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Screening maize genotypes for high quality protein based on assessment of protein and limiting aminoacids

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ABSTRACT : Promising in bred lines of maize genotypes were screened for their protein content and dye binding capacity (DBC) for identifying high lysine lines. The variability in quality was assessed based on its protein content and limiting amino acid lysine. Among the 55 maize genotypes screened, the maize genotypes - UMI 328, IC 361398 IC 552815, IC 447501, and IC538788 were identified as elite one of superior quality. The selected elite genotypes were subjected to a field trial and they were further evaluated for quality parameters. Based on quality index, the maize genotypes IC 361398 and IC 538788 were considered as good quality one as they have QI ratio more than 3.5 as 3.75 and 4.48, respectively. Moreover, the maize genotypes IC 538788 and IC 361398 were found to have 2.28 and 2.04 per cent lysine in protein, respectively and DBC value of 44.43 and 40.47 mg per unit weight of protein, respectively. The genotype IC 538788 reported 0.51 per cent tryptophan in protein. The genotype IC 361398 recorded high crude protein content of 10.79 per cent. Hence, the maize genotypes IC 361398 and IC 538788 with high QI ratio, DBC value and lysine content were identified as elite one for development of QPM.

KEY WORDS : Maize, Quality protein, DBC, Lysine, Tryptophan

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The major maize seed storage proteins, zeins are deficient in lysine and tryptophan content, which contribute to the poor nutritional quality of corn. (Huang *et al.*, 2006). Cereal proteins contain on an average about 2 per cent lysine, which is less than one-half of the concentration recommended for human nutrition by the Food and Agriculture Organization (FAO, 2005) of the United Nations. Owing to low nutritional value, mainly with respect to protein, many efforts have been made to improve the biological utilization of the nutrients it contains. Rapid and reliable determination of lysine content is one of the major limiting factors for QPM breeding programmes worldwide. Lysine measurements made by conventional amino acid analysis are expensive and slow, making them prohibitive for most breeding. The addition of 0.30 per cent L-lysine and 0.10 per cent L-tryptophan increases the protein quality of maize by 150 per cent (Bressani *et al.*, 1968). Owing to genotypic differences, screening genotypes for high protein content and evaluating its quality by assessing the amino acid lysine content will help in identifying the most promising maize genotype suitable for developing quality protein maize and breeding strategies aimed at improving the protein profile of maize will go a long way in reducing

prevalence and persistence of malnutrition in developing world (Sofi *et al.*, 2009).

RESEARCH PROCEDURE

The experiment was carried out in Agricultural College and Research Institute, Killikulam, Thoothukudi Dt, Tamil Nadu during 2010-2012. Promising in bred lines of maize genotypes received from Department of Millets, Centre for Plant Breeding and Genetics, TNAU, Coimbatore (30 accessions) and Germplasm Evaluation Division, National Bureau of Plant Genetic Resources, NBPGR (25 accessions) were utilized for the study. The variability in quality was assessed based on protein content, identification of high lysine lines based on dye binding capacity and quality index.

Protein content :

The crude protein content in the maize kernel of the genotypes was estimated by Microkjeldahl method (Pellet, and Young, 1980). One gram of the sample was digested with 30ml conc. H_2SO_4 for 3 hrs in the presence of catalyst mixture (copper sulphate and potassium sulphate in the ratio of 2:1) to convert the nitrogen in protein to ammonium sulphate. By steam distillation of the salt in the presence of a strong alkali, ammonia is liberated and collected in 4 per cent boric acid solution with mixed indicator (Methyl red and Methylene blue) as ammonium borate. 15 ml of distillate was titrated against standard acid 0.02N HCl by titration. Since 1 ml of 0.1N HCl is equivalent to 1.401 mg N, calculation was made to arrive at the nitrogen content of the sample. On an average most proteins have 16 per cent nitrogen in their composition and hence nitrogen content multiplied by the factor 6.25 gave the crude protein content.

