

Assessment of quality protein parameters of *Zea mays* L. through high throughput biochemical analysis

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Maize is one of the world's mostly cultivated crops which are consumed by the humans. The quality of maize protein is a vital factor in combating human malnutrition, a problem that is rapidly increasing among children. This study focuses on enhancing maize's protein quality by increasing lysine and tryptophan concentrations while reducing zein content. Seventeen in-house maize lines were analyzed for key biochemical characteristics. Among these, the CML161 line stood out, with a total protein content of 11.831%. The tryptophan and lysine contents in CML161 were 0.68% and 3.3%, respectively. Additionally, the total starch content in CML161 was found to be 37.8%. The endosperm modification test showed 100% opaqueness in the CML161 variety. Based on these findings, it can be concluded that CML161 contains a quality protein content of 5.74%, making it a promising candidate for further studies aimed at addressing malnutrition.

Keywords: Lysine, Maize quality index, Tryptophan, *Zea mays* L.

Zea mays L. is one of the widely cultivated cereal crops which is used as a staple food in many parts of the world. Maize is also used as a fodder for the livestock and poultry¹. Among the total protein of maize, 70% is occupied by the prolamin which is also termed as zein. Presence of excess zein content shows the limitation of two essential amino acids tryptophan and lysine. The amino acid deficient maize protein does not meet the balanced nutrition of the humans and livestock². 60% of the daily protein requirements of humans are supplied by the maize^{3,4}.

Many maize cultivars grown globally lack the essential amino acids *i.e.*, tryptophan and lysine which are the crucial factors in the growth and development of livestock, poultry and humans⁵. Research reports show that the maize quality protein reduces the risks of malnutrition and various diseases⁶. Deficiency of lysine and tryptophan causes depression, loss of appetite, retarded growth, anxiety and stress in children⁷. In the scenario, development of tryptophan and lysine rich maize varieties are much encouraged⁸. Daily maize consumers without protein, lysine and tryptophan

supplementation suffer from malnutrition. Therefore, maize food along with high quality protein, lysine and tryptophan are much needed.

India is the first country to develop hybrid variety with high protein quality. Selections of recurrent locate and the recurrent parent will lead to the generation of new desired variety⁹. Developing quality protein maize varieties is time consuming as only 50% of the recurrent parent alleles are recovered from the back cross. To deal with the above stated drawback, use of molecular markers is brought into the lime light which is an easy tool to select the interested genotypes. Single sequence repeats (SSR) markers are widely used to map opaque-2¹⁰. After the discovery of the opaque-2 (o2) allele into the normal maize cultivars showed increase in the maize quality protein^{11,12}. Research reports states that mutation in the o2 allele improves the lysine content in the endosperm of maize compared to the wild cultivar¹³. Similarly, homozygous state of the o2 allele decreases the production of α -zein and increases the production of lysine¹⁴.

The present study focuses on screening the high lysine and tryptophan content maize variety and studying the other biochemical parameters.

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Material and Methods

Collection of maize lines and processing the seed materials

The maize lines were collected from the Maize Research Centre (MRC) of Nuziveedu Seeds Ltd. Hyderabad, India. The samples were cleaned and screened on the basis of opaqueness. The material was discovered to be nearly uniform. Kernels were separated from maize cobs and dried in the sun to reduce the moisture content to 13%. These kernels were milled using a grinder and the resulting powder was sieved using standard 30 mesh sieve. Fine flour of each genotype's was packed gamma irradiated HDPE bags and stored at 4°C before being processed for further studies.

Estimation of total protein (micro-kjeldahl)

The total protein content was estimated using kjeldahl method. The fine powder of each maize line was digested using concentrated sulfuric acid, which serves as an oxidizing and dehydrating agent. As a result, the sample's carbon is oxidized to carbon dioxide, which escapes as a gas. Ammonium sulphate, which is a form of ammonium, retains nitrogen in the sample. By adding sodium hydroxide, it is transformed into ammonia, which is then distilled off, absorbed in boric acid solution, and titrated with regular hydrochloric acid. To prevent moisture-related protein quality analysis contamination, the powdered samples were stored in a desiccator. A conversion factor of 6.25 was used for the conversion of nitrogen to protein content¹⁵.

