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Genetic diversity among maize (*Zea mays*) inbred lines for methionine and yield associated traits

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Maize (*Zea mays* L.) is a globally important cereal having the highest genetic yield potential and occupies third place after rice and wheat. In India, about 60% (18 million metric tonnes) of maize produced is used for livestock and poultry feed, 20% (7 million metric tonnes) as food, and the rest is used as fuel and for industrial purposes (CROPS 2018). India contributed around 2.80% of maize production with a quantum of 31.51 million metric tonnes in 2019–2020 (FAO 2022). In India, a major segment (60%) of maize is used as poultry feed, so a demand exists to develop varieties with better field performance, higher yield and methionine content. High genotypic variability for various traits was reported for different genotypes (Magar *et al.* 2021), laying the scope of genetic improvement of maize for high yield and protein quality. Genotypic correlations have been used to determine the relationships among agronomic traits in genetically diverse populations for enhanced progress in crop improvement (Binodh *et al.* 2008). Methionine is one of the limiting amino acids that is mainly coded by zein proteins (prolamin), but its correlation with field parameters is not reported till now.

An experiment was conducted during winter (*rabi*) and rainy (*kharif*) seasons of 2020–21 at the research farm of ICAR-Indian Institute of Maize Research, Ludhiana, Punjab. A total of 150 inbred lines were screened and 22 inbreds were selected for further studies. The elite maize inbreds, procured from the International Center for Maize and Wheat Improvement (CIMMYT) and ICAR-Indian Institute of Maize Research, Ludhiana, Punjab were sown in a plot of 100 m^2, in randomized block design (RBD) in triplicate. A total of 50 seeds of each genotype were sown in three rows (3 m length) and were self-pollinated. Field parameters including plant height (PH), cob height (CH), anthesis and silking interval, germination, cobs per plant, rows per cob, seeds per row, 100-kernel weight (HKW), seed colour, specific gravity, moisture content, and methionine content were recorded during two consecutive seasons. A minimum of 5 well filled ears were sampled at 45 days after pollination (DAP) and screened (Bjarnason and Vasal 1992) to observe kernel opaqueness, seed colour, HKW, specific gravity, moisture content (Doehlert *et al.* 1993), and methionine (Gupta and Das 1954). The mean value of both seasons' data was subjected to analysis of variance (ANOVA) and significant means were compared using the least significant difference at $P<0.05$ and Tukey's test was done (Statistix 10.0 software). Correlation analysis (Statgraphics 18, Statistical graphics Corp. Manugistics Inc., Cambridge, MA), Genotypic (GCV) and phenotypic coefficient of variance (PCV), heritability (Broad-Sense), Genetic advance percent (GA%) (OP Stat software), PCA (STAR) and cluster analysis (agglomerative clustering method) were evaluated.

Based on screening data, 12 inbred lines with higher methionine content (1–3%) and 10 lines with lower methionine were selected for genetic diversity analysis. The experimental lines and their seed parameters are presented in Supplementary Table 1. Kernel opaqueness did not show any correlation with methionine. Kernel colour varied from yellow to dark orange. It was observed that high methionine lines possessed light yellow or orange seed colour, whereas low methionine lines have a yellow, light orange, orange, and dark orange seed colour, indicating that kernel colour showed a significant correlation with methionine content and can act as a visual marker to distinguish low and high methionine lines. However, screening of large sample sets under multiple environments and seasons is required to further validate the results. Maximum methionine content was observed in 174705 (3.29%) and 194010 (2.27%), whereas the lowest was recorded in E4-14-1 (0.312%) and E9-25 (0.30%). Moisture content varied from 10.70% (VS-64) to 33.06% (VS-26) (Supplementary Fig. 1 and Supplementary Table 2).

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Pre and post-harvesting field parameters were recorded to understand their correlation with methionine accumulation patterns. Genotype E7-11-1 showed the highest PH (165 cm), whereas, 174705 showed the lowest (84.3 cm). Maximum CH was observed in 194010 (91.7 cm) and minimum in VS-30 (40.03 cm). Three experimental lines, viz. E16-25-B-2 (89.70 cm, 41.30 cm), 174705 (84.30 cm, 44.30 cm) and E7-64 (99.70 cm, 49.0 cm) showed lower PH and CH, respectively. The line E4-14-1 showed maximum germination (76%), whereas, E9-25 showed minimum (5%). Only one line showed logging, as 6 out of 37 plants, whereas other lines performed well under field conditions. Most of the lines showed an anthesis interval from 55–67 days a silking interval from 55–68 days and an anthesis-silking interval (ASI) of only 1 or 2 days. Line E19-14-1 showed early anthesis (55 days) and silking (57 days) which is considered good for yield, whereas line E4-14-1 showed late anthesis (67 days) and silking (68 days) (Supplementary Fig. 2 and Supplementary Table 3). Late anthesis and silking lead to a longer period from germination to maturation of cobs thus affecting yield and harvesting.

