ORIGINAL ARTICLE

ANALYSIS OF GRAIN PROTEIN, TRYPTOPHAN AND LYSINE CONTENTS OF QUALITY PROTEIN MAIZE (QPM) LINES

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ABSTRACT

BACKGROUND: ***The nutritional well being and health of all people are vital prerequisites for the development of societies. However, malnutrition still remains a widespread problem, and is particularly severe in developing countries with low per capita income. Maize (Zea mays L.) plays a very important role in human nutrition in a number of developed and developing countries, worldwide. Maize proteins, however, have poor nutritional value for humans, because of reduced content of essential amino acids such as lysine, tryptophan and threonine. Maize proteins contain on an average about 2% lysine, which is less than one-half of the concentration recommended for human nutrition. Therefore, healthy diets for humans must include alternate sources of lysine and tryptophan. Significant advances have been made in genetic enhancement of maize for nutritional value. It is in this context that the value of Quality Protein Maize (QPM) assumes significance, as it signifies a breeding achievement of enhancing grain protein quality in maize. In view of the growing importance of QPM in human nutrition, the objective of this study was to analyze the protein, tryptpphan and lysine contents of QPM lines so as to utilize these genotypes in developing hybrid varieties and bringing its nutritional benefits to fruition.***

METHODS: ***The seeds for this work were obtained from a field experiment conducted in winter 2000 at Hyderabad and in summer 2000 at New Delhi. A total of 89, 50 and 31 genotypes including three checks were selected and evaluated for their endosperm protein, tryptophan and lysine contents using Microkjeldahl, Colourimetric and ELISA methods respectively.***

RESULTS: ***Endosperm protein content ranged from 6.9 to 11.3 (mg/100 mg flour) and genotypes were significantly different from each other ($p<0.01$). A large majority of the Indian as well as CIMMYT Quality Protein Maize (QPM) inbreds displayed higher levels of tryptophan per 100 mg protein in comparison with the non-QPM checks ($p<0.01$). The EF-1α concentration (estimator of lysine content) of a vast majority of the QPM genotypes analyzed was significantly superior to the non-QPM cultivars, except DMRQPM-56 x DMRQPM-44 (0.37) and DMRQPM-66 x DMRQPM-60 (0.39). Endosperm protein content showed a highly significant and negative correlation with tryptophan content in endosperm protein, whereas tryptophan content in flour and in protein showed highly significant and positive correlation***.

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C**ONCLUSION***: Among the genotypes analyzed, DMRQPM-66 could be considered particularly promising in view of its high tryptophan (1.09) and EF-1α (OD of 0.65) contents.*

KEY WORDS: **Quality Protein Maize, Lysine, Tryptophan**

INTRODUCTION

The nutritional well-being and health of all people are vital prerequisites for the development of societies. Unfortunately however, malnutrition still remains to be a widespread problem particularly in developing countries with low per capita income. Globally, nearly 200 million children under five years of age are undernourished for protein, leading to a number of health problems, including stunted growth, weakened resistance to infection and impaired intellectual development (1).

Maize is a major cereal crop for human nutrition, worldwide. Several million people, particularly in the developing countries, derive their protein requirements from maize. The maize grain accounts for about 15 to 56% of the total protein intake of people in about 25 developing countries, particularly in Africa and Latin America (1), where animal protein is scarce and expensive and consequently, unavailable to a vast sector of the population.

But maize proteins have poor nutritional value for monogastric animals including humans, because of reduced content of essential amino acids such as lysine, and tryptophan. Moreover, these animals including humans do not synthesize these amino acids in their body. Maize proteins contain on an average about 2% lysine, which is less than one-half of the concentration recommended for human nutrition by the Food and Agriculture Organization (FAO) of the United Nations (1). Therefore, maize based human diets must contain alternate sources of lysine and tryptophan. From the human nutrition viewpoint, lysine is the first most important limiting amino acid in the maize protein (2-7) followed by tryptophan (8).

This problem has been mainly dealt by supplementing grain with essential amino acids produced by bacterial fermentation although it is highly expensive. Thus, it is valuable to adopt a genetic enhancement strategy in which essential amino acids are either incorporated or increased in grain proteins. Significant advances have been made in genetic enhancement of crop plants for nutritional value. It is in this context that the value of Quality Protein Maize (QPM) assumes significance. QPM is rich in lysine and tryptophan, which are vital to the growth of children.

