Line X Tester Analysis for Protein Content in Quality Protein Maize (QPM) (*Zea mays* L.) Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. Author AM has Designed the study, wrote all the protocols and drafted the manuscript. Author TSB has carried out the Analysis and sorted out the tables and discussed the results. Author IUM has played the role as research assistant author MSNA this author contributed as sours of seed for the study served as laboratory technician that carried out germination test. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: To determine superior cross combination between QPM and tester (normal maize) genotypes in protein, tryptophan and lysine content so to enhanced protein content in locally adopted Maize Varieties (Tester) through Line X Tester method aimed in reducing protein deficiency in the study area and sub-Saharan Africa at large.
Study Design: Randomized Complete Block Design (RCBD) with three replicates and two border rows were used at the end of each replicate to minimize the border effect. Twenty-four 24 (12 x 2) crosses combinations were recovered through the Line X Tester Mating Method.
Place and Duration of Study: Field trial was conducted at Jega Teaching and Research farm of Kebbi State University of Science and Technology, Aliero (KSUSTA), Kebbi State Nigeria, during 2018 and 2019 rainy seasons.
Methodology: Experimental material comprised twelve quality protein maize (QPM) (female parents), two testers (male parents or normal maize) with diverse genetic base and one check for comparison (CML312/CML442 tester A and CML202/CML395 tester B and one check Yar acre C)

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were grown. The emasculation was carried out where the tassels of the female plants (seed parents or line) were removed immediately as soon as appeared, through the process called detasseling and Ear where put in selfing bag for 1 to 2 days after emergence and tassels of selected male parents (Tester) were covered with waterproof selfing bag one day after emergence, pollens from tester were dusted over the silk of line and care was taken in each stage to avoid contamination of pollen grains from tagged tester with foreign pollens.

Results: Analysis of Variance revealed that, genotypes CML503 (L4 x T1) recorded highest in crude protein in a combined mean performance with 9.1% but recorded lowest lysine and tryptophan of 3.1% and 0.4% respectively and there was drastic reduction in crude protein from 2018 (9.4%) to 2019 (8.8%), lysine from 2018 (4.1%) to 2019 (3.9%) and tryptophan from 2018 (0.8%) to 2019 (0.6%), However, local check recorded an increase in crude protein, lysine and tryptophan Crude protein increased from 2018 (1.4%) to 2019 (2.4%) and in combined mean performance (5.4%), lysine also increased from 2018 (0.2%) to 2019 (0.9%) and tryptophan 2018 (0.002%) to 2019 (0.90%).

Conclusion: Study concluded that highly significant differences among genotypes indicated the presence of inherent genetic differences among treatments and hybrid of the cross between QPM CML503 and the tester-A (L4 x T1) could be used for breeding programme aimed at protein improvement and therefore, could be grown by the maize producers for crude protein, lysine and tryptophan.

1. INTRODUCTION

Maize (*Zea mays* L. 2n=20) belongs to the grass family *Poaceae* and is the second leading crop after wheat Worldwide [1]. Maize had been the main staple food, particularly in tropical regions of Africa [2], mainly because it has high nutritional value, with high levels of starch, folic acid, and Vitamins and the grain is rich in magnesium, manganese, zinc, copper, iron and sodium and has large amounts of phosphorus and potassium [3]. However, maize is naturally deficient in lysine and tryptophan; the two are regarded as amino acids essential for humans, so it needs to be part of a balanced diet [4].

The menace of malnutrition in developing countries like Nigeria could be reduced through the increase in protein content in their diet but, these nutrients are not available and affordable to a common man [5]. Therefore, any low-cost and sustainable strategy that is capable of combating protein deficiency in Africa is of great benefit to our society.

Maize production estimation was 55'721,588 tonne worldwide, harvested from 40'935,896 hectares in 2016, with an average yield of 13,612 kg/ha [6]. And African total production for the year 2010 was 211'107,724 tonne harvested from 24'837,754 hectares at an average yield of 8,498 kg/ha while in Nigeria the total production was 4'784,100 tonne, harvested from 4'736,730 hectares at an average yield of 10,100 kg/ha [7].

In-plant breeding, various mating designs are used to generate improved plants; therefore, selection of suitable mating designs is key to any successful breeding programme and Line x tester design (breeding tool) is a design which involves the crossing between lines (f) and testers (m) in one to one fashion generating f x m = fm hybrids [8].

It is the simplest mating design that provides information on both full-sibs and half-sibs simultaneously as opposed to top cross which provides half-sibs information only [9]. It also provides the specific combining ability and general combining ability of both the lines and the testers of each cross [10]. Therefore, a study was conducted to evaluate protein content in locally adopted maize varieties suitable for commercial production.

