Revised version of Original Research Article

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

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 in 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 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 comparism (CML312/CML442 tester A and CML202/CML395 tester B and one check Yar acre C) 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 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.

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.

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

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 increasing 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 specific combining ability and general combining ability of both the lines and the testers of each cross (10). Therefore, a study was conducted in order to evaluate protein content in locally adopted maize varieties suitable for commercial production.

Material and Methods

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^{o}C during dry season and 27- 41^{o}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 in each year.

Composite soil samples were air-dried and sieved for physical and chemical analyses. Before the commencement of experiment, soil samples were collected from randomly selected points within the experimental site at 0-15 and 15-30cm 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 textural class was determined using United States Department of Agriculture (USDA) procedure. Total nitrogen was determined by Kjedhal 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 Walklay-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).

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 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.

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 (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 protein (%) using microKjeldahl 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 were calculated and used for the statistical analysis.

Data Analysis

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

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 and it is accounted for 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.

Table 3: The performance of 13 genotypes (inbred lines, tester, and check) grown in 2018 based on percentage of crud 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 dry weight basis

Table 4: The performance of 13 genotypes (inbred lines, tester, and check) grown in 2019 based on percentage of crud 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 dry weight basis

Table 5: The performance of 13 genotypes (inbred lines, tester, and check) grown in 2018 and 2019 based on percentage of crud 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 dry weight basis

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).

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 %) (Table 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 rude 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 vigor and frequency of favorable 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.

Therefore, CML503 and Tester-A (L4 x T1) could be selected for 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, amount of crude protein content does not indicate the amount of lysine and tryptophan in maize grain Table 3, 4 and 5. The results were commemorated the other findings reported by (24) which said" an increasing in the probability of obtaining a superior hybrid requires to increase the frequency of superior genotypes in a population.

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.

Acknowledgements

We wish to acknowledge the Tertiary Education Trust Fund (TETfund) of Nigeria that fully sponsored the research and Institute for Agricultural Research (IAR) Zaria, Nigeria for supplying the plant materials used in the research. We also wish to express our sincere gratitude and appreciation to all researchers for their valuable contributions used in the text as references.

References

1. Swapan K. T. Dinesh M. Ithape, M. Maharana and A. M. Prusty (2017). Quality protein maize (QPM) Genetic basis and breeding perspective. Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India. 4(1): 145–152

2. Rahman, H., Z. Arifuddin, S. Shah, A. Shah, M. Iqbal and I.H. Khalil, (2006). Evaluations of maize S2 lines in test cross combinations I: flowering and morphological traits. *Pakistan J. Bot*. 42(3): 1619-1627.
3. Tasfaye T., Alemu T., and Hussein M. (2011). Association between morphological traits and yield component in the durra Sorghum of Ethiopia. *Hereditas,* Department of agronomy, Kansas State University, U. S. A., 148: 98-109
4. Poehlma K. and A. J. Sleeper (2015). Rank comparisons of unadapted maize population by testers and per se evaluation. *Crop Sci.* 31: 650-656.
5. Bressani, R. (2014). *High Protein Quality Maize*, Dowden, Hutchinson and Ross, Stroudsberg, pp. 38–57.
6. FAOSTAT Data (2010). (Food and Agriculture Organization of the United Nation) Year book I. 51-85.
7. FAOSTAT. (2016) (Food and Agriculture Organization of the United Nation) Year book, I. 51,85pp.
8. Sharma, J.R. (2006). Statistical and biometrical techniques in plant breeding. 1 ed. New Age International. New Delhi.India: Genetica. 90: 73-79.
9. Acquaah, G. 2012. Principles of plant genetics and breeding. 2nd ed. Wiley-Blackwell, Oxford.
10. Hallauer, A.R. and J.B. Miranda, (1988). *Quantitative genetics in maize breeding*. 2nd ed. Iowa State pp 132
11. NIMET, (2017). Nigerian Meteorological Agency Report.
12. Anonymous, (2012b). Annual report of Kebbi State Environmental Protection Agency. 12pp
13. Boyoucus, G.HN (1951). A recalibration of hydrometer method for making mechanical analysis of soils. Agronomy Journal. 43: 434-438 Bray, R.S. and L.T. Kurtz (1945). Determination of total and available P in soil science. 42: 225-229.
14. Bremner, J.M (1965). Total Nitrogen In: method of soil analysis, part 1. American society of Agronomy Madison, pp 415-524.
15. Bray, R.S. and L.T. Kurtz (1945). Determination of total and available P in soil science. 42: 225-229.
17. Thomas, G.W (1996) Soil PH, Soil acidity, in: method of soil analysis part 3. Chemical method, L.D. sparks (ed.) Book series pp 159-165.
18. IBPGR and ICRISAT (1993). International Brue for Plant Genetics Resources and International Crop Research Institute for Semi-Arid Tropics Descriptors for Maize Maize (*Zea mays* L.). Int. Board Plant Genet. Resour., Rome, Italy. – ICRISAT, Patancheru, India. 1-18pp.
19. Hornandez H, Bates LS. 1969. A modified method for rapid tryptophan analysis of maize. Res. Bull. No. 13. CIMMYT.
20. AOAC., (2006). Official Methods of Analysis. 18th Edn., Association of Official Analytical Chemists, Gaithersburgs, MD2.
21. Cakmark, A. (2008). Combining ability of transitional high land maize inbred lines. *East African J. Sci.* 2(1): 19-24.
22. Maria, E., G. Ayres and P. Zagatto, (2001). Use of heterosis in maize breeding: history, methods and perspectives review. *Crop Breed. Appl. Biot.* 1(2): 159-178.

