Original Research Article

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

Abstract

Field trial was 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. The study was planned to determine specifically 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 in sub-Saharan Africa at large. 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 comparism (CML312/CML442 tester A and CML202/CML395 tester B and one check Yar acre C) were grown in an ~~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 Line X Tester Mating Method. ~~Analysis of Variance~~The results 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~~On the other ~~-there~~hand, 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 %)~~,~~. ~~,~~ 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 %)~~,~~. ~~,~~ In the ~~study concluded that, Highly~~study, highly significant differences among genotypes indicated the presence of inherent genetic differences among treatments. ~~The study was planned to determine specifically 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 in sub-Saharan Africa at large.~~

Key words: *Breeding, Protein, Genotype, Maize, Quality*

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Introduction

Maize (*Zea mays* L. 2n=20) belongs to the grass family *Poaceae* and is the second leading crop after wheat Worldwide (Kempthome, 2014). Maize had been the main staple food, particularly in tropical regions of Africa (Rahman *et al.*, 2006), mainly because it has high nutritional value,

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with high levels of starch, folic acid, and Vitamins (Tasfaye, *at el.*, 2011).~~,~~ ~~it is also~~The grain ~~rich~~is rich in magnesium, manganese, zinc, copper, iron and sodium and has large amounts of phosphorus and potassium (Poehlman and Sleper, 2015). 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 (Bressani, 2014). The menace of malnutrition in d~~D~~eveloping countries ~~example~~ like Nigeria could be reduced through the increasing in protein content in their diet but, these nutrients are not available and affordable to a common man (Njuguna, 20015). 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 ton worldwide, harvested from 40'935,896 hectares~~.~~ in 2016, with an average yield of 13,612 kg/ha. The African total production for the year 2010 was 211'107,724 ton harvested from 24'837,754 hectares at an average yield of 8,498 kg/ha. In Nigeria the total production was 4'784,100 ton, harvested from 4'736,730 hectares at an average yield of 10,100 kg/ha (FAOSTAT, 2016).

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 (Khan *et al*., 2009). 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 (Sharma, 2006). 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 (Acquaah, 2012). It also provides specific combining ability and general combining ability of both the lines and the testers of each cross (Hallauer and Miranda, 1988). Therefore, a study was conducted in order to evaluate……..~~The Objective of the study is to enhanced~~ protein content in locally adopted ~~Maize~~ maize ~~Varieties~~ varieties suitable for commercial production.

Material and Methods

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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 on latitude 13^0 08'N and longitude 5^0 15'E on an altitude of about 350 m above sea level, Nigeria Metrology (NIMET, 2017). The climate of the Jega area is characterized by annual rainfall ranges from 700-900 mm, and average temperature of 14-30 ^oC during dry season and 27- 41 ^oC during the rainy season and the relative humidity ranges from 21-47% in the dry season and 51-79% during the rainy season. 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 (Anonymous, 2012b). 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 in each year. Composite soil samples were air-dried and sieved for physical and chemical analyses.

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Planting materials and their source

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Twelve quality protein maize (QPM) (female parents), 2 testers (male parents or normal maize) of maize with diverse genetic base and one check for comparism (CML312/CML442 tester A and CML202/CML395 tester B and one check Yar acre C) were used the experiment (Table1). The 24 (12 ×2) cross combinations were recovered through Line × Tester Mating Method so far identified on 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.

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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 stand for tester and C for check.

Experimental set up

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 in each year. Composite soil samples were air-dried and sieved for physical and chemical analyses. 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 water proof 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 in accordance with 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.

Protein content was determined using micro-Kjeldahl method []. Data on tryptophan and lysine content was determined by using procedures described in Hernandez, (1969) and Mertz et al. (1964), and Doll and Koie (1996) and using official methods of Analysis 18th Edn. described by the Association of Official Analytical Chemists, standard method (Method 982.18 E (a, b, c), AOAC, 2006).

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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.