It is indicated that total protein concentration ranges from 7.95% to 8.2% in QPM lines, and 10.5% to 11.79% in normal maize lines. QPM protein contains significantly higher amount of lysine, argenine, tryptophan and cysteine than normal maize (9). Lysine contents of opaque-2 (the mutant which QPM has been developed) is more than twice that of protein from the normal maize (10). Mean total protein of 7.5%, mean lysine value of 4.5 mg per 100 mg of protein and mean tryptophan value of 0.67 mg per 100mg of protein have been also reported (11). Protein, tryptophan, and lysine contents of 12.5 and 9.9mg, 0.21 and 0.37, per 100 mg of flour, and 1.7 and 3.7 mg per 100mg of protein were reported for normal and o2 genotypes respectively (12). Analysis of the endosperm flour of 93 genotypes also indicated a wide range of variations in

protein and lysine contents of *opaque-2* and normal maize genotypes (13).

In view of the growing importance of QPM in human nutrition, the objective *of* this study was to analyze the protein, tryptophan and lysine contents of QPM lines aimed at developing hybrid varieties and bringing its nutritional benefits to fruition.

MATERIALS AND METHODS

Thirty QPM inbred lines: 14 developed in India, and 16 developed by the International Wheat and Maize Improvement Center (CIMMYT), Mexico were provided as part of the collaborative research and were involved to generate crosses using a diallel crossing system. The seeds for this work were obtained from a field experiment conducted in winter 2000 at Hyderabad and in summer 2000 at New Delhi. A total of 89, 50 and 31 genotypes including three checks were selected and evaluated for the following characters: (a) Protein content per 100 mg endosperm flour; (b) Tryptophan content per 100 mg flour and per 100 mg grain protein, and (c) EF-1α content per 100 mg endosperm flour, which provides an estimate of lysine content in the grain proteins using Microkjeldahl, Colourimetric and ELISA methods respectively.

Laboratory analysis:

a) Grain Protein Content

Total endosperm protein content of grains was analyzed by the standard MicroKjeldahl method (14). Flour samples were prepared from 100 randomly selected seeds of each genotype. The nitrogen percent in the flour was determined by digesting 0.5g of endosperm flour using concentrated H_2SO_4 and 2gm of catalyst mixture (K_2SO4, HgO, and $CuSO_4$ in a ratio of 10.0: 0.4: 0.1), kept for 1.5h in a digestion chamber at 398^0C. The nitrogen in the form of ammonium sulfate from the digested samples was distilled with an automatic distiller (Gerhadt GmBH, Germany) in the presence of 40% NaOH, and the liberated ammonia was collected by 0.1N H_2SO_4 which was estimated by titration against 0.1N NaOH.

The nitrogen values were calculated using the formula:

$$\% N = \frac{(B-S) \times N \times 1.401}{W}$$

where B is the amount of NaOH used for the titration of H_2SO_4 in the control; S is the amount of NaOH used for the titration of H_2SO_4 in the sample; N is the normality of NaOH used in titration; and W is the weight of sample used (Note that 1ml of 0.1N acid is equal to 1.401mg N). Two independent measurements were taken for each genotype to derive the mean values. The nitrogen (N) values were multiplied by 5.7 (1mg of nitrogen equals 5.7 mg of endosperm protein) to estimate the total endosperm protein content (13, 15).

b) Tryptophan content

Nineteen inbred lines, 28 crosses and 3 checks were analyzed for tryptophan content in endosperm flour. Tryptophan content was estimated by the colourimetric method (16). Degermed kernels were course ground, defatted in a sexlet extractor with hexane, and fine ground with an amalgamater. A 100mg flour sample with 4ml papain enzyme (4 mg/ml in 0.1M sodium acetate, pH 7.0) was incubated at 65^oC overnight. One ml of the hydrolysed sample was transferred into a test tube containing 'reagent C' (1:1 v/v of 30N Sulfuric acid and FeCl-glacial acetic acid), and incubated at 65^oC for 15 minutes for colour development. Then, the optical densities (OD) values of the samples were read using spectrophotometer (Systronic-117) at a wavelength of 545 nm. The tryptophan content was determined using a standard curve of a known check. Values

are the average of two independent measurements.

