ORIGINAL ARTICLE

PROTEIN, TRYPTOPHAN AND LYSINE CONTENTS IN QUALITY PROTIEN MAIZE, NORTH INDIA

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ABSTERACT

BACKGROUND: ***Maize is a major cereal crop for human nutrition world wide. Cereal proteins including maize contain about 2% lysine, which is less than one-half of the recommended concentration for human nutrition recommended by Food and Agriculture Organization in maize. Efforts for improved protein quality in maize began in mid-1960s with the discovery of mutants that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in maize endosperm protein. This study was designed to determine the protein, tryptophan and lysine contents of quality protein maize grown in North India.***

METHODS: ***The protein, tryptophan and lysine contents of improved genotypes of quality protein maize and two local checks were studied between June 2005 and January 2006 on seeds of Maize grown in three localities in North agro-ecological zones of India. Protein content was determined using micro-Kjeldahl method. Data on tryptophan and lysine content was determined by using procedures described in Hernandez and Bates, Mertz et al., and Doll and Koie. Analysis of variance was computed and statistical variations were determined as highly significant at 0.01 using MSTAT-c procedure version 5.1.***

RESULTS: ***Analysis of variance indicated highly significant differences among treatments for all the characters under all the environments. The genotypes gave different percentage protein, tryptophan and lysine ranges for all locations. P4 gave the highest vale of protein content in all locations. The highest tryptophan and lysine values were obtained from P1 at Gorekhpur, Pantnagar and Kashipur.***

CONCLUSION: ***This investigation confirmed the earlier reports that quality protein maize varieties contain two-fold lysine and higher tryptophan. Besides, variations in protein content do exist among cultivars. This study revealed the fact that genetic factor influences the protein, tryptophan and lysine contents of quality protein varieties. Therefore, the QPM genotypes could be incorporated in the breeding program targeted in developing cultivars with high lysine and tryptophan contents to meet protein and aminoacid requirement in developing countries. Alternatively P1 could be grown by the producers and can be used as sources of protein for both children and adult.***

KEY WORDS: ***Protein, tryptophan, lysine contents, QPM, Nothern India.***

INTRODUCTION

Maize (*Zea mays Linaeus.*) is a major cereal crop for human nutrition, worldwide. With its high content of carbohydrates, fats, proteins, some of the important vitamins and minerals, maize acquired a well-deserved reputation as a nutria-cereal. Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. It is cultivated on an area of 147 million hectares with production of 692 million tones in the world. India alone accounts for 7.61 million hectares with production of 14.87 million tones where maize follows wheat and rice in importance (1).

The maize grain accounts for about 15 to 56% of the total daily calories in diets of people in about 25 developing countries, particularly in Africa and Latin America, where animal protein is scarce and expensive and consequently, unavailable to a vast sector of the population (2).

Efforts to improved protein quality in maize began in mid-1960s with the discovery of mutants that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in maize endosperm protein (3). International Center for Wheat and Maize Improvement's (CIMMYT) success in developing quality protein maize (QPM) genotypes have been widely reported (4-7). Quality protein maize contains nearly twice amount of lysine and tryptophan, the two amino acids essential for protein synthesis in humans and a generally more balanced amino acid content that greatly enhances its nutritive value. Research suggests that QPM can help reduce protein deficiencies, particularly in young children where maize dominates in the diets (8).

In North India there is little or no report on study of protein, tryptophan and lysine composition of quality protein maize varieties (9).

The tryptophan content of eleven superior QPM genotypes was much higher than those of wheat,

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barley, sorghum and normal maize. Their values for tryptophan ranged from 0.6-1.0%. Further, this value is two-fold greater than those reported for normal maize (5).

The crude protein content varies in different crops. Intermediate levels of 9-10 % are encountered in maize, sorghum and barley. Rice is quite low in protein (7%). Wheat, oats and triticale exhibit a high protein content of 12% and more (10)..This value is lower than that of values indicated in the previous reports (10). The same researcher also reported that normal maize is deficient in tryptophan and lysine amino acids (10).

