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Research Note

Study of quality parameters among quality protein (QPM) and normal maize inbreds

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Abstract
The present investigation was undertaken to identify the high kernel protein and tryptophan content lines among forty maize inbred lines for utilization in the breeding programme. Analysis of variance revealed significant genetic variation for both protein and tryptophan contents among these inbreds with mean protein and tryptophan content ranging from 7.83-10.67 per cent and 0.38-1.02 per cent, respectively. Among all inbreds, QPM line CML163 showed highest protein content (10.67 %) whereas, two QPM lines CML163 and CML189 showed highest tryptophan content (1.02 %). Thus the inbreds possessing high protein and tryptophan content in maize kernels can be effectively utilized as potential donors in the future breeding programmes for improving the biological value of normal maize inbreds.

Key words
Maize, Inbred lines, Kernel protein, Tryptophan content, Quality Protein Maize

Maize (*Zea mays* L., 2n=20) plays an important role in human and animal nutrition in a number of developed and developing countries and accounts for 15 to 56 per cent of the total daily calories of people in about 25 developing countries (Prasanna *et al*. 2001). Therefore, enhancement of its nutritional status remains a high research priority for agricultural scientists. Normal maize endosperm consists of approximately 9-12 per cent protein, however, it is deficient in two essential amino acids *viz*., lysine and tryptophan. The discovery of nutritional value of the *opaque2 (o2)* mutation in maize was a significant breakthrough which led to the 2-3 folds increase in lysine and tryptophan content (Bressani, 1992). Thus the low nutritive value of maize is genetically corrected in the biofortified form known as Quality Protein Maize (QPM) in which *o2* has been incorporated along with associated modifiers. QPM maize has specific features of balanced amount of amino acids with high content of lysine and tryptophan along with low contents of leucine and isoleucine. The balanced proportion of all these essential amino acid in QPM enhances the biological value of protein as compared to the normal maize.

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. Hence, breeding maize for nutritional quality will serve as a potential tool in addressing micronutrient malnutrition, often called "hidden hunger" which is one of the alarming problems in the developing world, afflicting an estimated three billion people (UNSCN, 2004).
The QPM is 'nutricereal' bred with improved biological value and digestibility. This can serve protein requirements in a person diet, especially children, young, pregnant women and lactating mothers. Thus altering the amino acid profile of maize proteins and making them more balanced, will improve nutrition of hundreds of millions of people without changing their food habits and preferences. Keeping in view the importance and nutritional value of maize the present study was undertaken to analyze the protein and tryptophan content of diverse inbred lines.

The experimental material consisted of 40 maize inbred lines which comprised of 14 QPM and 26 non-QPM lines including 4 checks *viz.,* CML193, CML180 (QPM lines) and CML429, KI 30 (non-QPM lines). These lines along with checks were evaluated for different morphological and quality traits in α-RBD design during *kharif* 2011 in a plot size of 3.0 x 1.2 m^2 with the spacing of 60 cm and 20 cm between rows and plants, respectively (having 2 rows/plot) with 3 replications, 5 blocks/replication and 8 entries/block. The present investigation was carried out at the Experimental Farm of the Department of Crop Improvement, CSKHPKV, Palampur situated at 32°8´ N latitude and 76°3´ E longitude and at 1290.8 m above mean sea level (amsl), representing mid-hill zone of Himachal Pradesh, characterized by humid sub-temperate climate with rainfall of 2500mm per annum having acidic soil with pH ranging between 5.0 to 5.6. Standard agronomic practices were followed for raising a good crop and to maintain the population.

Biochemical analysis: Biochemical analysis of forty maize inbred lines (14 QPM and 26 non-QPM lines) was done to estimate the protein and tryptophan content. After kernel maturation and plant dried, the cob with husk were manually harvested and were dried under the shade to lower the post-harvest grain moisture to 14 per cent.

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Representative grain samples were drawn in duplicate and the individual samples were ground into fine powder. The crude protein content was calculated by Micro-Kjeldhal Method (AOAC, 1965). Two hundred fifty mg of grounded sample of each entry was taken and digested with H_2SO_4. The digested sample volume was made 50ml by adding distilled water and then 5ml of aliquot was taken and equal quantity of 40 per cent NaOH was added and distilled using distillation flask. The distilled material was titrated against acid (HCl, 0.025N). Per cent nitrogen was calculated by using the formulae: N (%)= [Sample titre (ml) – Blank titre (ml)] x 0.014 × Volume of the digest x Normality of the acid by Aliquot taken × Weight of sample (g) × 100. Protein (%) = N (%) × 6.25

