



RESEARCH ARTICLE

Genetic variation for methionine, tryptophan and lysine in maize (*Zea mays* L.) inbred lines

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Abstract

A lack of key amino acids, including lysine, tryptophan and methionine causes nutritional imbalance in maize (*Zea mays* L.) grain protein. The present investigation was carried out to determine the genetic variation for kernel methionine, lysine and tryptophan content in 25 promising maize inbred lines. ANOVA revealed significant differences between the genotypes for methionine (1.56–2.96%), lysine (1.90–3.68%), and tryptophan (0.51–0.92 %) content. QIL-4-2831 (2.96%), QIL-4-2829 (2.60%), QIL-4-2830 (2.44%), QIL-4-2311 (2.42%) and QIL-4-3080 (2.39%) had the highest mean methionine content. The present findings also indicated that there was no significant correlation between methionine and lysine ($r=0.14$), nor between methionine and tryptophan ($r=0.09$). However, lysine and tryptophan were shown to have a positive correlation ($r=0.84^{**}$). The high methionine lines can be used for developing high methionine cultivars in future crop improvement programs.

Keywords: Maize, high methionine, lysine, tryptophan, grain yield

Introduction

Maize (*Zea mays* L.) being an “a-maize-ing” crop, is one of the most productive, efficient, and widely adapted food-grain crops, globally feeding around 900 million people with 193.7 mha area under cultivation producing 1147.7 million MT (FAO 2020). Due to its exceptional genetic yield potential in comparison to all other cereals, it is frequently referred to as the “Queen of Cereals”. Maize originated and domesticated in south-central Mexico. The semi-domesticated maize from here traveled to the Americas and other parts of the world (Singh 2023). Despite its widespread use, normal maize protein is deficient in certain amino acids such as lysine, tryptophan, and methionine in the prolamine fraction, which comprises the largest proportion of storage proteins, causing a nutritional imbalance in maize grain protein (Darrigues et al. 2005). Neither animals nor humans are capable of synthesizing these amino acids. Therefore, nutritionally balanced maize is of utmost importance given that a significant portion of the maize produced serves

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as both feed and food. To achieve nutritional balance, legumes are frequently incorporated into animal feed mixes along with maize. However, methionine continues to be a limiting amino acid in these mixtures since no legume can fully compensate for the deficit (Scott et al. 2004). Hence, in an animal feed mixture, the concentrations of methionine alongside lysine, and tryptophan hold greater significance.

In 1920, a recessive mutant known as *opaque-2* (*o2*) was identified in maize fields in the USA by Singleton and Jones and this mutant resulted in increased lysine and tryptophan content in maize endosperm. Eventually, many QPM lines/cultivars have been developed using this “*o2* mutant” with increased lysine and tryptophan content. However, major efforts were limited only to improve the lysine and tryptophan in maize kernels whereas, limited efforts for breeding methionine-rich maize are reported. Traditional maize grains with the dominant *O2* allele accumulate low amounts of lysine (1.5–2.0% of protein) and tryptophan (0.3–0.4% of protein) whereas lysine (3.0–4.0% of protein) and tryptophan (0.7–0.9% of protein) content is almost double in grains carrying only recessive *o2* alleles (Hossain et al. 2019).

Methionine is a limiting amino acid in livestock feed especially standard maize-soy diet intended for laying hens and lack of methionine in their diet leads to low egg yield and reduced poultry size (Bertram and Schutte 1992). Methionine, besides being utilized as an animal feed additive, is also a vital amino acid for human consumption with the mean daily methionine need of 12.6 mg/kg of body weight/day (Di Buono et al. 2001), while in the context of India, it is 15 mg/kg (Kurpad et al. 2023). Methionine plays a crucial role in protein synthesis and is essential for proper development and growth in humans (Finkelstein 1990). A deficiency in methionine is linked to fatty liver, atherosclerosis, neurological problems, and tumor development (Fukagawa 2006).

The poultry diet is generally supplemented with synthetic methionine. The supplementation of a maize-soya diet to make up for methionine deficiency leads to increased feed costs for which inexpensive supplements are lacking (Darrigues et al. 2005). Globally, the sales of synthetic methionine to animal producers exceed one billion dollars annually (Lai and Messing 2002). In the context of declining fossil resources and strengthening environmental pressures, there is a growing interest in alternative, more sustainable, natural resource-based processes. Crop improvement requires understanding the genetic diversity of targeted traits. The availability of suitable donor lines in germplasm helps breeders develop desired cultivars (Hossain et al. 2022). There are very limited studies revealing genetic variation for kernel methionine along with lysine and tryptophan. Therefore, the present study was aimed at the assessment of genetic variation and identification of promising maize

inbred lines rich in methionine and its relationship to lysine and tryptophan.

