ºC and 30ºC.

2.1 Experimental Design

The experimental material consisted of two varieties of maize; improved yellow maize variety (premium maize seed) and quality protein maize (foundation seeds from the Department Crop Production and Protection, Faculty of Agriculture Federal University Wukari, Taraba State). The experiment was laid out in Randomized Complete Block Design (RCBD), with a gross area of 405 m^2 which was divided into three blocks of 135 m^2 and each block was further divided into six plots of 22.5 m^2. Seeds were sown at two per hole, with a planting distance of 25 cm × 50 cm intra-row and inter-row spacing.

2.2 Cultural Practices

At the course of the experiment, the following cultural practices were duly observed, to obtain high yield; land clearing, land preparation (in ridges), sowing (two seeds per hole), thinning, earthen-up, fertilizer application (side placement, of NPK 15:15:15 at 2WAP and UREA at 6WAP respectively), weed control using hand hoe (two and five) weeks after sowing and pests were controled using lambda-cyhalothrn 15 g/l + Dimethoate 300 g/l which is systemic in action.

2.3 Data Collection

The following data were collected at different stages of growth and after harvest;

Days to tasseling (DTT): Number of days from sowing to emergence of the tassels
Days to silking (DTS): Number of days taken to the visible expression of silk
Height at maturity: Measured in centimeter (cm) using meter rule
Ear height: Measured in centimeter (cm) using meter rule
Width of ear leaf: Was measured using measuring tape, in centimeter (cm)
Length of ear leaf: Measured in centimeter (cm) using measuring tape.
Ear length: Measured in centimeter (cm) using meter rule.
Ear diameter: Obtained, by dividing value for ear circumference by 2. The circumference was measured in centimeter, using the tape rule.
Ear weight: measured in gram (g) using sensitive weighing scale.
Ear insertion angle: measured in degree (^{0}) using protractor
Weight with husk: measured in gram (g) using sensitive scale.
Weight without husk: measured in gram (g) using sensitive scale
Weight of 100 seeds: measured in gram (g) using sensitive scale.
Number of nodes: all the nodes in the stand were counted and recorded.
Length of internodes: measured in centimeter using the meter rule
Number of tassel branches: a count of tassel number of branches was done and recorded.

2.4 Tagging

In other to have uniformity in the work without biasness in obtaining the needed information of the experiment, five plant stands were randomly picked and tagged per plots using numeric tagging method and attached to the stem with the help of a masking tape, for easy identification in all the replication.

2.5 Ear Bagging for Control Pollination

Ear shoot bag was manipulated or improvised to cover or bag the newly shooting ear, for preventing the undesirable pollination due to actions of insects and wind unwanted sources. As soon as the maize plant started coming up with tassel (tasseling), a proper inspection of the field was carried out in other to cover any ear shoot that has shootout, and it was covered firmly in other to prevent falling off during wind.

2.6 Tassel Bagging and Pollen Grains Formation

In order to obtain pollen grains for pollination, the tassel bag was also improvised and was used in covering the tassel by inserting the tassel inside the readily provided tassel bag and base of the tassel was fold from each corner and staple at the tassel peduncle to hold it in place. This

operation was normally done in the morning before breezing. To collect the pollen grain, the bagged tassel was bend and one hand holding the plant to bend and remove the clip and tassel was gently and removed from the bag that was used and the anther that fall into the bag was removed leaving only the pollen grain, which is in pale yellow colour.

2.7 Pollination

After the collection of pollen grain, the ear was covered to prevent unwanted pollination. The ear shoot bag was removed from the ear as the silk is already developed and ready to receive pollen for fertilization of the ovule. To have the pollen get into the ear properly, the elongated silk was cut away using scissor and the space was created artificially at the tip region of the ear, and the collected pollen grain was poured into the silk through the space that was made artificially. Appropriate measure was taken to ensure that the pollen get into the silk, having all of them pollinated. This was done by ensuring all the silk were touched by pollen grains that was poured into the silk. The pollination was done very quickly so as to cover it back since the silk is still receptive.