Tryptophan content: Tryptophan content for each entry was calculated by the method given by Mertz *et al.* (1975). Hundred mg of powdered sample of each entry was taken in test tubes and 5ml of papain solution (0.4 gm of technical grade papain was added to 100 ml of 0.1 N sodium acetate buffer, pH 7.0, prepared fresh on the day of use) was added and then incubated at 65^0C overnight. The supernatant was collected by cooling and centrifuging the digested material and to one ml supernatant four ml of reagent C (prepared by dissolving 135 gm of $FeCl_3$ .6 H_2O in 0.25 ml of water and diluting to 500 ml with glacial acetic acid containing 2 % acetic anhydride + 30 N H_2SO_4) was added. The mixture was then incubated at 65^0C for 15 minutes and cooled at room temperature. Then, the optical densities (OD) values of the orange-red colour filtrate were read using spectrophotometer at a wavelength of 545 nm. Standard tryptophan solution [5 mg tryptophan in 100 ml hot water (50µg/ml)] was used as blank. The tryptophan concentration in endosperm protein was calculated as:

$$\text{Tryptophan in protein (\%)} = \frac{\text{\% Tryptophan in sample}}{\text{\% Protein in sample}} \times 100$$

Statistical Analysis: Data was statistically analysed using the softwares PROC GLM SAS (Parsad *et al.* 2007).

Analysis of variance revealed that the inbreds were found to be significantly different for kernel protein and tryptophan (in protein) contents ($p<0.05$) (Table 1), suggesting the presence of wider genetic variability among the inbred lines that could be utilized for the genetic improvement of kernel quality traits in maize. Lal and Singh (2014) also reported the presence of significant variations among the maize genotypes for the kernel protein and tryptophan content.

Kernel protein content ranged from 7.83-10.67 per cent with a mean value of 8.90 per cent and tryptophan content from 0.38-1.02 per cent with a mean value of 0.58 per cent (Table 2). Among all inbreds a QPM line, CML163 showed the highest protein content (10.67 per cent) however, two QPM lines CML163 and CML189 had high tryptophan content (1.02 per cent). Jompuk *et al.* (2007) reported that protein content in endosperm of ten inbred lines derived from QPM populations ranged from 7.76 to 8.61 per cent. Lata *et al.* (2014) reported that levels of tryptophan content among normal maize inbreds ranged from 0.66 to 0.85 per cent.

Analysis of correlation among quality traits: Pearson correlation (r) among biochemical parameters of the QPM genotypes in this trial revealed that crude protein was significantly positively correlated with tryptophan (r = 0.429**) suggesting that selection between these traits would be effective. Bello *et al.* (2012) also found significant and positive association between the kernel protein and tryptophan contents. It could be concluded that even though some of the analyzed parameters can help in assorting QPM lines for further breeding process it is still necessary to complement them with agronomical characteristics that play an important role in developing desirable genotypes. The superior QPM lines CML163 and CML189 with high tryptophan content can be used as donor parent in future hybridization programme(s) for converting normal lines into QPM lines after further evaluation.

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Table 1. Analysis of variance of maize inbred lines for protein and tryptophan contents

Traits	Source	Mean sum of square			
		Replication	Blocks within replication	Lines	Error
d. f.		2	12	39	66
Protein content (%)		0.0490	0.0710	1.6320*	0.0400
Tryptophan content (%)		0.0003	0.0005	0.1030*	0.0004

*Significant at P≤0.05, p=parameter

Table 2. Protein and tryptophan content (%) of the analyzed normal and QPM inbred lines

S. No.	Inbreds	Protein content	Tryptophan content	S. No.	Inbreds	Protein content	Tryptophan content
1	VQL 1	9.67	0.74	21	CM 212	7.87	0.49
2	VQL 2	8.83	0.94	22	CL02450	7.93	0.56
3	CML 162	9.63	1.02	23	CML 429**	8.53	0.44
4	CML 163	10.67	0.71	24	CML 451	8.00	0.50
5	CML 169	9.37	0.73	25	CML 470	8.63	0.45
6	CML 170	9.33	0.88	26	CML 472	8.07	0.50
7	CML 171	10.17	0.91	27	CML 473	8.60	0.42
8	CML 180*	10.03	0.96	28	CML 474	8.70	0.47
9	CML 189	9.97	1.02	29	CML 481	8.10	0.54
10	CML 192	9.40	0.77	30	CML 496	9.23	0.43
11	CML 193*	9.30	0.87	31	BAJIM-08-26	7.87	0.46
12	HKI-1348	7.97	0.72	32	BAJIM-08-27**	9.83	0.57
13	CML 451Q	8.67	0.94	33	BAJIM-11-1	8.57	0.41
14	CL02450Q	8.13	0.81	34	BAJIM-11-2	9.10	0.38
15	CM 126	9.27	0.41	35	BAJIM-11-3	8.37	0.46
16	CM 127	9.73	0.40	36	BAJIM-11-4	7.97	0.46
17	CM 128	8.60	0.43	37	KI-16	9.80	0.38
18	CM 129	8.57	0.46	38	KI-18	9.13	0.42
19	CM 145	7.83	0.54	39	KI-29	8.40	0.47
20	CM 152	8.50	0.51	40	KI-30	9.73	0.40

* and ** indicates QPM and non-QPM checks, respectively