Estimation of tryptophan and lysine

The colorimetric method was used to determine the tryptophan content¹⁶. In a 15 mL Falcon tube, 80 mg of defatted powder (hexane washed) was added along with 3 mL of papa in solution. Samples were thoroughly vortexed and incubated for 16 h at 64°C in an oven (overnight). Take out the tubes after incubation and allow them to cool at room temperature. The tubes were vortexed and centrifuged for 5 min at 3600 rpm. To 1 mL of supernatant, add 3 mL of the colorimetric reagent (0.9205 g of glyoxylic acid in 100 mL of 7N H₂SO₄) to each tube. After thorough vortexing the tubes, they were placed in an oven set at 64°C for 30 min to allow for colour development. Cool the tubes and measure the absorbance using a BIO-RAD X-Mark Microplate spectrophotometer at 560 nm. With known tryptophan concentrations ranging from 0 to 30 g/mL, a standard calibration curve was

created to calculate the tryptophan content¹⁷. Similarly, lysine content was estimated using the standard procedure¹⁸.

Calculation of maize quality index (QI)

Maize quality index was calculated using the tryptophan to protein ratio in the sample which is also stated as maize quality index (QI). Maize quality index was calculated using the following formula:

$$QI = 100 \times \text{tryptophan content} / \text{total protein content}^{19}.$$

Determination of total soluble sugars in the maize seeds

The anthrone method was used to measure the amount of total soluble sugar (Laboratory Protocols - 2008, CIMMYT). Each sample contained 20 mg of dried powder in a 15 mL Falcon tube and added 4 mL of deionized water and thoroughly vortexed. Samples were thoroughly mixed by vortex for every 15 min during a 45min incubation period at 70°C in a water bath. Samples were cooled on ice packs after incubation and centrifuged at 3000 rpm for 10 min. Supernatant was collected and diluted 1:20 deionized water. Transfer 2.5 mL of into separate tube and under cold conditions 5 mL of anthrone reagent. The tubes were boiled in a water bath for 7.5 min at 70°C. Cool the tubes and record the absorbance values at 630 nm using BIO-RAD XMark Microplate spectrophotometer. The obtained absorbance values were calculated using the standard graph and results obtained were measured in mg/g²⁰.

Endosperm modification studies

Endosperm modification studies were carried out by light box screening test. The modification score is referred to as the degree of opaqueness of the maize seeds. The seeds for the test were collected from the centre of the cob. Normal maize seeds have 0% opaqueness and the modification score is 1. The quality protein maize has the modification score in between 2-4 which has almost 100% opaqueness²¹.

Results and Discussion

Maize is considered as one of the staple foods for the humans, livestock and cattle all over the globe. Through maize contains around 70-75% of the total starch content, it also contains 8-13% of total protein²². In general, the maize protein contains high amount of zein and trace amounts of lysine and tryptophan. As majority of the population depends on the maize, the deficiencies of lysine and tryptophan lead to malnutrition in the humans¹⁵. To address the

issue of increasing the lysine and tryptophan in the maize protein, the present study focussed on screening the in-house maize lines of Nuziveedu seeds private limited, Hyderabad for the high protein, lysine and tryptophan content.

Generally, maize contains 3% of globulins, 3% of albumins and 60% of zein in the endosperm. Other than zein, rest of the protein content has abundant amount of lysine and tryptophan. High content of zein may be the cause for poor content of lysine and tryptophan which is the key parameter for the quality maize protein. Reduction in the zein protein content proportionately increases the tryptophan and lysine contents⁶.

Prior assessing the lysine and tryptophan content in the maize endosperm, it is important to assess the total protein content. Total protein in all the in-house maize lines were assessed using Kjeldahl apparatus. Total 17 maize lines were tested for the total protein content among which the maize lines CML161 showed high protein content *i.e.*, 11.831% followed by 3003 with 11.047% (Fig. 1).

The protein content results obtained in the present research are in coordination with the results obtained for the drought tolerant maize varieties (11.2-14.29%) from the Maize research centre of Yugoslavia²³. Similar results were recorded for the maize varieties of Pantnagar, Kashipur and Gorakhpur which range from 4.36-23.04%²⁴. The maize lines were tested for the total tryptophan content which is key essential amino acid in a quality protein. Among all the maize lines tested, CML161 showed 0.68% followed by 3003 (0.64%) (Fig. 2).

The drought resistant maize lines from the Maize research centre of Yugoslavia also showed lower values ranging from 0.065 – 0.088%²³. Whereas, maize lines from Pantnagar, Kashipur and Gorakhpur

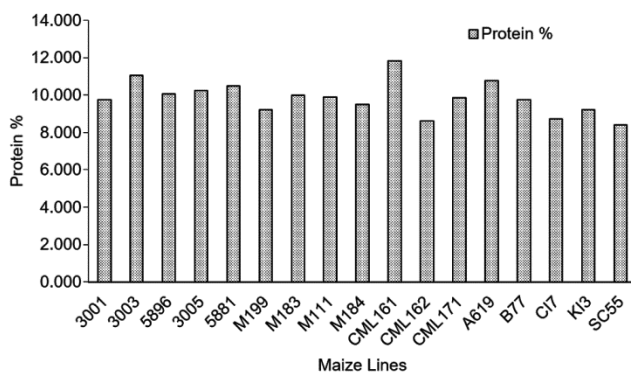


Fig. 1 — Maize lines showing the total protein content in the maize seeds

showed similar values of tryptophan content *i.e.*, 0.57 – 0.86%²⁴. Similar research results were obtained for the inbred maize lines *i.e.*, 0.38-0.67%²⁵. In the present study the maize quality protein of CML161 is estimated to be 5.74%.