2.8 Laboratory Analysis

Harvested seeds were analysed (Proximate Analysis and Amino Acids Profile) to compare the nutritional values of the hybrid with the two varieties used for the experiment.

2.9 Procedures for Proximate Analysis

Moisture: The sample was mixed thoroughly. And the moisture content was determined by weighing 2 g of feeding stuff with a silica dish which has been previously ignited and weighed. Genlab MINO/30 UK was dried in an oven for 24 hours at 100ºC to constant weight. Thereafter it was allowed to cool in a desiccator before weighing.

Calculation:

% Moisture = 100 x (wt of dish + feedstuff Before Drying)–(wt of dish + Feedstuff after Drying)/ wt. of Feed stuff taken

Ash: A silica dish was heated at 600ºC, cooled in desiccators and weighed. 3 g of dried, milled feeding stuff was transferred into the dish. Weigh dish and feeding stuff. Dish and feedstuff was taken to a heater in a fume cupboard to burn off the less volatile organic materials. This is pre-ashing. Stop when smoking stops. The dish was place in a cool muffle furnace Vecstar ECF3, UK. The temperature of the furnace was increased to 600°C. And maintain this temperature until whitish-ash remains. The dish was placed in a desiccator, and allowed to cool before weighing it.

Calculation

% Ash = wt. of dish + ash – wt. of dish x 100/ wt. of feeding stuff used

Protein (Nitrogen) determination (Kjeldahl method): Nitrogen in the sample was converted to ammonium-nitrogen by digestion with sulphuric acid using a catalyst. The ammonia librated when this digest is reacted with sodium hydroxide is removed by steam distillation and collected with 1% Boric Acid-indicator mixture. This is then titrated with O.OIN HCI to give % Nitrogen in the sample.

2.10 Preparation of Sample

The sample was grind to small particles (to pass a sieve of 1mm mesh).

Appearatus

i. kjeldahl flask – 300 ml
ii. Macro kjeldahl distillation unit Markham 230 foss usa

Reagents

i. Sulphuric acid conc 90%
ii. Kjeldahl catalyst tabs – 3 tabs or mixture of sodium sulphate
iii. And cooper sulphate in the ratio of 3.1,
iv. 4 gms of mixture is to be used.

2.11 Digestion Procedure

2 g of dried sample was transfered to a kjeldahl flask and catalyst tablets or 4 gms mixture of $Na_2SO_4/CuS0_4$ was added, 25 – 30 mls concentrated sulphuric acid was also added. After wish it was Swirl gently and taken to the heater. Which was heated gently at first untiled frothing stops; after that it was heated more strongly until a near clear solution was achieved. It was allowed to cool and digest quantitative was transfered into a 250 ml volumetric flask.

2.12 Determination of Ammonium Nitrogen

Apparatus

Distillation unit, Markham 230 macro distillation type foss usa

Reagents

i. Boric acid solution
ii. Methyl red-methylene blue: dissolve 1.25 gm of methyl red
iii. And 0.825% of methylene value in 1 litre of ethanol 90% v/v
iv. Sodium hydroxide 50-60%
v. Hydrochloric acid 0.oin/m

2.13 Distillation

The distillation apparatus was steam out for about 10 mins. While this was going on the volume of the digest up was mark. The flask was shaked properly and 25 mls pipette of sample digest was put into Kjeldahl flask and mixed with 25 mls of 40% sodium hydroxide solution the mixture was mounnted unto the diastillation unit, and heated with constant flow of water and the liberated ammonia collected with 10 ml boric acid-indicator mixture in a conical flask placed at the condenser and of the markham distillation unit. When the boric acid-indicater mixture turns green, the distillation was allowed to go on for another 5 mins.