Materials and methods

Experimental material

About 25 maize inbreds procured from ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana were selected for the present research investigation (Table 1). The germplasms were selected from the materials received from CIMMYT, Mexico during the year 2016-17. The EC numbers of the germplasm are presented in Table 1. The segregating populations received from CIMMYT were bred for methionine in the QPM background. Over the year selection for *per se* performance was done and selected genotypes were selfed to derive the homozygous inbred lines followed by biochemical estimation for methionine content. The selected genotypes (25) were sown in a randomized block design (RBD) in two replications at four locations *viz.*, (i) ICAR-IIMR Ludhiana (Punjab) (28.985N, 77.225E), (ii) HAREC-Bajaura (Himachal Pradesh) (31.835N, 77.171E) (iii) CCS-HAU-Karnal (Haryana) (29.142N, 75.703E) and (iv) OUAT-Bhubanesvar (Odisha) (20.266N, 85.810E) during Kharif 2023. A total of 40-50 seeds of each experimental line were sown in two rows (2-meter length), 70 cm row to row, and 20 cm from plant to plant and selfing was done. To raise a quality crop in each of the four locations, standard agronomic procedures were adhered to. The seeds of selfed cobs were used for the estimation of methionine, lysine and tryptophan.

Estimation of methionine, lysine and tryptophan

Methionine was estimated as per the methodology given by Gupta and Das, 1954. Tryptophan was estimated using the papain hydrolysis method (Hernandez and Bates 1969). The lysine was estimated using the papain hydrolysis method which involves the release of protein-bound lysine through enzymatic hydrolysis of the sample protein (Tsai et al. 1972).

Evaluation of yield-related components

Grain yield (GY) and yield related components *viz.*, days to 50% anthesis (DA)-duration from planting to 50% of the pollen shedding; days to 50% silking (DS)-duration from planting to 50% silk emergence were recorded; plant height (PH)-five plants were randomly selected, and their height from the base to the tassel-bearing node at maturity was measured in centimetres using a measuring rod, and the average plant height was calculated; ear height (EH)-measured at the dry silk stage in centimetres from the base of the plant to the base of the highest cob on the same randomly selected 5 plants whose plant height was recorded, and the average was calculated; cob length (CL)-measured in centimetres from the base to the filled grain point of all the cobs collected from the 5 plants; cob girth

(CG)-measured in centimetres at the centre of each cob from all the cobs collected from the 5 plants, and the mean was calculated; number of kernels/row (NKR)-number of seeds per row in each ear of 5 plants were counted and averaged; number of rows-the number of kernel rows in each ear of 5 plants were counted and averaged and grain yield (GY)-calculated in q/ha by considering the plot size and yield per plot. Furthermore, using the above lines the developed hybrids were further evaluated for grain yield.

Statistical analysis

To analyze the data collected for various parameters *i.e.* methionine, lysine, and tryptophan, the R software “agricolae” package (version 4.2.1) was used (Mendiburu and Simon 2009). Analysis of variance (ANOVA) for each of the amino acids was also performed in the Agricolae package in R software. The R software “variability” package (version 4.2.1) was used for the estimation of variability parameters. Correlation among the amino acids was computed using Pearson’s procedure (Benesty et al. 2009).