Protein related characteristics of maize grains were studied on QPM genotypes which reported 8.0-11.0% protein (10). These values were almost more than those values reported earlier (10,11). Moreover, some QPM varieties were obtained from CIMMYT and protein content was found to be 8.9-10.2% (11, 12). Similar values were reported elsewhere (13). Another study showed a protein content of maize ranging 8.0-11% (10). These values were greater than those reported for rice. Nevertheless, the values were similar with those reported for sorghum and barely by the same researcher. A complete analysis of many cultivars of legume crop was done and per cent values for protein was found to be 22.7-29 per cent (14), which is similar to values reported by other worker (15).

In QPM varieties, leucine: isoleucine ratio was improved and become better balanced which intern considered beneficial as it helps to liberate more tryptophan for more niacin biosynthesis, thus helping to combat pellagra (2). Tryptophan analysis of thirteen QPM genotypes from CIMMYT QPM gene pool showed 0.94-1.06%, which is almost similar to the value reported by other study (12). However, this value is less than the value reported by other researchers (5). Nine superior white QPM hybrids were tested across fifteen locations in El Salvador, Guatemala and Mexico in 1998 that showed a tryptophan values of 0.07-0.10% (12), which is less than the value reported by other researchers (11).

Cereal proteins contain on an average about 2% lysine, which is less than one-half of the recommended concentration for human nutrition by Food and Agriculture Organization (FAO) ((1). From the human nutrition view point, lysine is the most important limiting amino acid in the maize endosperm protein, followed by tryptophan. For this reasons, it is valuable to adopt genetic enhancement strategy in which essential amino acids are either incorporated or increased in grain proteins (2). Mutant gene that increases lysine content of maize endosperm were studied. In this genotypes lysine content was found to be 2.31 to 3.7% (3). Other studies reported 1.80 to 2.0% and 3.8-4.5% of lysine contents, respectively (10, 12). This value was less than the values reported by some researchers in wheat, rice, barley, oats and sorghum (10).

The present study aimed at determination of protein, tryptophan and lysine contents of QPM genotypes grown in different locations of North agro-ecological zone of India. It is hoped that the knowledge obtained through this study will serve as part of important information required for future research activities in QPM varieties development.

MATERIALS AND METHODS

The locations selected for field experiment were Govadin Belabh (G.B.) Pant University of Agriculture and Technology, Pantnagar (E_1), Maize Research Centre, Belipar, Gorakhpur (E_2) and Sugarcane Research Centre, Kashipur (E_3). Pantnagar is geographically situated at 29.0°N latitude and 79.3°E longitude and at an altitude of 243.84 m above sea level. Whereas Kashipur is situated 17°N, 78°E and 587 m latitude, longitude and altitude, respectively. Gorakhpur is situated at 81.36°E and 27.34 °N and at an altitude of 130 m sea level. Moreover, Pantnagar falls under humid subtropical climate zone and is located at the foothills of the Shivalik range of the Himalayas in a narrow belt called 'Tarai'. The basic experimental material comprised of 10 elite genotypes (Table 1).

Twelve genotypes of QPM varieties so far identified on yield basis and morphologically were used in this study. The seed samples were grown in three agro-ecological zones in India; namely Pantnagar, Gorakhpur and Kashipur during 2005 cropping season which will be abbreviated as E_1, E_2 and E_3, respectively on the following pages.

In all the locations, randomized complete block design with three replications was used with a plot size of 3.75 sq. m. (5 m x 0.75 m) in three replications. Two seeds per hill were planted at a space of 25 cm within each row, after about 30-35 days of sowing thinning was done to ensure uniformity in stand at one plant per hill. Crop management practices such as weeding, watering, fertilizer application, disease and insect pest control were applied as per recommendations of Directorate of Maize Research in all locations. After 45 days of sowing the seeds were hand harvested, threshed on canvas, kept in labeled plastic bags and taken to laboratory for chemical analysis.