23. Banziger, M., G.O. Edmeades, D. Beck and M. Bellon, (2000). Breeding for drought and nitrogen stress tolerance in maize: From Theory to Practice. Mexico, 190 pp
24. Betran, F.J., M. Banziger, D. Beck, J.M. Ribaut and G.O. Edmeades,(2004). Breeding approaches to develop drought tolerant maize hybrids. In: D. Polland, M. Sawkins, J.M. Ribaut and D. Hoisington (eds.). Resilient Crops for Water Limited Environments: Proceedings of a Workshop Held at Cuernavaca, Mexico. 24-28 May 2004, CIMMYT, Mexico D.F., Mexico. pp. 88-89.

Journal Name:	Asian Journal of Advanced Research and Reports
Manuscript Number:	**Ms_AJARR_54077**
Title of the Manuscript:	**Line X Tester Analysis for Protein content in Quality Protein Maize (QPM) (Zea mays L.) Genotypes**
Type of the Article	**Original Research Article**

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty'**, provided the manuscript is scientifically robust and technically sound.
To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline)

PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	• During screening, 28% plagiarism was detected which is not acceptable. Authors should revise the plagiarized text and prepare a new manuscript. • Please cite original paper for each citation like very first sentence "Maize (*Zea mays* L. 2n=20) belongs to the grass family *Poaceae* and is the second leading crop after wheat Worldwide (Kempthome, 2014)."This is not the correct reference for the statement. Please update this reference. Kempthorne, O., (2014). An introduction to genetic statistics. John Wiley, New York. 302pp. • Cross-check all the citations and make sure they are consistent with the stated text. • I am not happy with the description of discussion. Authors should improve this section. • Try to cite some recent literature to support your study in result and discussion. • The authors need to work on the manuscript more carefully and in detail to improve the quality and to make it relevant to the scientific community. • I strongly recommend authors to prepare entirely new, condense, sound and attractive submission and to resend it to the journal. •	• The manuscript. was revised to the acceptable level of plagiarism. • The original Author. was Kempthorne 2014 cited by Swapan *et al.* (2017). Therefore, the Author on the Journal (Swapan *et al.,* 2017) replaced Kempthorne 2014. • All references and citations were properly Cross-checked and updated • Seriously improved • Very recent were cited • It is now well articulated and suited for publication • The paper is highly modified and will be submitted
Minor REVISION comments	• Please revise the English	• The gramma, American and U.K. languages

	language from a native speaker and use one style throughout the manuscript (American or British English). There are many grammatical errors. ● Please cross-check all tables and make sure that all are cited in the correct place and order. ● Prepare references according to journal guidelines.	were justified ● All the tables were cross-checked and put them in order ● References in text and in list were arranged according to journal guidelines.
Optional/General comments		

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	