Results

Genetic variation for lysine, tryptophan and methionine content

A significant variation in methionine content was recorded varying from 1.56 to 2.96% with a mean of 2%. The QIL-4-2831 (2.96%) had the highest mean methionine content followed by QIL-4-2829 (2.60%), QIL-4-2830 (2.44%), QIL-4-2311 (2.42%) and QIL-4-3080 (2.39%). There was significant variation for tryptophan content which varied from 0.51 to 0.92% with a mean of 0.71%. The QIL-4-3080 (0.92%) had the highest mean tryptophan content followed by QIL-4-2836 (0.86%), QIL-4-2828 (0.81%), QIL-4-2830 (0.76%), QIL-4-2160 (0.75%). There was significant variation for lysine content which varied from 1.90 to 3.68% with a mean of 2.88%. The QIL-4-3080 (3.68%), QIL-4-2836 (3.42), QIL-4-2306 (3.30%), QIL-4-2828 (3.24%) and QIL-4-2192 (3.21%). Out of 25 lines, there were some lines rich in all three amino acids. The promising inbreds are rich in methionine, tryptophan, and lysine content includes QIL-4-2784 (methionine-2.35%, tryptophan-0.74%, lysine-3.18%), QIL-4-2800 (methionine-2.36%, tryptophan-0.73%, lysine-3.03%), QIL-4-3080 (methionine-2.39%, tryptophan-0.92%, lysine-3.68%), QIL-4-2825 (methionine 2.38%, tryptophan-0.71%, lysine-2.95%), QIL-4-2829 (methionine-2.60%, tryptophan-0.72%, lysine-2.92%), QIL-4-2830 (methionine-2.44%, tryptophan-0.76%, lysine-3.13%), QIL-4-2831 (methionine-2.96%, tryptophan-0.73%, lysine-3.05%) and QIL-4-2311(methionine-2.42%, tryptophan-0.70%, lysine-2.89%) (Table 1).

Analysis of variance for methionine, tryptophan, and lysine content

It revealed significant differences among the genotypes at a

0.1% level of significance for all the traits studied. Significant differences ($p < 0.05$) were observed in all three traits across different environments. The interaction between genotype and environment ($G \times E$) also exhibited a significant difference ($p < 0.01$) for all three amino acids (Table 2).

Study on genetic variability parameters

The estimates of variability parameters like the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2), and genetic advance as a percentage of the mean for methionine, lysine and tryptophan have been given in Table 3. This information helps the breeders to identify stable traits to be considered during effective selection for crop improvement.

The GCV for methionine, tryptophan, and lysine was observed to be 38.49, 24.50, and 29.13% respectively, whereas, the PCV for methionine, tryptophan, and lysine was observed to be 38.53, 24.58, and 29.19%, respectively. The PCV and GCV estimates were high for all these traits. Additionally, it was noted that the PCV values were slightly higher than the GCV values, with minimal fluctuations for all traits across replications. Heritability indicates the heritable portion of the total phenotypic variation in the population. It reflects the extent to which a certain trait is transmitted from parent to offspring. The estimates of heritability (broad sense) for methionine, tryptophan and lysine have been presented in Table 3. All traits were found to have a high degree of heritability (>95 %).

In the current study, the genetic advance as a percentage of the mean was 79.22, 50.30 and 59.86 for methionine, tryptophan and lysine content. The genetic advance represents the amount of improvement for a particular trait in a new population after selection as compared to the original population from which the selection was made. It mainly depends on three parameters, namely, variability in the original population, the heritability of the concerned trait, and the selection intensity. Genetic advance as a percent of the mean (GAM) is generally used to estimate effective selection indices.

Correlation analysis

The findings of the study indicated that there was no significant correlation between methionine and lysine ($r = 0.14$), nor between methionine and tryptophan ($r = 0.09$). Lysine and tryptophan were shown to have a positive correlation ($r = 0.84^{**}$), suggesting that the two amino acids are related. The correlation coefficients between methionine, tryptophan and lysine have been presented in Fig. 1.

Variation for yield and its related traits

The highest grain yield was recorded in QIL-4-2306 (30.65 q/ha) with a mean value of 25.8 q/ha. Among the 25 inbreds, QIL-4-2306 (30.65 q/ha), QIL-4-2300 (29.53 q/ha), QIL-4-2295

Table 1. Mean methionine, tryptophan and lysine content of the lines used in the experiment