At the end of the time, the conical flask was removed and the content was titrated with O.OIN Hydrochloric acid until the original colour of the boric acid-indicator mixture is restored.

Calculation

% n = 0.00014 x titre x 50 x 100/Wt. of sample taken

% Protein = % n x 6.25

Determination of Fat in Feeds: Oil was extracted from sample with petroleum spirit under controlled conditions.

2.14 Determination of Oil

Apparatus

i. Extraction thimbles – Double thickness, 22 x 80mm.
ii. Flasks – 150 ml to fit Soxhlet extractors, flat bottomed.
iii. Heater unit for extraction – With a controller for each heating element.
iv. Soxhlet extractors – All glass, of size suitable for the thimbles, fitted with condensers.

Reagents

i. Cotton wool, oil-free
ii. Petroleum spirit- Boiling range 40-60ºC.

Procedure

The flask was dried in an oven at 100ºC, and allowed to cool in a desiccator and weighed. 3-5 g, weighed was transfered to the nearest mg. The sample was grind, and passes through a 1mm mesh sieved into a thimble and plug with cotton wool. The thimble was placed with its contents into the extractor. Extract with petroleum spirit for about 4 hr. The residue was transferred from the thimble to a small mortar and grind lightly then returned to the thimble, to the extraction apparatus. The mortar was washed with a small quantity of petroleum spirit. And the washings of the flask were added. Extraction continued for an hour. The thimble was removed and most of the solvent was distiled from the flask into the extractor. The flask was disconnected, and place in an oven at 100ºC for 2 hr, cooled and weighed.

Calculation:

The increase in weight was mutilied by 100 and divided by the weight of the sample taken. The result gives the percentage w/w of oil in the sample.

2.15 Determination of Fibre in Samples

Apparatus

i. Beakers – 600 ml,
ii. Borosilicate glass,
iii. Tall form without spouts, with round bottomed flasks fitted as condensers, or conical flasks – 500 ml,
iv. Borosilicate glass, with cold finger condensers.
v. Buchner funnels.
vi. Filter crucibles – 50 ml,
vii. Vitreosil, porosity No. 1.

Reagents

i. Alcohol – Industrial methylated spirit is suitable.

ii. Diethyl ether.
iii. Hydrochloric acid, approx. 0.1 M- Dilute 1 vol. of hydrochloric acid, approx. 36% w/w HCl, to 100 vol.
iv. MS Antifoam A solution, approx. 2% w/v in carbon tetrachloride.
v. Petroleum spirit, boiling range 40-60ºC.
vi. Sodium hydroxide, 0.313 M.
vii. Sulphuric acid, 0.128 M.

Procedure

The oil was removed from 2 g of sample, and grind to pass a 1 mm mesh sieve, by Soxhlet extraction, setting and decanting was done three times with petroleum spirit. The air-dried fat-free material was transfered into a flask. 100 ml, measured at room temperature, of TCA (Trichloroacetic acid) were added. Boiled by refluxing gently for 30 min, constant volume by the flow of water was maintained. 11 cm Whatman No. 541 filter paper into a Buchner funnel was fitted, at the end of the 30 min boiling period, the acid mixture was allowed to stand for approximately 1 min and pour into the prepared funnel. The insoluble matter was washed with boiling water until the washings are neutral to litmus paper. The filter paper and the residue was transfered into a crucible. The crucible was dried and its contents at 100ºC. And allowed to cool in a desiccator and weighed. The crucible was placed in a cool muffle furnace, and the temperature was increased to 500ºC, and maintained until ashing is completed. The crucible was removed from the muffle furnace, cooled in a desiccator and weighed.

Calculation:

The loss in weight on ignition was multiplied by 100. The result gives the percentage of fibre in the sample.