Lysine content in the maize lines was tested, among which CML161 showed high value with 3.3% and 2.6% in 3003 variety (Fig. 3). Similar results were also recorded in the maize lines of Pantnagar, Kashipur and Gorakhpur which recorded the values in between 0.11 – 3.44%²⁴. The result obtained in the present research is also in par with the Chicken Itza and Sac Beh maize varieties which showed the values in between 1.99 – 2.91%¹⁵.

Starch content was also assessed in the maize seeds in addition to the total protein, tryptophan and lysine contents. Among all the in-house maize lines tested, CML161 showed high starch content 37.8% followed by 27.8% in 3003. Whereas drought tolerant varieties from the Maize research centre of Yugoslavia showed starch content in between 67.5- 71.3%²³.

The endosperm modification studies were carried out using light screen test, which showed opaqueness score of 4 for CML161 maize variety followed by

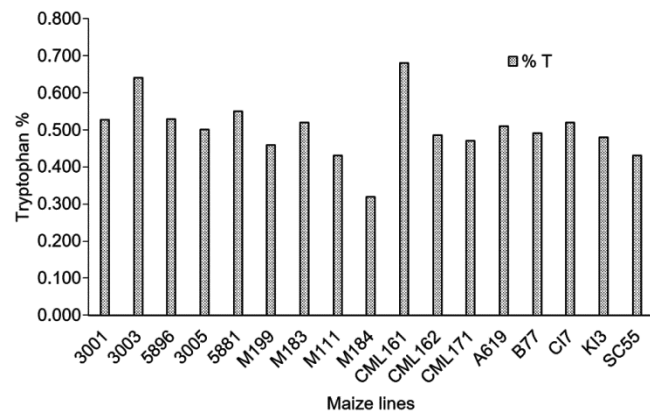


Fig. 2 — Maize lines showing the total tryptophan content in the seeds

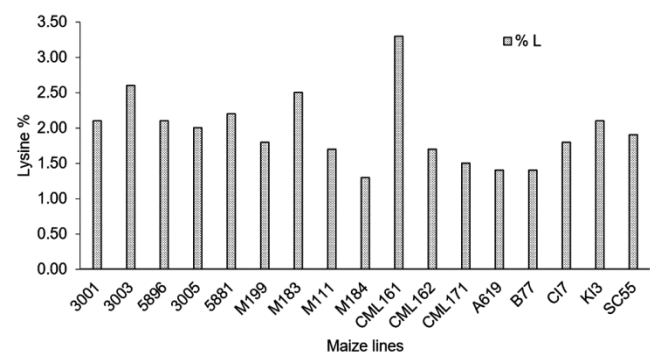


Fig. 3 — Maize lines showing the total lysine content in the seeds

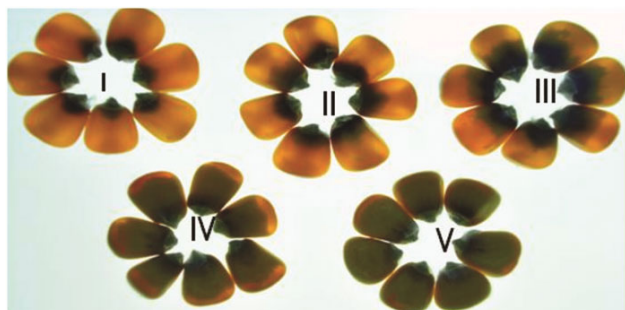


Fig. 4 — Maize lines showing various degrees of opacity, I – 0% opacity; II - 10% opacity; III - 25% opacity; IV - 75% opacity (3003 variety); V – 100% opacity (CML161)

3003 with score 3. This shows that both the varieties have quality protein in the maize endosperm (Fig. 4).

Conclusion

The QPM lines and commercial lines analysed in this study show significant lysine and tryptophan content. Notably, CML161 demonstrates superior levels with 3.3% lysine and 0.68% tryptophan, along with a protein content of 11.831%. As a result of these outcomes, CML161 has been identified for further utilization in back-cross breeding programs.

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Conflict of interest

The authors have no conflict of interests

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