Observations were recorded on protein (%), tryptophan (g/16 gram of nitrogen) and lysine (g/16gram of nitrogen) and 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.

Seed samples from each plot were cleaned by hand and grounded. Flour samples were digested by micro-Kjeldahl method (16). Maize flour samples of 0.5 gm were weighed in tarred scoop and transferred to boiling tubes.

A catalyst tablet, selenium was dropped into each tube and about 25ml of concentrated sulfuric acid was added. The tubes then placed in an automatic controlled heater set at 200 ^{o} c. The mixtures were heated until the color changes to light blue. For samples which have digest color of light brown or yellow, the digestions were repeated two or more times. Thirty milliliter of distilled water was added into the digestion tube carefully. During these events, the organic matter of QPM flour is oxidized, and the nitrogen in the protein is converted to ammonium by sulfuric acid (17, 13, 14).

Ammonium in the digestion mixture was determined by distillation and titration (14). The digestion tube was placed on to the Tecator steam distillation apparatus. The distiller was set, the digestion tube inserted in the system and 150 ml ammonia was collected in the receiver flask containing 50 ml 4% boric acid solution. Then ammonia was titrated against a standard acid (0.1N 10%HCl). Since 1 ml of 0.1 N HCl equivalent to 1.401 mg N, calculation is made to arrive at the nitrogen content of the sample.

$$\%N = \frac{(\text{m1HCl-ml black}) \times \text{normality} \times 14.007 \times 100}{\text{mg sample}}$$

$$\%\text{ Protein} = \%N \times 6.25$$

Because of the simplicity of the estimation of the tryptophan, its content has been used as a criterion for screening materials with superior protein quality. For estimation of tryptophan of opaqe-2 maize materials, papain hydrolysis method was used (18).

A single step papain hydrolysis is utilized for protein solubilization. The iron ions oxidise acetic acid to glyoxylic acid in the presence of sulphuric acid. The indole ring of free tryptophan as well as that bound in soluble proteins reacts with glyoxylic acid and a violet-purple compound is produced. The intensity of the violet- purple color is measured at 545 nanometer with spectrophotometer. By drawing a standard curve of optical density vs. tryptophan concentration, percent tryptophan in sample is recorded

$$\%\text{ trypotophan in protein} = \frac{\%\text{ trypotophan in sample}}{\%\text{protein in sample}}$$

Because of the relationship observed by various researchers (3, 18, 19,) between tryptophan and lysine in the maize endosperm protein, the tryptophan could be used as a single parameter for protein quality evaluation. So by increasing the value of tryptophan by 4 times the value of lysine is obtained.

Means of the various observations were subjected to analysis of variance for drawing appropriate conclusions from the present investigation. The analysis of variance for each character was carried out for the randomized complete block design (20) using the procedure of version 5.1 MSTAT-c. Observations recorded for different characters were averaged for each entry in three-replications separately in each environment.

RESULTS

The analysis of variance for different characters in the three environments (E_1, E_2, E_3) indicated highly significant differences among treatments for all the characters under all the environments (Table 2).

Table 1. Quality Protein Maize genotypes selected for study, North India, 2007.

S.No.	Pedigree code	Present pedigree code
1.	DMRQPM-75-⊗-⊗-#	P_1
2.	DMRQPM-17-⊗-⊗-#	P_2
3.	DMRQPM-18-⊗-⊗-#	P_3
4.	DMRQPM-28-5-⊗-⊗-#	P_4
5.	DMRQPM-03-101-#	P_5
6.	DMRQPM-03-102-#	P_6
7.	DMRQPM-03-121-#	P_7
8.	DMRQPM-03-103-#	P_8
9.	DMRQPM-28-3-#-⊗-#	P_9
10.	DMRQPM-03-125-#	P_{10}

Table 2. Analysis of variance for important economic characters in quality protein maize at E_1, E_2 and E_3 environments, North India, 2007.