Carbohydrate by difference in feeds

%CHO=100%-(%moisture+%ash+%protein+%fat +%cf)

Procedures for Amino acids Profile

The defatted flours samples were utilized to estimate amino acids. The sample (30 mg) was hydrolyzed with 6N HCl at 110ºC for 24 h. Amino acid analysis was performed on reverse phase-high pressure liquid chromatography (HPLC) (Buck scientific BLC 10/11 USA).The post column samples were derivatised with o – phthaldialdehyde. Analysis was performed by injecting 20 ul of the prepared sample into the HPLC equipped with UV 338 nm detector. A C18, 2.5 x 200 mm, 5 um column and a mobile phase of 1:2:2 (100 mM sodium phosphate, pH 7.2: Acetonitrile: methanol) at a flow rate of 0.45 mL/minute and an operating temperature of 40ºc. A 0.1 mg/ of each mixed amino acid standards were analysed in a similar manner for identification. Peak identification was conducted by comparing the retention times of authentic standards and those obtained from the samples. Concentrations were calculated using a four point calibration curve and data were integrated using peak simple chromatography data system (Buck SCI. chromatopac data processor) [15].

3. RESULTS AND DISCUSSION

3.1 Descriptive Statistics of Vegetative Characters

There was no signicant different between the days of tasseling and silking in the yellow maize (YM), quality protein maize (QPM) varieties and quality protein maize x yellow maize (QYM) respectively as seen in Table 1. There is significant difference in the numbers of tassel branches with YM having the least value (4.25) and QYM having the highest value at 12.6. There exist no signicant difference in the height at maturity. Width of ear leaf is significantly different with the QPM having the highest values of 9.43 cm and the yellow maize having the least value (8.58).

3.2 Descriptive Statistic of Reproductive Traits

There exist no significant different in all the traits of QPM,YM and QYM except in the weight without husk were the QPM having the highest value at (142.1 cm) while QYM having the least value at (123.4 cm) as shown in Table 3.

3.3 Descriptive Statistic of Proximate Analysis (Nutrient Content)

The statistical analysis shows that the data are significantly different as shown in Table 3, the dry matter is significantly different with the QYM having the highest dry matter (89.5%) content followed by YM (88.5%). The CP is high in the YM with the value of 13.5% followed by the QPM at 12.9% and QYM recorded 11.99%. The EE is the least at YM with 3.0% while QPM is highest

(4.760%). The CHO has high percentage in QYM (69.72%) while QPM has the least value (64.17%). QPM has the highest value (2.450%) of CF as compared to YM (1.025%).

3.4 Descriptive Statistic for Amino Acid Profile

Table 4 shows that the amino acids profile was significantly different, except in valine, argispateand alalanine. The lysine of the hybride (1.370%) is higher than the QPM (1.330%) and YM (1.355%) the lysine content in the varieties is higher compare to the researched of Osei *et al* (1998) QPM which is (0.27%) and QPM (0.25%) of the work Padan, et al (2011). Tryptophane of the hybride QYM (0.540%) is also higher than the YM (0.485) and the QPM is lessr at (0.435%). By comparing with other work the hybride maize was higher than the QPM of Padan, et al. (2011) which is (0.08%). Also the Methionine of the hybride (QYM) was of greater value than the YM and QPM, QYM is 0.875%, YM is 0.850% and QPM 0.785%. QYM is numerically higher in methionine than the QPM in the work of Padan, et al. (2011) which is (0.18%). The isoleucine (1.46%) and leucine (2.75%) of QYM are higher than that of the Isoleucine QPM (1.375%) and YM (1.155%), leucine QPM (2.045%) and YM (2.140%) which was relatively higher than the work of osei, et al. (1998). From the results gotten from the researched indicated that the hybridized maize (QPM, fortified with beta carotene) is most preferable for food and feed as compared to the white QPM and the local yellow maize. This indicates that if QYM is use in dietary it will give a better result than the other varieties of maize as pointed in the results.