S. No.	Genotype	Source/Pedigree	EC no.	Methionine (%/100g protein)	Tryptophan (%/100g protein)	Lysine (%/100g protein)
1	QIL-4-2784	E2-7	EC 552077	2.35	0.74	3.18
2	QIL-4-2800	E3-25-1-1	EC 658478	2.36	0.73	3.03
3	QIL-4-3080	E4-1-1	EC 658478	2.39	0.92	3.68
4	QIL-4-2825	E5-18-2-1-2	EC 551974	2.38	0.71	2.95
5	QIL-4-2828	E6-16-1-B	EC 698197	1.70	0.81	3.24
6	QIL-4-2829	E6-20-1-1-C1	EC 698197	2.60	0.72	2.92
7	QIL-4-2830	E6-21-1	EC 698197	2.44	0.76	3.13
8	QIL-4-2831	E6-21-1-2	EC 698197	2.96	0.73	3.05
9	QIL-4-2836	E6-36-1-C2	EC 698197	1.56	0.86	3.42
10	QIL-4-2840	E7-11-2-1	EC 698163	1.83	0.61	2.17
11	QIL-4-2841	E7-11-2-2	EC 698163	1.62	0.74	3.12
12	QIL-4-2047	HQPM 1-1-1-1-1-1	HQPM 1	1.67	0.62	2.38
13	QIL-4-2124	HQPM5-2-1-1-1-1-1	HQPM5	1.75	0.73	2.63
14	QIL-4-2160	CLQRCY-47- O2-B6-1-1-1-1	CLQRCY-47- O2-B6-1-1-1-1	1.61	0.75	2.85
15	QIL-4-2169-1	Temp x Trop (Ho) QPM- 23-B-B-B-1-1-1	Temp x Trop (Ho) QPM-23-B-B-B-1-1-1	1.60	0.71	2.84
16	QIL-4-2170-2	CML 151	CML 151	1.74	0.63	2.12
17	QIL-4-2192 (Check)	Su2Su2O2O2 Comp(R)- BBB-1-1-1	Su2Su2O2O2 Comp(R)-BBB-1-1-1	1.65	0.70	3.21
18	QIL-4-2209-1 (Check)	WNCQPM 10033-2-1	WNCQPM 10033-2-1	2.09	0.65	2.47
19	QIL-4-2234	WNCQPM 10256-1	WNCQPM 10256-1	1.71	0.73	3.00
20	QIL-4-2243	WNCQPM 10342-1	WNCQPM 10342-1	1.84	0.60	2.78
21	QIL-4-2295	DML 1824	WNC 18745	2.07	0.51	1.90
22	QIL-4-2297	DML 1824-1-1	WNC 18745	2.12	0.60	2.70
23	QIL-4-2300	DML 1278	Bio 9637-2-1-1-1-1-1	1.80	0.72	3.10
24	QIL-4-2306	DML 2062	HQPM 1-3-2-1-1-1-1-1	1.74	0.68	3.30
25	QIL-4-2311	DML 2087	HQPM 7-4-1-1-1-1-1-1	2.42	0.70	2.89
	CD			0.22	0.04	0.04
	Mean			1.93	0.68	2.77
	CV			5.41	2.98	0.74

(28.66 q/ha), QIL-4-2311 (27.26 q/ha) and QIL-4-2297 (27.00 q/ha) emerged as the promising inbred for grain yield (Table 4). ANOVA revealed significant differences among the genotypes for grain yield and its component traits as detailed in Table 5. Among identified high methionine lines, some lines also had high grain yield viz., QIL-4-2831 (25.71q/ha), QIL-4-2829 (25.48 q/ha), QIL-4-2830 (25.61 q/ha), QIL-4-2311 (27.26 q/ha) and QIL-4-3080 (25.25 q/ha). Hybrids were further developed from 25 lines used in this experiment. Further, we have contributed hybrid IQPMH 2310 for AICRP

testing in zones II (North Western Plain Zone) and III (North Eastern Plain Zone) during *kharif* 2023. The parental lines of this hybrid were QIL-4-2784 and QIL-4-2800. This hybrid was promoted to AVT-1 for testing during *kharif* 2024. It showed a yield potential of 8.31 t/ha (zone II) and 5.78 t/ha in zone III. These genotypes can be used in future breeding programs for developing methionine-rich high-yielding cultivars or may be used as such as parents in the hybrid breeding for methionine content in maize.

Table 2. ANOVA for methionine, tryptophan and lysine content in maize inbred lines

Source of variation	Df	Met	Lys	Trp
Genotype	24	1.1865***	1.4123***	0.06***
Replication	1	0.0052	0.0006	0.00011
Environment	3	0.0001	0.0039	0.00002
Genotype:Environment	72	0.0009	0.0028	0.00014
Residuals	99	0.0013	0.0037	0.00021