2. MATERIALS AND METHODS

2.1 Experimental Site

Field trials were conducted during 2018 and 2019 rainy seasons at Jega Teaching and Research farm of Kebbi State University of Science and Technology, Aliero (KSUSTA), Kebbi State Nigeria. Jega is located in Kebbi State, in the Sudan Savanna agro-ecological zone of Nigeria at 14˚13.029'S, 033˚48.019'E with an elevation of 1187 metres above sea level Nigeria Metrology [11].

The climate of the Jega area is characterized by annual rainfall ranges from 700-900 mm, and average temperature of 14-30°C during dry season and 27-41°C during the rainy season and the relative humidity ranges from 21-47% in the dry season and 51-79% during the rainy season and the area is characterized by long dry season with cool air during hammattan period (November- February), followed by a short rainy season May/June – September/October [12].

Soil samples were collected from randomly selected points within the experimental site at 0-30 cm depth using soil auger before planting and after harvesting in each trial and each year.

Composite soil samples were air-dried and sieved for physical and chemical analyses. Before the commencement of the experiment, soil samples were collected from randomly selected points within the experimental site at 0-15 and 15-30 cm depth using soil auger after and before land preparation in each trial. The composited soil samples were air-dried and sieved to pass through 2.00 mm sieve before subjecting them to physical and chemical analyses.

The Particle size analysis was conducted using the hydrometer method [13] and the textural class was determined using the United States Department of Agriculture (USDA) procedure. Total nitrogen was determined by Kjeldahl digestion method [14]; while Bray 1 method as outlined by Bray and [15] was used for the determination of available P.

Organic carbon was determined by Walkley-Black method [16], while exchangeable cations of Na and K were determined by flame photometer, Mg and Ca were determined by EDTA titration method, cation exchange capacity (CEC) was determined using the normal ammonium acetate solution. Soil reaction (pH) was determined using 1:2 soil liquid (water) ratio [17].

2.2 Planting Materials and Their Source

Twelve quality protein maize (QPM) (female parents), 2 testers (male parents or normal maize) of maize with diverse genetic base and one check for comparison (CML312/CML442 tester A and CML202/CML395 tester B and one check Yar acre C) were used the experiment (Table 1).

The 24 (12 ×2) cross combinations were recovered through Line × Tester Mating Method so far identified on a yield basis and morphologically were used in this study. The lines were obtained from an International Maize and Wheat Improvement Center (CIMMYT-Mexico), and two testers from Institute for Agricultural Research (IAR) Zaria, Nigeria.

2.3 Experimental Setup

Soil samples were collected from randomly selected points within the experimental site at 0-30 cm depth using soil auger before planting and after harvesting in each trial and each year. Composite soil samples were air-dried and sieved for physical and chemical analyses.

Table 1. Descriptions of quality protein maize (QPM), tester, check and Crosses (Line x tester method)

Ent.	Pedigree material	Stock ID	Origin	Crosses 1-7	Crosses 8-12
1	CML144	AF10B-5481-20	Mexico	L1 x T1	L8 x T1
2	CML159	AF15A-011-1	Mexico	L1 xT2	L8 x T2
3	CML491	AF15A-011-3	Mexico	L2 xT1	L9 x T1
4	CML554=CLQRCWQ131	AF13A-482-10	Mexico	L2 x T2	L9 x T2
5	CLQ6315	AF10A-218-12	Mexico	L3 x T1	L10 x T1
6	CML502	AF10A-481-5	Mexico	L3 x T2	L10 x T2
7	CML503	AF15A-011-2	Mexico	L4 x T1	L11 x T1
8	CML555=CLQRCWQ26	AF13A-482-11	Mexico	L4 x T2	L11 x T2
9	CML556=CLQRCWQ123	AF13A-482-12	Mexico	L5 x T1	L12 x T1
10	CML557=CLQRCWQ48	AF13A-482-13	Mexico	L5 x T2	L12 x T2
11	A	Tester A	Zaria	L7 x T1	Check
12	B	Tester B	Zaria	L7 x T2	Check
13	C	Checks	Argungu		

*1-10 are inbred lines with high protein content, A and B are tester with low protein content and C is a local check for comparison, L Stand for inbred line, T stands for tester and C for the check