3.5 Mean, Mean Squares of Coefficient of Variation (CV) of Vegetative Growth

In Table 5, the mean, mean square and coeffient of variation of the ten characters for vegetative growth parameters were measured for the two maize varieties were presented. The coeffient of variation ranges from 4.282% (days to silking) to 26.32% (ear insertion angle). Very high CV value was observed for ear insertion angle (26.32%) which range is wider compare to the other parameters showed in the table below. Number of tassel branches (19.12%), length of ear leaf (11.26%), height at maturity (11.97%), width of ear leaf (11.32%), number of nodes (11.28%), Length of internode (13.06%) and days o silking (4.282%) were coefficient of variation values recorded for vegetative parameters, however days to silking had the least variability (3.95%).

3.6 Mean, Mean Squares, Coefficient of Variation of Reproductive Characters

The mean, mean square and coeffient of variation of the six characters for reproductive

Table 1. Descriptive statistic of vegetative characters

	DT	DS	NTB	LI (cm)	NN	NT	HM (cm)	WEL (cm)	LEL (cm)	AI (^{0})
Mean	56.20^{ab}	58.50^{b}	12.5^{b}	13.37^{ab}	11.80^{ab}	0.100^{b}	209.1^{a}	9.430^{ab}	80.60^{b}	58.0^{ab}
Mean	56.20^{ab}	58.4^{b}	4.249^{ab}	12.83^{ab}	11.20^{ab}	0.100^{b}	193.0^{a}	8.580^{b}	83.00^{ab}	61.0^{ab}
Mean	56.50^{ab}	58.6^{b}	12.6^{b}	14.28^{a}	11.50^{ab}	0.700^{a}	207.0^{a}	8.60^{b}	81.6^{ab}	67.0^{a}
Mean	56.30	58.50	9.783	13.49	11.5	0.30	203.0	8.87	83.5	62.0
SD	2.997	2.505	2.619	1.756	1.297	0.436	24.3	1.004	9.177	16.32

QPM= Quality protein maize, YM= Yellow Maize, QYM= Quality Protein Maize cross with Yellow Maize, DT= Days to Tasseling, DS= Days to silking, NTB= Number of Tasseling branch, LI= Length of internode, NN= Number of Node, NT= Number of Tillers, HM= Height at Maturity, WEL= Width of Ear leaf, LEL= Length of Ear Leaf, AI= Angle of Insertion

Table 2. Descriptive statistic of reproductive traits

Genotype/Traits	ED (cm)	EL (cm)	EH (cm)	WH (g)	WWH (g)	100SW (g)	Seeds coat colour
QPM	4.41	14.70	78.4	173.0	142.0^{a}	34.05	White
YM	4.37	14.80	78.0	165.0	136.0^{ab}	36.28	Yellow
QYM	4.10	14.50	72.3	191.8	123.4^{b}	34.24	Pale yellow
Grand	4.29	14.65	76.3	176.6	133.9	34.86	
SD	0.35	1.843	9.43	39.4	37.7	4.107	

ED= Ear diameter, EL=Ear length, WH= Weight with husk, WWH= Weight without husk, EH=Ear height 100SW= 100 Seeds weight, SD= Standard deviation

Table 3. Descriptive statistic for proximate analysis

Genotype	CP%	EE%	CF%	ASH%	DM%	CHO%
QPM	12.9[b]	4.760[a]	2.450[a]	4.200[a]	88.57[c]	64.17[c]
YM	13.5[a]	3.0[b]	1.025[c]	3.500[b]	88.90[b]	68.28[b]
QYM	11.99[c]	2.950[c]	1.340[b]	3.510[b]	89.50[a]	69.72[a]
Grand Mean	12.80	3.57	1.605	3.737	88.99	67.39
SD	0.0024	0.0047	0.0024	0.0047	0.0024	0.0353

CP=Crude protein, EE= Ether extract, CF= Crude fiber, ASH= Ash content DM= Dry matter, CHO= Carbohydrate content, SD= Standard deviation