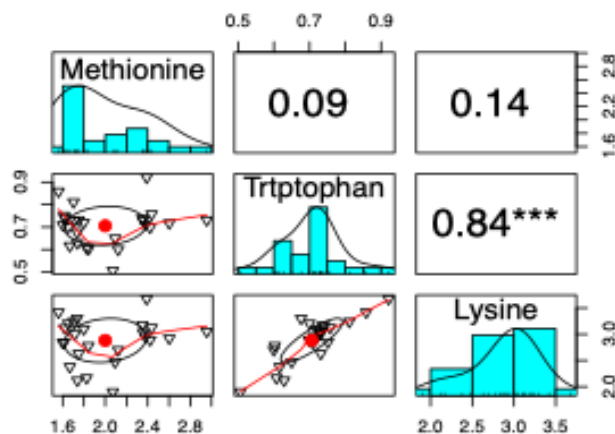
***, ** and * denote values that are significant at 0.1, 1, and 5% level of significance, respectively

Table 3. Descriptive statistics of methionine, lysine and tryptophan content in maize inbred lines

S. No.	Variability parameters	Methionine	Tryptophan	Lysine
1	Maximum (%/ protein)	2.96	0.92	3.68
2	Minimum (%/ protein)	1.56	0.51	1.90
3	Grand mean	2.00	0.71	2.88
4	Standard error of mean (SEm)	0.02	0.01	0.04
5	Critical difference (CD) 5%	0.07	0.03	0.11
6	Critical difference (CD) 1%	0.09	0.03	0.15
7	Genotypic coefficient of variance (%)	38.49	24.50	29.13
8	Phenotypic coefficient of variance	38.53	24.58	29.19
9	Heritability (Broad Sense) %	99.81	99.34	99.53
10	Genetic advance	1.58	0.36	1.73
11	Genetic advance as percentage of mean	79.22	50.30	59.86

Discussion

In the current study, ANOVA revealed significant differences between the genotypes for methionine, lysine and tryptophan indicating that sufficient genotypic variation existed to facilitate effective selection, germplasm improvement, and the development of superior hybrids (Ali et al. 2011). In the past study, a set of 46 QPM inbreds exhibited notable variability in lysine (0.223–0.444%) and tryptophan (0.056–0.111%) (Pandey et al. 2015). Hossain et al. (2018) documented fluctuations in tryptophan (0.067–0.082%) and lysine (0.277–0.373%) levels among QPM inbreds. The percentages of lysine and tryptophan in the progenies of QPM varied between 0.274 and 0.394; 0.071 and 0.084%, respectively (Zunjare et al. 2018). Scott and Blanco (2009) documented a comparable level of variability (lysine: 0.07–0.11%; tryptophan: 0.09–0.17%); Erdal et al. (2019) found lysine to vary between 0.26 and 0.32%. The study conducted

**Fig. 1.** Scatterplot matrices, histograms and Pearson's correlation between methionine, tryptophan and lysine (***)significance at $p < 0.01$)

by Scott and Blanco (2009) revealed that the concentration of methionine varied between 0.09 and 0.19%. Similarly, an association panel consisting of 513 inbreds revealed a mean concentration of 0.238% for methionine (Deng et al. 2017). Erdal et al. (2019) analyzed 73 inbreds and found that the mean concentration of methionine was 0.19%, with a range of 0.17 to 0.22%. Devi et al. (2024) studied the methionine concentration in 22 experimental lines at varying growth stages. The accumulation of methionine is much less at the initial stage of kernel development (15 DAP), it increased significantly at the intermediate stage (30 DAP) and remained constant until kernel maturity. The methionine content ranged from 0.30 to 3.29%. Duo et al. (2024) conducted a study using seven diverse inbred lines and postulated that methionine levels varied from 0.058 to 0.306% with a mean of 0.196%.

The majority of past research has concentrated on one or two amino acids; however, methionine in combination with tryptophan and lysine, has received relatively little attention. The study conducted by Scott and Blanco (2009) revealed that the concentration of methionine varied between 0.09 and 0.19%. Similarly, an association panel consisting of 513 inbreds revealed a mean concentration of 0.238% for methionine (Deng et al. 2017). Duo et al. (2023) revealed significant genetic variation for methionine, lysine and methionine content ranging from 0.031 to 0.305%, 0.014 to 0.090% and 0.118 to 0.389%, respectively. Our study involved the identification of lines with high levels of methionine, tryptophan, and lysine among the 25 lines of maize germplasm that were screened for these amino acids. Identified maize inbreds rich in methionine, lysine and tryptophan (QIL-4-2784 (methionine-2.35%, tryptophan-0.74%, lysine-3.18%), QIL-4-2800 (methionine-2.36%, tryptophan-0.73%, lysine-3.03%), QIL-4-3080 (methionine-2.39%, tryptophan-0.92%, lysine-