The maximum number of cobs was recorded in E7-11-1 and E7-64 (32 cobs), whereas, the minimum was in line 174705 (4 cobs). Number of rows in a cob was minimum in 174705 (6 rows only), whereas maximum (16 rows) in VS-26. The number of seeds in one row was maximum in E7-24-3 (29 seeds/row), whereas the minimum in 174705 (5 seeds/row). The kernel weight was minimum in E11-35 (9.42 g) and maximum in E9-25-1 (31.86 g). Genotype with a higher number of seeds along with higher kernel weight is a desirable trait for breeding high-yielding maize variety. Specific gravity varied from 0.923 (VS-30) to 1.91 g/cm^3 (E9-25) (Supplementary Fig. 3 and Supplementary Table 4).

The following pairs of variables have *P* values below 0.05: (i) protein and methionine, (ii) methionine and no. of seeds per row, (iii) PH and CH, (iv) no. of cobs and no. of rows per cob, (v) days to anthesis and days to silking, (vi) specific gravity and days to silking. Methionine showed a statistically significant negative correlation with no. of seeds per row ($r = -0.45$), indicating that methionine content will be higher in the lines with low-yielding maize inbred. It also showed a statistically significant negative correlation with no. of rows per cob ($r = -0.59$) indicating that methionine content decreased as the number of rows per cob increased and vice versa. Methionine showed a negative correlation with interval to silking ($r = -0.42$), indicating that early maturing lines will contain higher methionine content. PH and CH also showed a statistically significant correlation ($r = 0.83$) with each other. PH showed a positive correlation with the number of cobs ($r = 0.30$). Similarly, no. of rows per cob and no. of seeds per row showed a statistically significant positive correlation ($r = 0.76$) with each other (Supplementary Fig. 4 and Supplementary Table 5). Significant positive correlation is also reported between days to anthesis and silking, plant height and cob height, plant height and no. of cobs in maize (Ogunniyan and Olakojo 2014, Triveni *et al.* 2014, Khan *et al.* 2018). The Coefficient of variance (CV) values were low except for PH, CH and number of cobs. The CVs for days to anthesis and days to silking were zero, showing a high level of uniformity for inbred lines (Supplementary Table 6). Zero and low CVs of these traits may be expected because the lines had undergone inbreeding depression resulting in the fixation of recessive genes thus increasing homozygosity within the lines.

Genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) were higher for methionine, moisture, HKW, no. of cobs, no. of seeds/row, and germination rate. Parameters with higher CV values particularly GCV are used for selecting suitable parents for crosses or selecting lines for further improvement. GCV values for PH, CH, no. of cobs, and no. of rows/cob were lower than PCV value indicating, that characters were more influenced by their surrounding environment. Heritability ranged from 40.23 to 99.88% and GA mean percent ranged from low (9.45%) in interval of anthesis to high (130.05%) in methionine. High heritability showed that the environmental influence is minimal. Characters with high heritability can be used for the selection of plant material with desirable features. Similar results were reported by Rafiq *et al.* (2010) and Ogunniyan and Olakojo (2014), who reported high heritability for different yield controlling traits in maize. The first five principal components (PCs) based on 12 quantitative traits showed eigen values greater than 1. The contribution of these five PCs was 82.01% in the overall variability among the genotypes. The contribution of PC1 was found to be 21.98% in the total divergence of the studied population, in which the major contributing traits were CH, no. of cobs, methionine and germination. The PC2 was responsible for about 20.85% of the variation and was mainly contributed by PH, no. of cobs, no. of rows per cob, no. of seeds per row, and germination rate. The PC3 explained 18.37% of the variation and was associated with no. of rows per cob, no. of seeds per row, and moisture. The PC4 explained 10.82% variation and was contributed by PH, CH, no. of seeds per row, and specific gravity. The PC5 explained 9.98% variation and was contributed by no. of cobs, specific gravity, and HKW (Table 1).

Cluster analysis (CA) based on PCA scores was compared with the results of the PCA on a visual aid in desecrating clusters in the two-dimensional scattered diagram and the genotypes falling in the same cluster were present closer to each other. The diagram showed that the genotypes numbered 12 and 20 i.e. 194010 and E-25 scattered away from other genotypes indicating that these lines are genotypically far apart from each other (Supplementary Fig. 5). Similar results were reported by Shrestha (2016) and Mounika *et al.* (2018). Genotypes were grouped according to their variability and it was noted that inbred lines 174705 and 194010 showed higher methionine but poor field parameters. The second major cluster has five inbred lines i.e. E7-11-1, E7-24-3, E19-14-1, E2-52-1, and E11-35, with better methionine and other parameters. Hence, inbreds of this cluster can be used for

Table 1 Genotypic and phenotypic coefficient of variations, broad-sense heritability, genetic advance and principal component analysis for 12 parameters