Table 4. Descriptive statistic for amino acid profile

Genotype	Hi%	Iso%	Leu%	Lys%	Met%	Phe%	Thr%	Try%	Val%	Arg%
QPM	0.620[a]	1.375[b]	2.045[c]	1.330[c]	0.785[b]	0.880[ab]	0.705[c]	0.435[c]	1.400	0.420
YM	0.460[b]	1.155[c]	2.140[b]	1.355[b]	0.850[a]	0.910[a]	0.745[b]	0.485[b]	1.400	0.670
QYM	0.385[c]	1.460[a]	2.745[a]	1.370[a]	0.875[a]	0.860[c]	0.775[a]	0.540[a]	1.450	0.510
Mean	0.488	1.330	2.310	1.352	0.837	0.883	0.742	0.487	1.417	0.533
SD	0.021	0.028	2.31	0.028	0.028	0.014	0.012	0.014	0.097	0.238

SN=Serial numbers, Hi=Histidine, Iso= Isoleucine, Leu= Leucine, Lys=Lysine, Met= Methionine, Phe= phenylalanine, Thr = Threonine, Try=Tryptophan, Val= Valine, Arg=Arginine. SD= Standard deviation, m= mean, GM= Grand mean, QPM= Quality protein maize, YM= yellow maize, QYM =Quality protein maize cross with yellow maize

Table 5. Mean, mean squares of coefficient of variation of vegetative growth

Traits	Mean	Mean Square	CV (%)
DT	$56.3\pm_{0.948}$	14.08	5.323
DS	$58.5\pm_{0.972}$	14.82	4.282
NTB	$13.7\pm_{1.089}$	64.87	19.12
LI	$13.5\pm_{0.555}$	6.199	13.04
NN	$11.5\pm_{0.410}$	2.910	11.28
NT	$0.3\pm_{0.138}$	0.657	14.50
HM	$203\pm_{7.693}$	789.1	11.97
WEL	$8.87\pm_{0.477}$	2.656	11.32
LEL	$81.5\pm_{2.901}$	140.8	11.26
AI	$62.0\pm_{0.112}$	429.6	26.32

Table 6. Mean, mean squares of coefficient of variation of reproductive characters

Traits	Mean	Mean Square	CV (%)
ED	$4.293\pm_{0.112}$	0.322	8.246
EL	$14.65\pm_{0.332}$	12.16	12.58
EH	$76.3\pm_{2.98}$	85.32	12.36
WH	$144.2\pm_{12.46}$	12.88	27.34
WWH	$133.9\pm_{11.93}$	31.93	28.12
100SW	$34.86\pm_{1.299}$	31.8	11.78

traits parameters were measured for the two maize varieties as presented in Table 6 revealed that the coeffient of variation ranges from 8.246% (ear diameter) to 28.12% (weight without husk). Very high CV value was observed for weight without husk (28.12%) followed by weight with husk (27.34%).The rest parameters show low variabity, which are; ear diameter (8.462%), ear length(12.58%), ear height (12.36%), and 100 seeds weight (11.78). Ear diameter had the least variabity.

3.7 Correlation of Yield and Yield Related Traits

Table 7, shows the traits and their contribution to the yield and yield related parameters. From the table it was reviewed that days to tasseling was