Table 4. Mean of yield and yield related traits for the maize inbred lines used in the experiment

S. No.	Genotype	DA	DS	PH	EH	CL	CG	NKR	NR	GY
1	QIL-4-2784	54	55	171.3	76.4	13.3	29.8	16	24	24.7
2	QIL-4-2800	58	60	139.7	66.3	12.4	27.4	13	20	25.0
3	QIL-4-3080	59	61	123.8	64.6	11.4	25.7	15	16	25.3
4	QIL-4-2825	60	62	116.0	50.7	10.6	23.5	15	18	25.3
5	QIL-4-2828	61	63	91.7	43.7	11.6	21.5	14	16	20.3
6	QIL-4-2829	63	65	126.2	61.9	10.9	22.0	12	14	25.5
7	QIL-4-2830	58	60	122.5	60.7	11.5	23.3	15	16	25.6
8	QIL-4-2831	59	61	122.2	66.8	10.4	21.6	14	14	25.7
9	QIL-4-2836	58	60	112.2	48.1	10.4	23.9	15	18	20.9
10	QIL-4-2840	61	63	100.9	44.4	11.0	22.2	15	18	26.0
11	QIL-4-2841	57	59	143.8	58.7	12.7	26.3	15	20	21.6
12	QIL-4-2047	58	60	125.6	54.3	11.7	21.2	15	16	26.2
13	QIL-4-2124	61	63	133.0	68.9	11.1	26.1	15	16	26.3
14	QIL-4-2160	58	60	123.8	52.3	11.7	22.4	15	18	26.4
15	QIL-4-2169-1	57	58	98.4	47.0	10.6	22.0	14	14	26.5
16	QIL-4-2170-2	58	60	111.2	55.6	11.1	23.4	15	18	24.1
17	QIL-4-2192	59	60	135.7	70.5	11.3	25.8	12	18	26.6
18	QIL-4-2209-1	56	58	145.2	76.5	11.4	26.3	13	18	26.7
19	QIL-4-2234	56	57	163.2	81.2	12.6	28.5	13	22	26.8
20	QIL-4-2243	55	57	179.4	94.9	12.6	29.4	18	20	26.9
21	QIL-4-2295	59	60	139.2	72.6	10.9	27.2	14	20	28.7
22	QIL-4-2297	61	63	131.0	68.4	13.0	23.8	14	18	27.0
23	QIL-4-2300	59	61	127.2	67.9	10.6	24.5	17	18	29.5
24	QIL-4-2306	58	60	117.1	54.7	11.1	26.4	16	18	30.7
25	QIL-4-2311	59	62	120.9	60.4	9.9	26.8	16	16	27.3

DA = Days to 50% anthesis ; DA = Days to 50% silking; PH = Plant height; EH = Ear height; CL = Cob length; CG = Cob girth; NKR = Number of kernels/row; NR = Number of seeds per row and GY = Grain yield

Table 5. ANOVA for yield-related traits in maize inbred lines

Source of variation	Df	DA	DS	PH	EH	CL	CG	NKR	NR	GY
Genotype (G)	24	34.8***	38.8***	3509***	1223***	6.379***	52***	13.11***	45***	43.8***
Replication	1	15.1	10.1	52	70	0.162	27	24.5*	73.2*	161.8***
Environment	3	1118.5***	1030.5***	25041***	13395***	17.304***	3939***	192.918***	1611.3***	689.6***
G:E	72	9.2***	9.8***	641***	238***	2.665***	19**	9.42**	21.2*	8.1***
Residuals	99	4.1	4	138	65	1.085	11	5.31	13.7	1.8