c) **Lysine (EF-1α) content**

Protein extraction was done following the procedure described by Wallace et al (17). Four inbreds and 24 experimental crosses, besides three checks (Trishulata, Parkash and Shakti-1), were analyzed using ELISA (Enzyme Linked Immunosorbent Assay), following the standard procedure (18). Degermed kernels were defatted and fine ground with an amalgameter. The total protein extract was diluted 300-fold in carbonate coating buffer (CCB). Fifty µl of this dilution was mixed with 100µl of CCB ($NaCO_2$ and $NaHCO_2$) in the well of an ELISA plate (Immuno2; Dynex Technologies, Inc., Chantilly, VA, USA), following the protocol suggested by Habben et al (18). After all samples were loaded, a multi-channel pipette was used to make four, three-fold dilutions into the adjacent wells containing CCB. The antigen was allowed to bind by incubating the plate overnight at 4°C. Subsequently, the antigen was removed, the wells were washed twice using TTBS (25mM Trish, pH 7.5; 9g/l NaCl: 0.15 ml/l Tween-20). Then 100 µl primary antibody (rabbit anti-EF-1α serum), diluted 1:1000 in TTBS, was added and allowed to react for 3 hours. The primary antibody was removed and the wells were washed twice with TTBS. The secondary antibody, goat anti-rabbit-IgG alkaline phosphatase conjugate (Sigma) with a dilution ratio of 1:1000 in TTBS was added and allowed to bind for two hours. The secondary antibody was removed and the wells were washed twice with TTBS, and 200µl of alkaline phosphatase substrate (Sigma) diluted in diethanolamine substrate buffer was added. The colour was allowed to develop for 1hour. The optical density (OD) values were read at 410nm using Dynatech Technologies ELISA plate reader.

The ANOVA for protein and tryptophan contents, and the correlation between the two, and mean comparison (Duncan's Multiple Range Test) for protein content were analyzed using SAS statistical package.

RESULTS

In the present study, we assessed the variability in protein, tryptophan and lysine contents from a set of inbred lines and their crosses obtained from India and CIMMYT (Mexico). The genotypes were found to be significantly different for their protein and tryptophan (in protein) contents ($p<0.01$).

Protein content: Protein content in endosperm flour, which ranged from 6.9 to 11.3 (mg/100mg flour) with significant variability among the genotypes. DMRQPM-58 showed the highest protein content (11.3 mg/100mg flour) among the genotypes analyzed (Table 1).

Tryptophan content: The tryptophan content in the endosperm flour and in 100mg protein of genotypes analyzed is presented in table 2. CML150 among the inbred lines, and DMRQPM-65 x DMRQPM-57 among the crosses showed the highest tryptophan values, 1.18 and 1.16 (mg/100mg protein) respectively, indicating the potential of these genotypes for future use.

EF-1α (lysine) levels: The data presented in table 3, show the OD values of the EF-1α content (level of lysine) for the inbred lines and their crosses. Differences in lysine content among the maize genotypes are mostly dependent on the content of non-zein proteins(13).

Table 1. Mean grain protein content of QPM crosses and their parental lines.