Table 7. Correlation of yield and yield related traits

Traits	DT	DS	NTB	LI	NN	NT	HM	WEL	LEL	AI	ED	EL	EH	WH	WWH	100SW
DT		.956**	.205	.045	.101	.441	.069	.318	-.167	-.187	.215	.639	.478	.847	.153	.810
DS			.221	.038	.108	.411	.073	-.229	-.105	-.019	-.075	-.088	-.055	.005	-.154	-.019
NTB				.016	.310*	-.064	.234	.254	.088	-.266*	-.050	-.054	.141	-.140	-.095	-.064
LI					.224	.221	.620**	.035	.370**	.120	-.034	.095	.113	.339**	.188	-.056
NN						.248	.666**	.135	.122	-.029	.042	-.091	.248	.023	-.061	.032
NT							.263*	-.059	.001	.266*	-.017	-.042	-.093	.136	-.016	-.058
HM								.180	.419**	.148	.146	-.064	.335**	.115	.092	-.047
WEL									.257*	-.100	.137	.118	.155	.149	.207	-.118
LEL										.1080	.050	.202	.136	.221	.222	.159
AI											.1450	.015	.093	.234	.168	.162
ED												.162	-.054	.351**	.601**	.330*
EL													.045	.460**	.526**	.041
EH														.004	.118	-.105
WH															.706**	.058
WWH																.233
100SW																

Qpm= quality protein maize, YM= yellow maize, QYM= Quality protein maize cross with yellow maize, DT=Days to tasseling, DS= Days to silking, NTB= Number of tasseling branch, LI= Length of internodes, NN= Numbers of node, NT= Number of tillers, HM= Height at maturity, WEL= Width of ear leaf, LEL= Length of ear leaf AI= Angle of insertion

high and correlated to the days of silking positively and negatively correlated to the length of ear leaf and angle of insertion and positively correlated to the ear diameter, ear length, ear height, weight without husk, weight with husk and 100 seed weight. Days to silking was negatively correlated to the yield and yield related traits. Number of tasseling branch is negatively correlated to the ear length, weight without husk, weight with husk and 100 seed weight. Length of inter node is negatively correlated to ear diameter and 100 seeds weight, but highly correlated to weight with husk positively. Height at maturity correlated positively to the ear height and negatively correlated to 100 seeds weight. Length of ear leaf and angle of insertion are positively correlated to the yield and yield related traits. Ear diameter is and ear length are highly correlated to weight with husk, weight without husk and 100 seeds however ear height is not highly correlated to 100 seeds weight . But ear diameter was negatively correlated to the ear height.ear height is negatively correlated to 100 seeds weight. Weight without husk is highly correlated to weight with husk. Weight without husk was positively corrected to 100 seeds weight.

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

QPM varieties are being crossed with high carotenoid varieties and traditionally bred to contain an enhanced overall nutritional profile. These high pro vitamin A carotenoid QPM varieties may be nutritionally advantageous for preventing or treating exophthalmia, night blindness, and mortality related to vitamin A deficiency, as opined by NRC, 1988; Rolfes *et al.,* 2009. With the successful cross, yellow QPM would have a greater impact on health and nutrition for target countries such as developing countries of Africa and this would provide the estimated average requirement of vitamin A to children who consume 200 g/day dry maize, thereby supporting the initial claim of Harvest Plus, 2007. The aim of the researched was achieved since the quality protein maize (QPM) was improved with beta carotene which was the major source of vitamin A. The addition of beta carotene to quality protein maize makes it more nutritious for food and feeds compare to other varieties of maize.

4.2 Recommendation

- Continuous supply of QPM grains for the industry needs to be ensured through effective backward linkages and contract farming to ensure sustainable supply of QPM.
- Better demonstration, promotion and also awareness creation of stakeholders, on QPM fortifying with beta carotene using proper channels to demonstrate it.
- To harness full potential of QPM, there is an urgent need to sensitize the food processing and value addition industries in Nigeria and other developing countries on nutritional benefits of QPM, so as to generate and deploy QPM-based value-added food products in both rural and urban markets especially with the improve one with beta carotene.
- The potential of QPM, especially yellow QPM should be promoted so as to strengthening the maize-poultry value chain and to properly utilize the sector
- Further research on the cost-benefit ratio of QPM to determine the value gained in terms of kg of meat and number of eggs and also the quality of the products by using QPM over conventional maize is required at each production settings
- Incentives should be available like premium price for the QPM producers and value addition over normal maize grains

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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