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RESEARCH ARTICLE

Genetic studies on Quality Protein Maize (QPM) for variability and diversity analysis under temperate conditions (*Zea mays* L.)

Ajaz A Lone[1*], Mohd Wassim Alie[2], Bipasha Datta[3], Sagar[4], Valeeta Marina Dsouza[5], Aman Tutlani[2], Amit Lohar[6], Dhamni Patyal[7], Shourabh Joshi[8], Vishva Deepak Chaturvedi[9], Sapna Jarial[10] & Zahoor A Dar[1*]

[1]Dryland Agriculture Research Station (DARS), Srinagar 190 001, Jammu and Kashmir, India
[2]Division of Genetics and Plant Breeding, Faculty of Agriculture (FoA), Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST-K), Wadura 193 201, Jammu and Kashmir, India
[3]College of Post Graduate Studies in Agricultural Sciences, Umiam 793 103, Meghalaya, India
[4]Department of Genetics and Plant Breeding, Faculty of Agricultural Sciences, GLA University, Bharthia 281 406, Uttar Pradesh, India
[5]Department of Biotechnology, St. Joseph's University, Bengaluru 560 027, Karnataka, India
[6]Department of Horticulture, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Gajapati 761 211, Odisha, India
[7]Department of Agronomy, Sher-e-Kashmir University of Agriculture Sciences and Technology Jammu 180 009, Jammu and Kashmir, India
[8]Department of Plant Biotechnology, College of Agriculture Nagaur, Agriculture University, Jodhpur 341 001, Rajasthan, India
[9]Department of Genetics and Plant Breeding, College of Agriculture, Chandrashekhar Azad University of Agriculture and Technology, Kanpur 262 802, Uttar Pradesh, India
[10]Department of Agricultural Economics and Extension, School of Agriculture, Lovely Professional University, Phagwara 144 411, Punjab, India

*Correspondence email - ajaz999@gmail.com; zahoorpbg@gmail.com

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Abstract

Despite significant progress in maize improvement, information on the extent of genetic variability and trait relationships in Quality Protein Maize (QPM) germplasm under temperate conditions remains limited, constraining effective selection for yield and nutritional quality. The present investigation was conducted to assess genetic variability, heritability, character associations and genetic divergence among 50 QPM genotypes, including five checks. The experiment was laid out in an augmented block design (ABD) without replications at the Dryland Agriculture Research Station (DARS), Budgam and observations were recorded on 13 morphological and quality traits. A wide range of variability was observed for all traits. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were in a moderate range for most of the characters except for the number of days to maturity, shelling percentage and protein content. High heritability of characters like grain yield, kernels cob^{-1}, number of cobs plant^{-1} and 100-seed weight, along with extensive genetic advance, suggests that additive gene effects are playing a predominant role and direct selection will be effective for improvement of these traits. After correlation analysis, it was observed that grain yield was robustly and positively allied with plant height, ear height, number of cobs plant^{-1}, cob length, kernel rows cob^{-1}, kernels cob^{-1} and 100-seed weight, whereas protein content showed negative association with yield. Genetic divergence assessed through Mahalanobis D^2 statistics classified the genotypes into eight clusters, with the highest inter-cluster distance observed between cluster IV and cluster VIII with 341.59, indicating scope for heterotic hybrid development. The mean grain yield was highest in cluster II, while superior protein content was observed in clusters III and cluster VIII. The current findings identified ten early-maturing genotypes with commendable yield and protein content, which can be effectively used in QPM breeding programs aimed at improving productivity and nutritional quality.

Keywords: correlation; genetic divergence; genetic variability; heritability; Quality Protein Maize

Introduction

Maize (*Zea mays* L.) is a widely cultivated cereal crop of the *Poaceae* family that originated in Mexico approximately 6000-7000 years ago (1). It is the only species in the genus *Zea* with a 2n=20 chromosome number and its closest relatives are *Teosinte* and *Tripsacum*, with the former considered the closest. Maize is a monoecious annual grass varying in height from 1 to 4 m (2). It was predicted that by 2020 maize would surpass wheat and rice to become the most widely grown cereal (3). This adaptability is largely attributed to climate change, as maize can thrive across a wide range of environments. In addition, its genetic diversity surpasses that of other cereal crops, primarily due to its high outcrossing potential (4).