The soluble protein content in the maize kernel of the genotypes was estimated by the spectrophotometric method (Lowry *et al*., 1951). The method developed by Lowry *et al*. (1951) is sensitive enough to give a moderately constant value. The blue colour developed by the reduction of the phosphomolybdic phosphotungstic acid components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the Biuret reaction of the protein with the alkaline cupric tartrate were measured in the Lowry's method.

Dye biding assay :

Dye binding capacity (DBC) of the genotypes were determined by dye binding assay using acrilane orange G for identifying high lysine lines (Mertz *et al.*, 1975). When a solution of the acid diazo dye Acrilane Orange G was mixed with the ground sample, it was bound quantitatively by the basic imidazole, guanidino, and amino groups of the protein, that occur in the polypeptide chain on histidine, arginine, and Iysine or as free terminal groups. The unbound dye remaining in the solution was measured spectrophotometrically after filtration or centrifugation.

Quality index :

The quality of protein indicated by the quality index (QI) value was calculated by dividing the DBC value by the percentage of protein in the whole grain. Values above 3.5 represented good-quality protein.

Among the 55 maize genotypes screened based on quality assays, selected five maize genotypes as elite one. They were subjected to a field trial in the cropping season. Crop management practices weeding, watering, fertilizer application, disease and insect pest control measures were followed as per the recommendations. The seeds were hand harvested, threshed, kept in labeled plastic bags and taken to laboratory for analysis. They were evaluated for quality parameters crude protein, dye binding capacity, quality index, lysine and tryptophan content.

Lysine content :

The low protein quality of maize stems mainly from the deficiency in the protein of the essential amino acids lysine and tryptophan. The limiting aminocid lysine content in the selected five maize genotypes were quantified using dinitropyridine reagent by spectrophotometric method. (Mertz *et al.*, 1975). The papain digest of the sample was treated with a solution of 2-chloro-3,5-dinitropyridine (DNP). 100 mg of the sample was digested with 5ml of papain solution and incubated at 65^0 C overnight. Cooled and centrifuged at 3000g for 5 minutes and collected the supernatant. To 1 ml of the supernatant added 0.5ml of carbonate buffer and 0.5 ml of copper phosphate. Centrifuged followed by addition of 0.1 ml of pyridine solution and kept in shaker for 2 hrs at room temperature. Added 5 ml of 1.2N HCl and extracted 3 times with 5 ml of ethyl acetate using separating flask and discarded the ethyl acetate layer. Read the OD value at 390nm against reagent blank. Standard lysine solutions were also treated simultaneously and calculated lysine content as follows:

Lysine content of sample (g/16g N) = Lysine value in «g × 0.16/ per cent N in the sample

Lysine in protein (%) = % lysine / per cent of protein in sample × 100

Tryptophan content :

The limiting aminoacid tryptophan in the selected five maize genotypes was quantified using Glacial acetic acid reagent by spectrophotometric method (Hernandez and Bates, 1969). 100mg of powdered grain samples was mixed with 5ml of papain solution and incubated at 65°C overnight. Centrifuged and collected the supernatant. To 1ml of supernatant added 4ml of reagent C (Ferric chloride in glacial acetic acid and 30N H_2SO_4) and incubated at 65°C for 15 minutes. Cooled to room temperature and read the orange red colour developed at 545 nm. Standard tryptophan was also treated in similar way and calculated tryptophan content as follows:

Tryptophan content of sample (g/16g N) = Tryptophan value in «g × 0.096 / per cent N in the sample

Tryptophan in protein (%) = % Tryptophan in sample / % of protein in sample × 100

Means of the various observations were subjected to analysis of variance for drawing appropriate conclusions.