Genotypes	Protein content (mg/100 mg flour)	Genotypes	Protein content (mg/100 mg flour
DMRQPM-59	10.0	DMRQPM-37 x DMRQPM-66	10.3
DMRQPM-28	9.6	DMRQPM-37 x DMRQPM-57	9.2
DMRQPM-43	10.5	DMRQPM-37 x DMRQPM-44	9.3
DMRQPM-44	8.8	DMRQPM-37 x DMRQPM-56	9.0
DMRQPM-56	8.5	DMRQPM-37 x DMRQPM-60	9.3
DMRQPM-57	9.1	DMRQPM-43 x DMRQPM-65	9.0
DMRQPM-58	11.3 *	DMRQPM-43 x DMRQPM-58	9.4
DMRQPM-60	8.8	DMRQPM-43 x DMRQPM-37	9.9
DMRQPM-65	9.1	DMRQPM-56 x DMRQPM-44	8.6
DMRQPM-66	9.4	DMRQPM-43 x DMRQPM-56	9.4
DMRQPM-37	9.2	DMRQPM-44 x DMRQPM-56	9.1
CML 193	9.7	DMRQPM-65 x DMRQPM-28	7.4
CML 161	8.8	DMRQPM-44 x DMRQPM-28	9.7
CML 150	7.7	DMRQPM-44 x DMRQPM-59	9.5
CML 175	7.8	DMRQPM-57 x DMRQPM-66	9.2
DMRQPM-60 x DMRQPM-43	8.0	DMRQPM-60 x DMRQPM-65	8.7
DMRQPM-28 x DMRQPM-37	7.8	DMRQPM-58 x DMRQPM-55	7.1
DMRQPM-28 x DMRQPM-56	7.9	DMRQPM-56 x DMRQPM-43	9.2
DMRQPM-55 x DMRQPM-65	8.4	DMRQPM-66 x DMRQPM-65	10.8
DMRQPM-56 x DMRQPM-58	8.9	DMRQPM-44 x DMRQPM-37	9.5
DMRQPM-56 x DMRQPM-57	9.0	DMRQPM-56 x DMRQPM-37	9.2
DMRQPM-57 x DMRQPM-55	8.3	DMRQPM- 56 x DMRQPM-44	9.1
DMRQPM-56 x DMRQPM-55	8.4	DMRQPM-57 x DMRQPM-37	9.2
DMRQPM-56 x DMRQPM-65	8.9	DMRQPM-57 x DMRQPM-44	9.9
DMRQPM-56 x DMRQPM-60	9.9	DMRQPM-57 x DMRQPM-56	9.0
DMRQPM-58 x DMRQPM-60	9.0	DMRQPM-58 x DMRQPM-44	8.9
DMRQPM- 65 x DMRQPM-57	7.3	DMRQPM-58 x DMRQPM-56	9.1

Continued…

DMRQPM- 65 x DMRQPM-58	9.1	DMRQPM-58 x DMRQPM-57	9.1
DMRQPM- 65 x DMRQPM-60	8.7	DMRQPM-60 x DMRQPM-44	9.2
DMRQPM-37 x DMRQPM-58	9.6	DMRQPM-60 x DMRQPM-56	9.8
DMRQPM-66 x DMRQPM-37	10.0	DMRQPM-60 x DMRQPM-57	9.0
DMRQPM-66 x DMRQPM-44	8.3	DMRQPM-60 x DMRQPM-58	9.0
DMRQPM-66 x DMRQPM-56	9.1	DMRQPM-65 x DMRQPM-37	9.6
DMRQPM-66 x DMRQPM-57	9.2	DMRQPM- 65 x DMRQPM-44	9.0
DMRQPM-66 x DMRQPM-58	10.0	DMRQPM-65 x DMRQPM-56	8.9
DMRQPM-66 x DMRQPM-60	9.2	CML 181 x DMRQPM-65	7.3
DMRQPM-66 x DMRQPM-65	8.7	CML 175 x CML 188	7.4
DMRQPM-60 x DMRQPM-37	9.0	CML 161 x CML 175	10.0
DMRQPM-37 x DMRQPM-58	8.6	CML 176 x CML 175	8.9
DMRQPM-37 x DMRQPM-65	9.3	CML 184 x CML 180	7.3
CML 181 x CML 188	6.9*	CML 142 x CML 150	8.5
CML 181 x CML 175	7.7	Trishulata	9.2
CML 161 x CML 176	9.3	Parkash	10.0
CML 176 x CML 186	8.6	Shakti-1	9.3
CML 175 x CML 176	8.8		

* Significant at $P < 0.05$

Table 2. Mean tryptophan per 100 mg endosperm flour and per 100 mg protein contents of genotypes