Maize is widely grown in India, suggesting its wide adaptability and is the third most important cereal crop in India after rice and wheat. The worldwide production of maize exceeded 1067 Mt in 2016. Globally, the area under maize cultivation covered about 181.80 million ha and estimated production in 2017 was approximately 1031.86 Mt (5). In India, the harvested area for maize reached around 11.2 million ha in 2024-25, with production estimated at 42.3 Mt, reflecting a significant upward trend over previous years (6). In the Jammu & Kashmir region, the area under maize stands at 263000 ha with a production of 466000 quintals and a productivity of 1495 kg ha^{-1} (7). Maize is highly significant to Indian agriculture because of its numerous applications in human food and livestock feed. It also holds key industrial applications and a scope of diverse value-added products because of the enriched composition of starch, protein, fat, oil and sugars (primarily sucrose) in the seed.

Maize is an important source of macro (carbohydrates, fats and proteins) as well as essential micro (vitamins and minerals) nutrients. The kernel structure is comparable to other cereal grains, primarily comprising 82 % endosperm, 12 % germ and 6 % pericarp. The endosperm is primarily made up of starch, a complex carbohydrate that averages around 71 % of the grain and provides concentrated energy. While the majority of proteins in a mature maize kernel are distributed between the endosperm and the germ, the germ proteins are superior in both quantity and nutritional quality (8). The proteins are typically divided into four different groups based on their solubility as albumins (3 % soluble in water), globulins (3 % soluble in dilute salt solutions), zeins or prolamins (60 % soluble in 70 % ethanol) and glutelins (34 % soluble in dilute acids or bases) (9).

A natural mutation that resulted in soft, opaque kernels was discovered in a Connecticut maize field, USA, during the 1920s and subsequently brought to the Connecticut Agricultural Experiment Station (10). The mutant was later designated as *opaque-2* (*o2*) by a researcher in Connecticut (11). In the 1960s, Dr. Oliver Nelson at Purdue University, USA, provided seeds of the *opaque-2* maize to Dr. Edwin Mertz, enabling his research team to systematically evaluate maize accessions for enhanced protein quality (12). In 1961, researchers at Purdue University reported that maize homozygous for the recessive *o2* allele (carrying two copies of the mutation) exhibited a markedly higher lysine content in the endosperm, with an increase of about 69 % compared to normal maize (13). Further investigations revealed that this genotype also exhibited a parallel increase in tryptophan content. The elevated levels of these two essential amino acids typically deficient in maize endosperm significantly enhanced the nutritional profile, effectively doubling the biological value of maize protein (14), with the considerably advantageous result that only half the amount of *o2* maize (relative to normal maize) needs to be consumed to obtain the same biologically usable protein (15).

Among these developments, the most significant was the creation of Quality Protein Maize (QPM) following the discovery of the *opaque-2* mutant in the mid-1960s, which increased lysine and tryptophan levels in the endosperm protein. Tryptophan and lysine concentrations in maize have been found to be highly correlated (16). Lysine and tryptophan content, expressed as a percentage of total protein, in whole-grain flour of conventional and QPM (*o2o2*) maize genotypes (17). Mutations that affect grain protein synthesis also influence kernel texture. Early *opaque-2* (*o2*) mutants exhibited reduced α-zein levels, leading to smaller, unexpanded protein bodies, whereas the *o15* mutation, which decreases zein fractions, results in a reduced number of protein bodies (18). Other mutations, including *floury-2 (fl-2)*, *Mucronate (Mc)* and *defective endosperm (De B30)*, produce protein bodies with irregular shapes. In view of the above factors, the study was proposed with the objectives of characterizing QPM inbred lines and analyzing their variability and diversity.