Trait	Heritability	GCV	PCV	GA	GA% mean	PC1	PC2	PC3	PC4	PC5
Methionine	99.88	63.17	63.21	1.47	130.05	0.39	-0.30	-0.01	-0.20	-0.37
Moisture	99.79	30.16	30.20	12.89	62.07	-0.16	-0.05	0.06	-0.69	-0.32
Plant height	52.43	12.53	17.31	23.54	18.69	-0.01	0.10	-0.59	0.08	-0.25
Cob height	40.23	11.58	18.26	8.82	15.13	0.02	-0.07	-0.61	0.11	-0.21
Days to anthesis	78.74	5.17	5.83	5.66	9.45	-0.52	-0.25	-0.08	-0.03	-0.12
Days to silking	83.20	5.25	5.75	5.99	9.86	-0.57	-0.13	-0.01	-0.01	-0.08
Germination rate	99.68	53.63	53.71	46.87	110.29	0.04	0.36	-0.24	-0.20	-0.23
Number of cobs	57.05	29.82	39.49	7.68	46.40	0.11	0.31	-0.33	-0.14	0.51
No. of rows/cob	92.41	19.77	20.56	4.83	39.14	-0.26	0.50	0.13	-0.07	-0.07
No. of seeds/row	97.76	33.09	33.47	12.73	67.40	-0.21	0.49	0.09	0.10	-0.16
100-kernel weight	99.66	29.62	29.67	11.82	60.91	-0.13	-0.16	-0.22	-0.53	0.51
Specific gravity	97.74	16.08	16.27	0.40	32.75	-0.29	-0.24	-0.16	0.34	0.15
Eigen value						2.64	2.50	2.20	1.30	1.20
Variance percent						21.98	20.85	18.37	10.82	9.98
Cumulative variance per cent						21.98	42.84	61.21	72.03	82.01

breeding high-yielding, high-methionine lines. Another cluster having lines, E9-25, E4-14-1, VS-26, VS-5, and E4-5-2 showing better yield contributing traits, can be used as an acceptor line. These two clusters showed maximum variation (Supplementary Fig. 6).

A total of 6 high methionine lines performed very well in terms of yield-associated traits. Line E7-24-3 had 11 traits above average and the highest no. of seeds per row (29). Line E7-11-1 and E19-14-1 showed 10 traits above average. The line E7-11-1 had the two best traits, PH (165 cm) and the number of cobs (32), and E19-14-1 showed early flowering characteristics. Line E4-5-2 was a low methionine line but performed well in terms of field traits. Lines E2-52-1, E2-7 and E19-22-2 showed 9 traits above average, and E19-22-2 also showed the highest germination rate (76%). So, these above-mentioned lines may be used as acceptor lines in future breeding programs. Line 174705 has poor field traits having 4 traits with the lowest values but had the highest methionine content (3.29%). Although line 194010 had only 5 traits above average its methionine content (2.27%) was the second highest. These two lines may be used as donor in breeding high methionine maize hybrids. Lines 174705, 194010, E7-11-1, E7-24-3, E19-14-1, E2-52-1, E11-35 and E9-25 showed maximum inter-cluster distance. Hence, they can be used in breeding programs to develop high methionine hybrids in maize.

In conclusion, all the traits showed significant differences, showing wide genetic variability among the maize genotypes, which can be used for yield improvement. Line 174705 and 194010 with the highest methionine can be used for developing high-methionine maize inbreds. PCV values of PH, CH, no. of cobs, and no. of rows/cob were larger than GCV, showing that there was an environmental influence. Higher values of GCV, PCV, heritability, and GAM for traits, viz. methionine, moisture, 100KW, no. of cobs, no. of rows/cob, no. of seeds/row and germination rate indicated efficient indirect selection for higher grain yield.

SUMMARY

A study was carried out during winter (*rabi*) and rainy (*kharif*) seasons of 2020–21 at the research farm of ICAR-Indian Institute of Maize Research, Ludhiana, Punjab in which 22 maize inbred lines with varying content of methionine were evaluated to study various seeds and field parameters, and methionine content and further statistically analysed through genetic variability, principal components and hierarchical cluster analysis. Significant differences were noted among inbred lines for various traits. Broad-sense heritability analysis showed that traits exhibited more than 80% heritability, except plant height, cob height and no. of cobs. Principal component analysis showed five principal components having eigen value more than 1.00 and accounted for 82.01% of total variation. Cluster analysis distributed the inbred lines in 4 major clusters suggesting their use in breeding programmes. Correlation analysis was also studied to understand the correlation of field and yield parameters with methionine content. Plant height and cob height (0.83), no. of rows per cob and no. of seeds per row (0.76), anthesis and silking interval (0.89), specific gravity and silking interval (0.45) showed significant positive correlation. Traits including methionine, moisture, 100-kernel weight, no. of cobs, no. of rows/cob, no. of seeds/row and germination rate having higher genotypic and phenotypic coefficient of variation, heritability, and genetic

advance mean can be used for further crop improvement programme.

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