*** Trishulata and Parkash were used as non-QPM controls and Shakti-1 (*opaque-2* composite) as another check.**

Genotypes*	Tryptophan content (mg/ 100 mg)		Genotypes*	Tryptophan content (mg/ 100 mg)	
	In flour	In protein		In flour	In protein
DMRQPM-28	0.08	0.78	DMRQPM- 65 x DMRQPM-60	0.10	1.14
DMRQPM-37	0.10	1.07	DMRQPM-37 x DMRQPM-58	0.08	0.86
DMRQPM-43	0.08	0.71	DMRQPM-66 x DMRQPM-37	0.09	0.86
DMRQPM-44	0.09	1.02	DMRQPM-66 x DMRQPM-44	0.09	1.08
DMRQPM-56	0.08	0.93	DMRQPM-66 x DMRQPM-56	0.09	1.04
DMRQPM-57	0.10	1.09	DMRQPM-66 x DMRQPM-57	0.08	0.86
DMRQPM-58	0.10	0.85	DMRQPM-44 x DMRQPM-37	0.07	0.75
DMRQPM-60	0.07	0.73	DMRQPM-56 x DMRQPM-37	0.09	0.98
DMRQPM-65	0.08	0.83	DMRQPM- 56 x DMRQPM-44	0.08	0.91
DMRQPM-66	0.10	1.09	DMRQPM-57 x DMRQPM-37	0.09	0.94
CML142	0.08	0.90	DMRQPM-57 x DMRQPM-44	0.09	0.88
CML149	0.08	1.03	DMRQPM-57 x DMRQPM-56	0.07	0.82
CML150	0.09	1.18	DMRQPM-58 x DMRQPM-44	0.10	1.11
CML161	0.08	0.88	DMRQPM-58 x DMRQPM-56	0.10	1.06
CML175	0.08	1.02	DMRQPM-58 x DMRQPM-57	0.10	1.10
CML176	0.09	1.02	DMRQPM-60 x DMRQPM-44	0.10	1.07
CML186	0.07	0.74	DMRQPM-60 x DMRQPM-56	0.10	1.10
CML188	0.09	0.73	DMRQPM-60 x DMRQPM-57	0.09	1.05
CML193	0.07	0.69	DMRQPM-66 x DMRQPM-58	0.07	0.74
DMRQPM-60 x DMRQPM-58	0.08	0.88	DMRQPM-66 x DMRQPM-60	0.08	0.83
DMRQPM-65 x DMRQPM-37	0.09	0.98	DMRQPM-66 x DMRQPM-65	0.10	1.09
DMRQPM- 65 x DMRQPM-44	0.09	1.05	DMRQPM-60 x DMRQPM-37	0.09	1.06
DMRQPM-65 x DMRQPM-56	0.09	1.03	Trishulata	0.07	0.73
DMRQPM- 65 x DMRQPM-57	0.09	1.16	Parkash	0.07	0.65
DMRQPM- 65 x DMRQPM-58	0.10	1.05	Shakti-1	0.09	0.95

Table 3. Mean OD values for EF-1α content per 25mg endosperm flour in selected QPM experimental hybrids and their parental lines

Genotypes*	OD value	Genotypes*	OD value
DMRQPM-44	0.59	DMRQPM-60 x DMRQPM-58	0.57
DMRQPM-58	0.51	DMRQPM-66 x DMRQPM-65	0.51
DMRQPM-65	0.55	DMRQPM-65 x DMRQPM-44	0.40
DMRQPM-66	0.65	DMRQPM-65 x DMRQPM-56	0.61
DMRQPM-44 x DMRQPM-37	0.55	DMRQPM-66 x DMRQPM-58	0.58
DMRQPM-56 x DMRQPM-37	0.62	DMRQPM-65 x DMRQPM-58	0.47
DMRQPM-56 x DMRQPM-44	0.37	DMRQPM-37 x DMRQPM-58	0.59
DMRQPM-57 x DMRQPM-37	0.56	DMRQPM-66 x DMRQPM-37	0.48
DMRQPM-57 x DMRQPM-44	0.62	DMRQPM-66 x DMRQPM-44	0.55
DMRQPM-57 x DMRQPM-56	0.54	DMRQPM-66 x DMRQPM-56	0.48
DMRQPM-58 x DMRQPM-44	0.56	DMRQPM-66 x DMRQPM-57	0.55
DMRQPM-58 x DMRQPM-56	0.58	DMRQPM-66 x DMRQPM -60	0.39
DMRQPM-58 x DMRQPM-57	0.45	Trishulata	0.33
DMRQPM-60 x DMRQPM-44	0.58	Parkash	0.37
DMRQPM-60 x DMRQPM-56	0.64	Shakti-1	0.74
DMRQPM-60 x DMRQPM-57	0.58		

*Trishulata and Parkash were used as non-QPM controls and Shakti-1 (*opaque-2* composite) as another check.