***, ** and * denote values that are significant at 0.1, 1, and 5% level of significance, respectively

3.68%), QIL-4-2825 (methionine-2.38%, tryptophan-0.71%, lysine-2.95%), QIL-4-2829 (methionine-2.60%, tryptophan-0.72%, lysine-2.92%), QIL-4-2830 (methionine-2.44%, tryptophan-0.76%, lysine-3.13%), QIL-4-2831 (methionine-2.96%, tryptophan-0.73%, lysine-3.05%)

and QIL-4-2311(methionine-2.42%, tryptophan-0.70%, lysine-2.89%) will serve as valuable germplasm in developing multinutrient-rich cultivars. They can be used as donors for the introgression of methionine, tryptophan and lysine into the elite germplasm in one go. They may be used as such as

parents in the hybrid breeding for these traits. As explained earlier, most of the breeding efforts in the past were only limited to the breeding of maize for lysine and tryptophan. However, the breeding for methionine alongside lysine and tryptophan will lead to the development of multinutrient-rich cultivars in the future. Additionally, the beneficial effects of lysine and tryptophan-rich QPM on chickens and humans are well documented (Hossain et al. 2019). Therefore, inbreds that contain elevated levels of methionine, lysine, and tryptophan are of great importance as germplasm sources for the development of multinutrient-rich maize hybrids that aim to mitigate malnutrition in a sustainable and economically viable manner.

Furthermore, the correlation analysis was also done among methionine with lysine and tryptophan. In order to comprehend the manner in which various factors influence the genetic makeup of a crop, correlation studies are vital. The identification of strongly associated traits for direct selection based on those traits is possible by analyzing the relationships between these variables. For an effective selection program, therefore, understanding the nature and degree of the interdependence among various characteristics is essential. Similar to a previous study (Duo et al. 2023), the findings of our study also indicated that there was no statistically significant correlation between methionine either with tryptophan or lysine. The lack of correlation observed among methionine with lysine and tryptophan implies that it is potentially feasible to augment all three amino acids currently. The present findings are one of the first of its kind to assess methionine content in Indian maize inbred lines.

Author's contribution

Conceptualization of research (RK); Designing of the experiments (RK, AKD); Contribution of experimental materials (RK, VS); Execution of field/lab experiments and data collection (RK, AS, DPC, SN, BK, DS, NS, YK, SK, SS); Analysis of data and interpretation (AKD, CGK, JS); Preparation of the manuscript (RK, JS, PS, SR).