Source of variation	d.f.	Environment	Protein	Trypto-phan	Lysine
Replication	2	E_1	13.09	0.16	2.76
		E_2	10.62	0.07	1.25
		E_3	23.40	0.08	1.33
Treatment	56	E_1	5.09**	0.04**	0.72**
		E_2	4.12**	0.04**	0.75**
		E_3	4.96**	0.05**	0.85**
Error	112	E_1	0.01	0.03	0.01
		E_2	0.03	0.01	0.01
		E_3	0.01	0.01	0.01

E_1 = Pantnagar Kharif; E_2 = Gorakhpur Kharif, E_3 = Kashipur Kharif
**= $p<0.01$.

The protein content of 12 genotypes of QPM gave different values (6.87 to 12.02%) for all locations and ranks of genotypes varied considerably. At Pantnagar P4 and P1 gave the highest (12.02%) and the lowest (7.64%) values, respectively. Similarly, P4 gave the highest (10.82 and 11.88%, in order) at Gorakhpur and Kashipurt. P1 gave the lowest (6.87%) at Gorakpur and Kashipur (7.54%). Values obtained for Pantnagar samples were relatively higher than those obtained for Gorekhpur and Kashipur (Table 3).

The tryptophan contents ranged between 0.57 and 0.92. At Pantnagar the highest (0.86%) value was obtained from P1 and the lowest (0.57%) value was recorded from P7. At Gorakhpur the highest (0.87%) tryptophan value was recorded from P1 and P6, where as, the lowest (0.58%) was recorded from P7. At Kashipur P1 and P6 gave the highest (0.92%) values and P7 gave the lowest (0.62%). Generally, values obtained for Gorekhpur samples were higher than those obtained for Pantnagar and Kashipur (Tables 4).

Lysine content varied from 2.27-3.69% in the genotypes. At Pantnagar, P1 (3.44%) and P2 (3.44%) gave the highest value and P7 gave the lowest (2.27). At Gorakhpur P1 and P6 gave the highest (3.49%) and the lowest (2.30%) lysine values. At Kashipur P1 and P6 gave the highest (3.69%) and the lowest (2.43%) lysine value was obtained from P7. As oppose to tryptophan values, lysine values obtained for Kashipur samples are higher than from Pantnagar and Gorekhpur (Table 4).

Table 3. Percentage protein contents of 12 QPM genotypes grown at three locations on a dry weight basis, North India, 2007.

Genotypes	Pantnagar	Gorekhpur	Kashipur	mean
Protein content				
P_1	7.64	6.87	7.54	7.35
P_2	11.16	10.05	11.03	10.75
P_3	11.59	10.43	11.45	11.16
P_4	12.02	10.82	11.88	11.57
P_5	10.33	9.30	10.21	9.95
P_6	9.53	8.58	9.41	9.17
P_7	11.59	10.43	11.45	11.16
P_8	9.93	8.93	9.81	9.56
P_9	11.16	10.05	11.03	10.75
P_{10}	11.16	10.05	11.03	10.75
Check-1	10.33	9.30	10.21	9.95
Check-2	9.53	8.58	9.41	9.17
Grand mean	10.46	9.45	10.37	
CV(%)	0.64	0.63	0.87	
LSD	0.10	0.09	0.87	

Table 4. Percentage tryptophane and lysine contents of 12 QPM genotypes grown at three locations on a dry weight basis