RESEARCH ANALYSIS AND REASONING

The findings of the present study as well as relevant discussion have been presented under following heads :

Selection of promising elite maize genotypes based on screening tests :

In the selection of promising genotypes based on the screening tests, those genotypes with elevated levels of crude protein, soluble protein, and DBC value were identified as elite maize genotypes of superior quality (Fig. 1). Because the proportions of basic amino acids and terminal groups are reasonably constant in cereal proteins, the correlations between DBC and total protein content are high. The genotypes screened were found to have crude protein content ranged from 7.01 per cent - 12.25 per cent and mean protein content of 9.57 per cent. Vasal (2005 and 1993) and Villegas and Mertz (1970) also reported similar protein content in maize ranging from 8 - 11 per cent and 8.9 -10.2 per cent, respectively. Significant variation in protein content could be attributed to the variation in genetic makeup of the genotypes studied as reported by Sentayehu (2008) in a similar investigation on protein, tryptophan and lysine contents in quality protein maize. Moreover, the mean DBC value was found to be 37.97 mg per unit weight of protein. As elevated lysine content as observed by DBC and total protein content in maize are heritable, among the 55 maize genotypes screened, five maize genotypes with high protein content and DBC value - UMI 328, IC 361398, IC 447501, IC538788 and IC 552815 were selected as the promising genotypes of superior quality for further analysis.

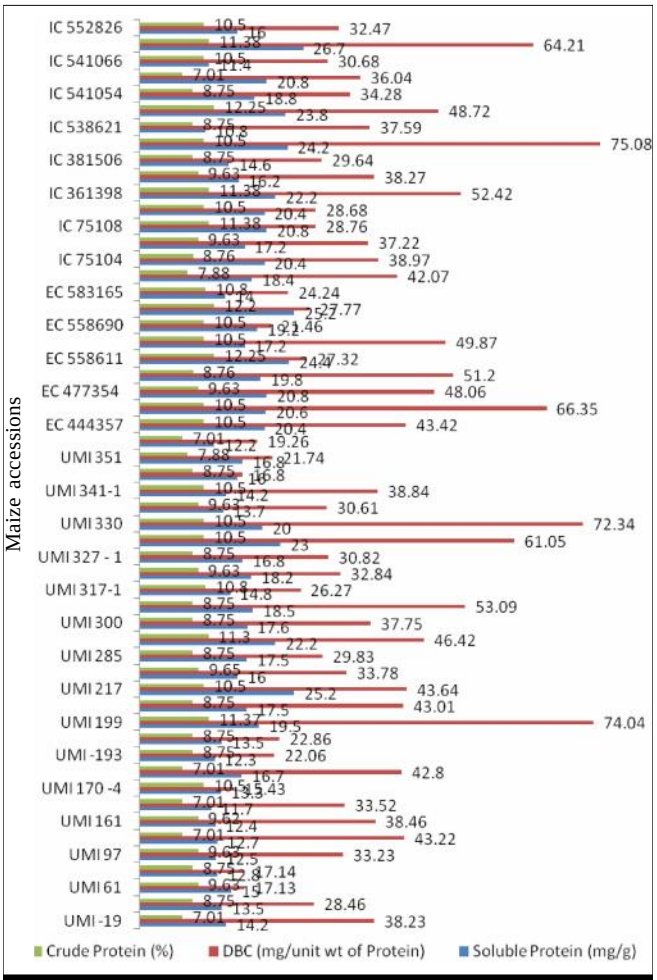

Fig. 1 : Screening maize genotypes based on protein content and dye binding capacity (DBC)

Assessment of promising maize genotypes based on quality traits :

Crude protein, dye binding capacity and quality index:

The crude protein, dye binding capacity and quality index of the selected five maize genotypes UMI 328, IC 361398, IC 447501, IC538788 and IC 552815 are given in Table 1. The mean protein content of maize genotypes was found to be 10.206 per cent. The maize genotypes -

IC 361398 and UMI 328 were found to have high crude protein content of 10.79 per cent and 10.49 per cent, respectively. The genotypes - IC 538788 and IC 361398 recorded high DBC values of 44.43 and 40.47 mg per unit weight of protein indicating high lysine lines. The genotypes IC 361398 and IC 538788 had high quality index ratio of 4.48 and 3.75, respectively. Generally those genotypes with QI above 3.5 were identified as good quality one. Hence, based on the ratio of quality index the maize genotypes - IC 361398 and IC 538788 were selected as the elite one.