References

- Ali M., Scott M. P. and Bakht J. 2011. Molecular mechanism of methionine differentiation in high and low methionine maize lines. *Afr. J. Biotechnol.*, **10**: 3747-52.
- Bertram H. L. and Schutte J. B. 1992. Evaluation of the sulfur-containing amino acids in laying hens. In *World's Poultry Congress*. Amsterdam, The Netherlands (**3**): 606-609).
- Chauhan J. S., Choudhury P. R., Singh K. H. and Thakur A. K. 2022. Recent trends in crop breeding, the varietal induction in seed chain and its impact on food grain production in India. *Indian J. Genet. Plant Breed.*, **82**(3): 259-279.
- Deng M., Li D., Luo J., Xiao Y., Liu H., Pan Q., Zhang X., Jin M., Zhao M. and Yan J. 2017. The genetic architecture of amino acids dissection by association and linkage analysis in maize. *Plant Biotechnol. J.*, **15**: 1250-1263.
- Devi V., Sethi M., Kaur C., Singh V., Kumar R. and Chaudhary D. P. 2024. Temporal profile of amino acids and protein fractions in the developing kernel of maize germplasm. *Scientific Reports*, **14**(1): p.27161.
- Di Buono M., Wykes L. J., Ball R. D. and Pencharz P. B. 2001. Total sulphur amino acids requirement in young men as determined by indicator aminoacid oxidation with L-[1-¹³C] phenylalanine. *A. M. J. Clin. Nutr.*, **74**: 756-60.
- Darrigues C., Buffard K. R. and Scott M. P. 2005. Variability and genetic effects for tryptophan and methionine in commercial maize germplasm. *Maydica*, **50**: 147-56.
- Duo H., Mishra S., Muthusamy V., Mishra S. J., Chhabra R., Chand G., Bhatt V., Mehta B., Devlash R., Guleria S. K., Pal D., Kumar R., Zunjare R. U. and Hossain F. 2023. Composition of methionine and association with lysine and tryptophan in subtropically adapted maize breeding lines. *Cereal Chem.*, **100**: 1336-1346.
- Duo H., Zunjare R. U., Mishra S. J., Muthusamy V., Thambiyanan S., Kumar S., Kasana R. K., Gopinath I., Sharma G., Chhabra R. and Sarma G. R. 2024. Genetic analysis on composition of sulfur-containing amino acids: methionine and cysteine in subtropical maize. *J Food Compos. Anal.*, **136**: 106774.
- Erdal S., Cengiz R. C. and Ozturk A. 2019. Breeding Doubled Haploid Maize Inbred Lines for Methionine and Lysine Amino Acid Composition. *Maydica*, **64**: 11.
- FAO. 2020. Statistical Database of the United Nation Food and Agriculture Organization (FAO) Statistical division Rome.
- Finkelstein J. D. 1990. Methionine metabolism in mammals. *J. Nutr. Biochem.*, **1**: 228-237.
- Fukagawa N. K. 2006. Sparing of methionine requirements: evaluation of human data takes sulfur amino acids beyond protein. *J. Nutr.*, **136**: 1676S-1681S.
- Gupta Y. P. and Das N. B. 1954. Estimation of methionine in wheat. *Sci. Cult.*, **20**: 90-91.
- Hefner W. 1998. Preparation of acrolein, acrylic acid or a mixture thereof from propane. U. S. Patent, 5705684.
- Hernandez H. and Bates L. S. 1969. A modified method for rapid tryptophan analysis of maize. *Res Bull 19th edn*, Pp 1-42. International Maize and Wheat improvement centre, Mexico.
- Hossain F., Bhat S. R., Mohapatra T. and Singh A. K. 2019. Genetics on a maize cob: A teaching tool for schools. *Indian J. Genet. Plant Breed.*, **79**: 340-366.
- Industry ARC. 2018. Methionine Market – Forecast (2024 - 2030). <https://www.industryarc.com/>
- Kurpad A. V., Regan M. M., Varalakshmi S., Vasudevan J., Gnanou J., Raj T., Young V. R. 2003. Daily methionine requirements of healthy Indian men, measured by a 24-h indicator amino acid oxidation and balance technique. *Am. J. Clin. Nutr.*, **77**(5): 1198-205
- Lai J. and Messing J. 2002. Increasing maize seed methionine by mRNA stability. *Plant J.*, **30**(4): 395-402.
- Mendiburu and Simon. 2009. *Agricolae - a free statistical toolbox for agricultural experiments*. ISTRC symposium. 15th Triennial Symposium of the International Society for Tropical Root Crops, Lima, Peru, Nov. 2-6, 2009
- Pandey N., Hossain F., Kumar K., Vishwakarma A. K., Muthusamy V., Saha S., Agrawal P. K., Guleria S. K., Reddy S. S., Thirunavukkarasu N. and Gupta H. S. 2016. Molecular characterization of endosperm and amino acids modifications among quality protein maize inbreds. *Plant Breed.*, **135**(1): 47-54.
- Scott M. P., Bhatnagar S. and Betran J. 2004. Tryptophan and methionine levels in quality protein maize breeding

- germplasm. *Maydica*, **49**: 303-11.
- Scott M. P. and Blanco M. 2009. Evaluation of the grain methionine, lysine and tryptophan contents of maize (*Zea mays* L) germplasm in the Germplasm Enhancement of Maize Project. *Plant Genet. Resour.*, **7**: 237-43.
- Sharma H., Sandhu S., Yousuf N., Oberoi H. K., Karnatam K. S., Bhavyasree R., Vikal Y., Kumar V. 2024. Analysis of genetic variability for nutritional quality traits among maize inbreds adapted to Punjab: A North-Western province of India. *Cereal Chem.*, 1-11.
- Singh A. K. 2023. Early history of crop presence/introduction into India: VII. American cereal, maize (*Zea mays* L.). *Indian J. Genet. Plant Breed.*, **83**(01): 1-7.
- Tome D. and Bos C. 2007. Lysine requirement through the human lifecycle. *J. Nutr.*, **137**: 1642-1645.
- Tsai C. Y., Hansel L. W. and Nelson O. E. 1972. A colorimetric method of screening maize seeds for lysine content. *Cereal Chem.*, **49**: 572-79.
- Ullman F. 2012. *Ullmann's Encyclopedia of Industrial Chemistry*. Vol 10, pp 673-710. Wiley Online Library, New Jersey.
- Zunjare R. U., Hossain F., Muthusamy V., Baveja A., Chauhan H. S., Bhat J. S., Thirunavukkarasu N., Saha S. and Gupta H. S. 2018. Development of Biofortified Maize Hybrids through Marker-Assisted Stacking of β -Carotene Hydroxylase, Lycopene- ϵ -Cyclase and Opaque2 Genes. *Front. Plant Sci.*, **9**: 1-12.