Materials and Methods

The present study was conducted at Dryland Agricultural Research Station, Budgam, during Kharif, 2016. The experimental material was sown in an augmented block design (ABD) without replications (Table 1). The test inbred lines were sown in 5 blocks, with each block containing 9 inbred lines and 5 checks. The observations were recorded for 13 characters, viz., days to 50 % tasseling, days to 50 % silking, plant height, ear height, number of cobs^{-1}, cob length (cm), kernel row cob^{-1}, kernels cob^{-1}, shelling percentage, 100-grain weight, grain yield (q/ha) and protein content (%). The grain protein content of inbred lines was estimated by NIR Technology instrument (Crop

Table 1. List of QPM inbred lines (50) including 5 checks

S. No.	Pedigree	Inbred lines
1	(CLQ-RCYQ31xCLQ-RCYQ35)	KDQPM1
2	(CLQ-RCYQ46=(CML150xCL-03618)	KDQPM2
3	(CML161xCLQ-RCYQ31)-B-12	KDQPM3
4	(CML165xCL-02843)-B-12-3-1-BB-	KDQPM4
5	(CML165xKI45)-B-11-3-BB-1-B*8	KDQPM5
6	(CML165xKI45)-B-14-1-B*4-1-B*7	KDQPM6
7	97P65BBB19BBB	KDQPM7
8	97P65BBB5BBB	KDQPM8
9	97P65BBB9BBB	KDQPM9
10	CLQ-6603-B-1-B*8	KDQPM10
11	CLQRCWQ15B*6	KDQPM11
12	CLQ-RCWQ50-B*12	KDQPM12
13	VQL1	VQL1
14	VQL17	VQL17
15	CLQ-RCYQ40	KDQPM15
16	CLQ-RCYQ54=(CML176xCL-G2501)	KDQPM16
17	CLQS89YQ04-B*8	KDQPM17
18	CLQY36	KDQPM18
19	CML137	KDQPM19
20	CML153	KDQPM20
21	VQL2	VQL2
22	CML171	KDQPM22
23	CML181	KDQPM23
24	CML189	KDQPM24
25	CML135	KDQPM25
26	CML189BBB	KDQPM26
27	CML 196	KDQPM27
28	CML136	KDQPM28
29	CML137BB	KDQPM29
30	CML138BB	KDQPM30
31	CML140	KDQPM31
32	CML144/CML176-B4-2-3-2B*6	KDQPM32
33	CML153	KDQPM33
34	CML154	KDQPM34
35	CML161	KDQPM35
36	CML161/CML165-BBB11BBB	KDQPM36
37	CML161/CML165-BBB12BBB	KDQPM37
38	CML161/CML165-BBB15BBB	KDQPM38
39	CML161/CML165-BBB16BBB	KDQPM39
40	CML161/CML165-BBB18BBB	KDQPM40
41	CML161/CML165-BBB7	KDQPM41
42	CML161/CML165-BBB8BBB	KDQPM42
43	CML162	KDQPM43
44	CML162-B*10	KDQPM44
45	CML164-B*8	KDQPM45
46	CML165BBB	KDQPM46
47	CML166	KDQPM47
48	CML169	KDQPM48
49	CML 163	CML163
50	CML193	CML193

Scan 200) in the seed processing unit in the division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences & Technology (SKUAST-Kashmir), Jammu and Kashmir.

Analysis of variance was performed for all the characters to test variation among genotypes. Genotypic variance (19) and phenotypic variance were calculated (20). The magnitude of genotypic (GCV) and phenotypic coefficients of variation (PCV) for each trait was estimated (21). Phenotypic coefficient of variation and GCV were classified (22). Broad-sense heritability [H^2(bs)], defined as the ratio of genotypic variance to phenotypic variance, was estimated (23). Genetic advance at a mean of 5 % (GAM) selection intensity was determined (24).

Correlation coefficients were calculated to assess the extent of association between traits and grain yield, as well as among yield components. Both genotypic and phenotypic correlation coefficients were estimated using variance and covariance components (19). Genetic divergence was computed (25, 26). Tocher's method for grouping varieties into various clusters was adopted.