Similar results have been obtained when dye binding capacity (DBC), complemented by Kjeldahl nitrogen determination was used to obtain the protein quality index (DBC/ % Protein) in samples of maize, barley and triticale. There was a significant correlation between QI and basic amino acid content, permitting the identification of grains with high lysine content.

Lysine and tryptophan content :

Among the selected five maize genotypes, the maize genotypes - IC 538788 and IC 361398 recorded high lysine content of 2.28 and 2.04 per cent lysine in protein whereas the maize genotypes - IC 552815, IC 538788 and IC 447501 were found to have high tryptophan content of 0.54, 0.51 and 0.50 per cent tryptophan in protein, respectively. Hence, based on the limiting aminoacid content, the genotype IC 538788 could be identified as the most promising genotype as it was reported to have high lysine as well as tryptophan content which is evident from Table 2. However, the lysine and tryptophan content showed no significant variation among the genotypes. It has been suggested that concentrations of lysine and tryptophan are correlated in maize grains (Hernandez and Bates, 1969).

Lysine and tryptophan are present in a roughly constant ratio of 4:1. Bressani *et al.* (1968) have stated that the addition of 0.30 per cent L-lysine and 0.10 per cent L-tryptophan increases the protein quality of maize by 150 per cent. Misra *et al.* (1975) have reported that lysine content in various inbred lines of corn are quite similar in the narrow range of 1.6 -1.8 per cent but varying levels of free aminoacids were observed. Vasal (2005) reported similar results for lysine content as 1.80 to 2.0 per cent in maize

Table 1 : Crude protein, DBC and quality index of the selected maize genotypes

Sr. No.	Genotypes	Crude protein (%)	DBC (mg per unit wt of protein)	Quality index
1.	UMI 328	10.49	27.45	2.63
2.	IC 361398	10.79	40.47	3.75
3.	IC 447501	10.20	29.47	2.89
4.	IC 538788	9.91	44.43	4.48
5.	IC 552815	9.62	32.60	3.39
Mean		10.205	34.8867	
S.E. ±		0.4178	5.1374	
C.D. (P=0.05)		0.9103 (NS)	11.1936*	

* indicate significance of value at P=0.05 NS=Non-significant

Table 2: Lysine and tryptophan content in the selected maize whole kernel

Sr. No.	Genotypes	Lysine in protein (%)	Tryptophan in protein (%)
1.	UMI 328	1.90	0.39
2.	IC 361398	2.04	0.44
3.	IC 447501	1.96	0.50
4.	IC 538788	2.28	0.51
5.	IC 552815	1.66	0.54
Mean		1.9687	0.4762
S.E. ±		0.3886	0.0644
C.D. (P=0.05)		0.0846	0.1402
		NS	NS

NS=Non-significant

genotypes. Scott *et al.* (2004) found that tryptophan levels were negatively correlated with endosperm translucence, a measure of kernel hardness suggesting the process of selection for hard kernels reduces tryptophan levels and they reported tryptophan levels in white normal inbreds as 0.534 per cent.

To conclude, the study revealed that genotypic variations in protein content and limiting aminoacids do exist among different maize accessions and genetic factor influences the protein, lysine and tryptophan contents of the quality protein maize genotypes. The findings of the study are also in agreement with earlier reports. As elevated lysine content as observed by DBC and total protein content in maize are heritable, the maize genotypes IC 361398 and IC 538788 with high QI ratio, DBC value and lysine content were identified as the elite maize genotypes of superior quality protein and they could be used for development of quality protein maize. The incorporation of QPM genotypes in the breeding programme targeted in developing cultivars with high lysine and tryptophan contents would meet protein and aminoacid requirement in developing countries.

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