Table 2. General analysis of variance for line x tester mating design

Source of variations	Degrees of freedom (Df)	Mean squares (MS)
Replicate (R)	r-1	
Blocks within rep (R)	Blk (r-1)	
Genotypes (g)	(lt+c)-1	MSg
Lines/Females (l)	(l-1)	MSl
Testers/Males (t)	(t-1)	MSt
Lines X Testers	(l-1) (t-1)	MSlxt
Checks (C)	C-1	MSC
Checks vs crosses (C vs Cr)	(l-1)(t-1)(y-1)	MSC v Cr
Error (E)	(g-1) (r-1)	MSe
Total	gmfr-1	

Two seeds were planted per hill; weed, pest and disease management was carried out throughout the growing seasons. Using Line X Tester, the emasculation was carried out where the tassels of the female plants (seed parents or line) were removed immediately as soon as appeared, through the process called detasseling and ear where put in selfing bag for 1 to 2 days after emergence and tassels of selected male parents (Tester) were covered with waterproof selfing bag one day after emergence, pollens from tester were dusted over the silk of line and care was taken in each stage to avoid contamination of pollen grains from tagged tester with foreign pollens.

The samples (5-10 in each cross combination) were collected by the procedure outlined in the International Brue for Plant Genetics Resources and International Crop Research Institute for Semi-Arid Tropics (IBPGR/ICRISAT) Maize descriptor in each cross combination [18]. Protein content was determined using micro-Kjeldahl method and data on tryptophan and lysine content was determined by using procedures described in Hernandez, [19] using official methods of Analysis 18th Edn. described by the Association of Official Analytical Chemists, standard method (Method 982.18 E [20]).

The experiment had one experimental factor (maize genotype) with 13 levels twelve quality protein maize (QPM) (female parents), 2 testers (male parents or normal maize) of maize with diverse genetic base and one check. The experiment was arranged in a Randomized Complete Block Design and replicated three times.

Observations were recorded on the protein (%) using micro Kjeldahl method., tryptophan (g/20 gram of nitrogen) and lysine (g/20 gram of nitrogen) by using procedures described in Hernandez ,1969 the data were calculated from composite seeds of 5 randomly selected plants from a plot for genotypes and checks. The average value of these plants for these characters was calculated and used for the statistical analysis.

2.4 Data Analysis

Analysis of variance was computed and statistical variations were determined as highly significant using MSTAT-c procedure version 5.1.

3. RESULTS AND DISCUSSION

In Africa, protein deficiency affects more than 80 million children and it is accounted for the death of about 10.8 million children and 600,000 women were also affected [5]. Lack of protein caused kwashiorkor to over 6.6 million children annually [21]. The menace of malnutrition in developing countries like Nigeria could be reduced through increasing the protein content in their diet but, these nutrients are not available and affordable to a common man [5]. Therefore, any low-cost and sustainable strategy that is capable of combating protein deficiency in Africa is of great benefit to our society.

The amount of crude protein, lysine and tryptophan in maize grain is directly controlled by the plant's capacity to take up and transfer nitrogen from roots and leaves (source) to the seed (sink) [22] and [5]. Therefore, the results for 2018, 2019 and combined indicated that there were significant differences among genotypes at 0.5% and this indicated the presence of inherent genetic differences among treatments, where genotypes CML503 (L4 x T1) recorded highest in crude protein content in a combined mean performance with 9.1% but recorded lowest lysine and tryptophan of 3.1% and 0.4% respectively (Table 5).

Table 3. The performance of 13 genotypes (inbred lines, tester, and check) grown in 2018 based on the percentage of crude protein, lysine and tryptophan contents

Ent.	Pedigree material	Stock ID	Crude protein (%)	Lysine (%)	Tryptophan (%)
1	CML144	AF10B-548120	9.5	3.7	0.6
2	CML159	AF15A-011-1	9.3	4.3	0.9
3	CML491	AF15A-011-3	8.9	3.9	0.8
4	CML554=CLQRCWQ131	AF13A-482-10	9.1	4.5	0.7
5	CLQ6315	AF10A-218-12	8.1	4.1	1.1
6	CML502	AF10A-481-5	8.8	4.2	0.9
7	CML503	AF15A-011-2	9.4	4.1	0.8
8	CML555=CLQRCWQ26	AF13A-482-11	8.6	3.8	0.7
9	CML556=CLQRCWQ123	AF13A-482-12	9.3	3.8	0.9
10	CML557=CLQRCWQ48	AF13A-482-13	9.3	4.6	1.06
11	A	Tester A	2.9	0.9	0.03
12	B	Tester B	2.1	0.7	0.02
13	C	Checks	1.4	0.2	0.002