~~**Treatments and Experimental Design**~~

~~Experimental material comprised twelve quality protein maize (QPM) (female parents), 2 testers (male parents or normal maize) of maize with diverse genetic base and one check for comparism (CML312/CML442 tester A and CML202/CML395 tester B and one check Yar acre C) (Table1). The 24 (12 ×2) cross combinations were recovered through Line × Tester Mating Method so far identified on yield basis and morphologically were used in this study.~~

~~The materials were grown in a Randomized Complete Block Design with three replicates and two border rows were used at the end each replication to minimize the border effect. Gross plot of 17 × 45 m and each plot will comprise 2 rows of 5× 2 m with the spacing of 0.75 m between rows and 0.30 m between plants.~~

~~Two seeds were planted per hill; weed, pest and disease management was carried out throughout the growing seasons. 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.~~

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~~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 water proof 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 in accordance with 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 as shown in the table below.

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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 stand for tester and C for check.

Data Analysis

Protein content was determined using micro-Kjeldahl method. Data on tryptophan and lysine content was determined by using procedures described in Hernandez 1969 and Mertz *et al.* 1964,

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~~and Doll and Koie 1996 and using official methods of Analysis 18th Edn. described by the Association of Official Analytical Chemists, standard method (Method 982.18 E (a, b, c), AOAC, 2006).~~ Analysis of variance was computed and statistical variations were determined as highly significant at 0.01 using MSTAT-c procedure version 5.1.

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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		

Results and Discussion

In Africa, protein deficiency affects more than 80 million children []. It accounted for death of about 10.8 million children and 600,000 women were also affected (Bressani, 2014). Lack of protein caused kwashiorkor to over 6.6 million children annually (Cakmark, 2008). The menace of malnutrition in d~~D~~eveloping countries ~~example~~ like Nigeria could be reduced through increasing the protein content in their diet but, these nutrients are not available and affordable to

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a common man (Bressani, 2014). Therefore, any low-cost and sustainable strategy that is capable of combating protein deficiency in Africa is of great benefit to our society.

Table 3: The performance of inbred lines, tester, and check in 2018

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	10.1	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 content in grain of maize genotypes

Table 4: The performance of inbred lines, tester, and check in 2019

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 content in grain of maize genotypes

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Table 5: The combined performance of inbred lines, tester, and check in 2018 and 2019

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 content in grain of maize genotypes

The mean performance for 2018, 2019 and combined for crude protein, lysine and tryptophan gGrain protein content can be considered as the amount of protein per seed or unit of weight of grain. It is directly controlled by the plant's capacity to take up and transfer nitrogen from roots and leaves to the seed.~~,~~ ~~therefore~~Therefore, ~~H~~highly ~~-~~highly significant differences among genotypes indicated the presence of inherent genetic differences among treatments, where ~~Analysis of Variance (ANOVA)~~the results revealed that, 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). ~~and~~ However, there ~~was~~ 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 %) (Table 3 and 4).~~,~~ ~~these~~ These may be as a result of inbreeding depression as it can increase homozygosity in population. 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 %) and may be as a result of heterosis known to occur in maize due to crossing between two dissimilar parents. This agreed with Miranda and Viegas, (1987) and Maria *et al.*, (2001) ~~whose carried out independent researches and came out with the same findings which~~

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~~indicated that,~~where an increase in vigor and frequency of favorable alleles in the populations is the function of an increase in heterozygosity and heterogeneity among genotypes in a population. ~~but~~ However, the findings ~~–~~disagreed with ~~a research conducted by~~ Larkins *at el.*, (1995), findings where ~~reported that,~~ some of the 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.

Therefore, CML503 and Tester-A (L4 x T1) could be selected for any breeding programme aimed at protein improvement and could contribute in reducing protein deficiency in the study area and sub-Saharan Africa at large. It also indicated that, crude protein content does not indicate the amount of lysine and tryptophan as in Table 3, 4 and 5. The results are ~~commemorating~~ similar to other findings reported by Betran *et al.*, (2004) ~~who mentioned that to~~where increasing~~e~~ the probability of obtaining a superior hybrid ~~it is necessary~~requires to increase the frequency of superior genotypes in the population.

Conclusion

~~In conclusion, this investigation~~The study has revealed that genetic factor influences the protein, tryptophan and lysine contents of the QPM and normal maize (tester) genotypes. The finding of this study is also in agreement with the earlier reports that, some of the 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 (normal maize). Moreover, increase in vigor and frequency of favorable alleles in the populations is the function of an increase in heterozygosity and heterogeneity among genotypes in a population. ~~and finally studies concluded that,~~ 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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