DISCUSSION

In the present study, the percent endosperm protein content of genotypes ranged from 6.9 (CML181 x CML188) to 11.3 (DMRQPM-58), with significant variability among the Indian (DMRQPM) experimental crosses. The percent endosperm protein content in many of the DMRQPM inbreds as well as CML lines was mostly on par with the non-QPM cultivars, Trishulata and Parkash, and Shakti-1, which were used as non-QPM and *opaque-2* 'checks', respectively. Only two inbred lines, DMRQPM-59 and DMRQPM-58, showed relatively higher levels of endosperm protein content in comparison with the popular single-cross hybrid, 'Parkash'. These results are in accordance with those obtained by many researchers who reported a range of 7.4 to 8.4 % (9, 19, 20). Similarly, protein content ranging from 8.3 to 9.7 (mg/100mg flour) was reported in a study involving both normal and *opaque-2* genotypes (21). Wider range of 6.7 to 13.5% for non-QPM genotypes and 6.5 to 11.9% for QPM genotypes was also reported (13).

Total protein content is not the criterion for the preference of QPM genotypes over normal maize as it does not indicate protein quality (level of lysine and tryptpphan in the protein), which is the main objective of QPM breeding. Hence, in the present study, we also analyzed the tryptophan and EF-1α (estimate of lysine) contents of endosperm protein. The analysis in the present study revealed that except for a very few genotypes, almost all QPM lines evaluated in the investigation had higher levels of tryptophan both per 100mg of endosperm flour and 100mg of protein as compared to the normal checks. Several other workers reported similar results (21,22).

The non-QPM or non-opaque genotypes show considerable reduction in the non-zein fraction in comparison with the *o2* mutants and EF-1α was found to be highly associated with the lysine content (18). A very high positive correlation (r^2 = 0.88) and (r^2 =0.91) between EF-1α levels in endosperm and the lysine content was found by many workers (12, 13). The results obtained in this study indicated considerable differences in the EF-1α levels among QPM and non-normal genotypes, with the QPM genotypes clearly showing superiority over the checks. Among the genotypes analyzed, DMRQPM-66 was having the highest OD value (0.65) next to the *opaque-2* composite (Shakti-1), which had 0.74 suggesting the potential of this inbred line for the development of QPM hybrids with high lysine content. These results are in conformity with those reported by others (18). Endosperm protein content showed a highly significant and negative correlation with tryptophan in protein. This is because tryptophan percent in protein is the ratio between tryptophan content in flour and protein content in flour and when the protein content increases the percentage of tryptophan decreases since the major component in the proteins is zein protein, which is devoid of tryptophan.

The endosperm of maize contains a group of four structurally distinct alcohol-soluble proteins called 'zeins'. Their function is to store N, C and S and supply these important elements to the germinating seedling. In normal maize genotypes, zeins usually account for 50 to 70% of the endosperm protein and are characterized by a high content of glutamine, leucine and proline. Since zeins are essentially devoid of lysine and tryptophan, they dilute the contribution of these essential amino acids from the other types of endosperm proteins, which are collectively called 'non-zeins'. In normal maize, proportions of various endosperm storage protein fractions, on an average,

are: albumins (3%), globulins (3%), zeins (60%) and glutelins (34%). Significantly, all fractions other than zeins are balanced in amino acid content and are quite rich in lysine and tryptophan.

As a result of the mutation of the dominant opaque-2 (O2) gene into the recessive gene (o2) in QPM genotypes, the proportion of lysine and tryptophan rich non-zein proteins such as EF-1α is increased and that of the lysine and tryptophan deficient zein proteins is reduced. Hence, reducing the zein fraction and increasing the non-zein proteins is a feasible approach to bring about improvements in the amino acid balance in maize grain.

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