Genotypes	Pantnagar	Gorekhpur	Kashipur	mean
Tryptophan content				
P_1	0.86	0.87	0.92	0.88
P_2	0.69	0.70	0.74	0.71
P_3	0.71	0.72	0.76	0.73
P_4	0.59	0.60	0.64	0.61
P_5	0.64	0.65	0.68	0.66
P_6	0.86	0.87	0.92	0.88
P_7	0.57	0.58	0.61	0.59
P_8	0.77	0.78	0.83	0.79
P_9	0.73	0.75	0.79	0.76
P_{10}	0.78	0.79	0.84	0.80
Check-1	0.79	0.81	0.85	0.82
Check-2	0.75	0.76	0.80	0.77
Grand mean	0.37	0.80	0.66	
CV(%)	2.22	0.84	1.35	
LSD	0.27	0.01	1.35	
Lysine content				
P_1	3.44	3.49	3.69	3.54
P_2	2.74	2.78	2.94	2.82
P_3	2.83	2.87	3.04	2.91
P_4	2.36	2.40	2.54	2.43
P_5	2.54	2.58	2.73	2.62
P_6	3.44	3.49	3.69	3.54
P_7	2.27	2.30	2.43	2.33
P_8	3.08	3.13	3.31	3.17
P_9	2.94	2.98	3.15	3.02
P_{10}	3.13	3.18	3.36	3.22
Check-1	3.17	3.22	3.41	3.27
Check-2	2.98	3.03	3.20	3.07
Grand mean	3.16	3.09	3.20	
CV(%)	2.20	0.81	1.29	
LSD	0.11	0.04	1.29	

DISCUSSION

The present study was planned to determine specifically superior QPM genotypes in protein, tryptophan and lysine content. Highly significant differences among genotypes indicated the presence of inherent genetic differences among treatments for all the characters studied similar with other finding (5).

Grain 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. In this study the genotypes gave different percent protein contents (6.87 to 12.02%) for all locations, and ranks of genotypes varied considerably. The considerable variation in protein content could be attributed to the variation in genetic make up of the genotypes studied. The genotypes studied showed the mean protein content of 9.45 %. Therefore, these genotypes represent the best potential sources of genes for protein. The mean values were generally higher than that of previously reported for normal maize (5). However, this finding is in agreement with protein contents of QPM varieties reported by previous researchers (10-11). P4 gave the highest percent protein content in all environments which is in agreement with the finding of earlier findings (11)**.**

The tryptophan content ranged from 0.50 to 0.92 %, which is lower than the values reported by earlier researchers (11, 12). However, the tryptophan contents of the studied genotypes is much higher than the values reported elsewhere (10). This may be due to difference in the genotypes studied..

Lysine values obtained in this study is similar with previous finding (3), but it is inconsistent with another finding reported by earlier researchers (11).

In the present study, there is a two-fold in the level of lysine and tryptophan contents compared to normal maize which is in agreement with previous finding (8). Normal maize, which is deficient in tryptophan and lysine (13), is the main sources of calorie in developing countries. The nutritional quality of the diet is usually considered unsatisfactory (both for adults and children) when more calorie is derived from cereals (such as normal maize), starchy roots and sugars (21). The high tryptophan and lysine contents of the studied genotypes could raise the quality of the protein and improves the consumers' dietary intake. Since QPM is like eggs and milk, both low in niacin, but offer protection from pellagra because its high level of tryptophan. Compared to skimmed milk, the nutritional value of the studied QPM genotypes is about 90%. This nutritional value of the genotypes meets the requirements of pre-school children for their protein needs (3,8) Moreover, in countries or communities where low protein and tuber crops make up an infant diet, QPM could offer better prospects.

The studied QPM genotypes could serve for different purposes. Clinical studies conducted in hospitals have demonstrated that QPM can prevent and cure severe protein deficiency disease in young children by simply using it as the only sources of protein (2). QPM could be a great weaning food when used alone in maize diets. QPM could be helpful in catch-up growth, particularly in the malnourished and those who are sick (8).

In conclusion, this investigation revealed that genetic factor influences the protein, tryptophan and lysine contents of the QPM genotypes. The finding of this study is also in agreement with the earlier reports that QPM genotypes contain two-fold lysine and higher tryptophan. Moreover, variations in nutritive values do exist among cultivars. Therefore, the QPM genotypes could be incorporated in the breeding program targeted in developing cultivars with high lysine and tryptophan contents to meet protein and aminoacid requirement in developing countries. P1 could be grown by the producers and can be used as sources of protein for both children and adult.

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