Percentage of crude protein, lysine and tryptophan contents on a dry weight basis

Table 4. The performance of 13 genotypes (inbred lines, tester, and check) grown in 2019 based on the percentage of crude protein, lysine and tryptophan contents

Ent.	Pedigree material	Stock ID	Crude Protein (%)	Lysine (%)	Tryptophan (%)
1	CML144	AF10B-548120	8.6	3.8	0.5
2	CML159	AF15A-011-1	8.3	3.9	0.9
3	CML491	AF15A-011-3	7.9	3.5	0.7
4	CML554=CLQRCWQ131	AF13A-482-10	9.9	4.1	0.9
5	CLQ6315	AF10A-218-12	8.7	4.1	1.3
6	CML502	AF10A-481-5	8.7	3.9	0.9
7	CML503	AF15A-011-2	8.8	3.9	0.6
8	CML555=CLQRCWQ26	AF13A-482-11	8.2	2.6	0.4
9	CML556=CLQRCWQ123	AF13A-482-12	8.8	3.9	0.6
10	CML557=CLQRCWQ48	AF13A-482-13	7.3	4.6	1.2
11	A	Tester A	2.6	0.9	0.09
12	B	Tester B	2.8	0.9	0.06
13	C	Checks	2.4	0.9	0.90

Percentage of crude protein, lysine and tryptophan contents on a dry weight basis

However, there was a drastic reduction in crude protein from 2018 (9.4%) to 2019 (8.8%), lysine from 2018 (4.1%) to 2019 (3.9%) and tryptophan from 2018 (0.8%) to 2019 (0.6%) (Tables 3 and 4) for almost all the genotypes. These may be as a result of inbreeding depression as it can increase homozygosity in a population.

However, local check recorded an increase in crude protein, lysine and tryptophan with crude protein increased from 1.4% for 2018 to 2.4% 2019 and to 5.4% in the combined results, lysine also increased from 2018 (0.2%) to 2019 (0.9%) and tryptophan 2018 (0.002%) to 2019 (0.90%). This may be as a result of heterosis known to occur in maize due to crossing between two dissimilar parents. This agreed with Maria et al. [22] who reported that an increase in vigour and frequency of favourable alleles in the populations is the function of an increase in heterozygosity and heterogeneity among genotypes in a population. However, the findings disagreed with [23] reported that some quality protein maize (QPM) hybrids performed equal to or better than some of the local checks and or open-pollinated varieties included in the trials.

Table 5. The performance of 13 genotypes (inbred lines, tester, and check) grown in 2018 and 2019 based on the percentage of crude protein, lysine and tryptophan contents

Ent.	Pedigree material	Stock ID	Crude protein (%)	Lysine (%)	Tryptophan (%)
1	CML144	AF10B-548120	8.9	3.9	0.7
2	CML159	AF15A-011-1	9.0	3.3	1.4
3	CML491	AF15A-011-3	8.6	3.4	0.9
4	CML554=CLQRCWQ131	AF13A-482-10	7.8	3.6	0.6
5	CLQ6315	AF10A-218-12	9.0	3.8	1.5
6	CML502	AF10A-481-5	7.9	4.9	0.9
7	CML503	AF15A-011-2	9.1	3.1	0.4
8	CML555=CLQRCWQ26	AF13A-482-11	7.9	3.5	0.9
9	CML556=CLQRCWQ123	AF13A-482-12	8.8	3.9	0.9
10	CML557=CLQRCWQ48	AF13A-482-13	8.6	4.4	1.9
11	A	Tester A	3.3	1.3	0.4
12	B	Tester B	3.6	0.8	0.4
13	C	Checks	5.4	0.9	0.7

Percentage of crude protein, lysine and tryptophan contents on a dry weight basis

Therefore, CML503 and Tester-A (L4 x T1) could be selected for breeding programme aimed at protein improvement and could contribute to reducing protein deficiency in the study area and sub-Saharan Africa at large. It also indicated that the amount of crude protein content does not indicate the amount of lysine and tryptophan in maize grain Tables 3, 4 and 5. The results were commemorated the other findings reported by [24] which said" an increase in the probability of obtaining a superior hybrid requires to increase the frequency of superior genotypes in a population.

4. CONCLUSION

The study has revealed that genetic factor influences the protein, tryptophan and lysine contents of the QPM and normal maize (tester) genotypes. Hybrid of the cross between QPM CML503 and the tester-A (L4 x T1) could be used for any breeding programme that aimed at protein improvement and therefore, could be grown by the maize producers to be used as sources of protein for both children and adult.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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