The average intra- and inter-cluster distances were calculated (26). Genetic divergence between genotypes can also be estimated using Mahalanobis's D^2 statistics. All the above computations were carried out using Windostat Version 9.1.

Results and Discussion

Analysis of variance and components of variability

Analysis of variance for an experiment involving 50 QPM inbred lines, including five checks, across 13 quantitative traits revealed highly significant mean squares for all characters, indicating substantial variation among the genotypes (Table 2). Analysis of variance for dispersion of QPM inbred line genotypes revealed a highly significant mean sum of squares for genotypes (Table 3). In the present study, 45 maize genotypes were evaluated to determine their genetic potential for yield and its components. Analysis of variance revealed significant variation among the genotypes. The significant mean squares observed for all the studied traits were comparable with the results of previous studies (27, 28).

Mean performance of the genotypes

A wide range of variability was observed among the genotypes for all the studied traits, with their mean and range tabulated in Table 4. The mean value for days to 50 % tasseling was 77.91 days, with genotype KDQPM35 (68.33 days) being the earliest and KDQPM26 (82.66 days) being the latest. Similarly, for days to 50 % silking, genotype KDQPM35 (71.33 days) was the earliest and KDQPM44 (87.00 days) was the latest. For agro-morphological traits, the average value for plant height was 136.82 cm, with the VQL1 (154.00 cm) genotype being the tallest and KDQPM38 (92.33 cm) being the shortest. Ear height ranged from 46.66 cm (KDQPM38) to 77.66 cm (KDQPM35), with an average of 69.28 cm. Cob length averaged 15.11

Table 3. Analysis of variance for dispersion of QPM inbred lines

Source of variation	Degree of freedom	Mean squares
Genotypes	49	12.45**
Error	107	2.16
Total	**161**	**0.00**

* Significant at 5 % level; ** significant at 1 % level (Wilk's criterion = 129.5)

Table 2. Analysis of variance for different morphological, maturity and yield component traits in maize

		Mean Sum of Squares												
Source of variation	**Degree of freedom**	**Days to 50 % tasseling**	**Days to 50 % silking**	**Plant height (cm)**	**Ear height (cm)**	**No. of cobs plant^{-1}**	**Cob length (cm)**	**Kernel rows cob^{-1}**	**Kernels cob^{-1}**	**Shelling %**	**100-seed weight (g)**	**Grain yield (q/ha)**	**Days to maturity**	**Protein content**
Block	4	12.89	12.69	363.6**	103.99**	0.03588**	3.664**	2.721**	586.5	15.970**	15.768**	11.473	21.0	0.039164
Genotypes	49	29.14**	33.85**	135.0**	37.83**	0.02612**	3.945**	2.097**	7109.8**	8.635**	7.395**	16.589*	89.3**	0.184744**
Treatment: Check	4	74.24**	62.50*	85.4**	21.43**	0.03040**	2.730**	4.267**	20.29113	9.047**	14.466**	29.103*	368.6**	0.1681885
Treatment: Test and Test\check	45	25.13**	31.31**	139.4**	39.29**	0.02574**	4.053**	1.904**	14.69*	8.599**	6.766**	15.477*	64.5**	3.19**
Residuals	16	7.61	8.11	3.6	1.12	0.00440	0.106	0.322	285.9	0.779	0.808	6.794	12.9	0.019644

* Significant at 5 % level; ** significant at 1 % level

Table 4. Estimates of mean, range, phenotypic and genotypic variance, phenotypic and genotypic coefficient of variation for different morphological and quality component traits in maize

Parameters	Plant height (cm)	Ear height (cm)	Day to 50 % tasseling	Days to 50 % silking	No. of cobs plant^{-1}	Cob length (cm)	Kernel rows cob^{-1}	kernels cob^{-1}	Shelling percentage	100 grain weight (g)	Grain yield (qha^{-1})	Maturity days	Protein content (%)
Mean	136.82	69.29	77.91	82.11	0.52	15.11	14.08	298.33	73.95	21.91	20.99	146.75	12.38
Range	(92.33-154.0)	(46.67-77.67)	(68.33-82.66)	(71.33-87.0)	(0.40-1.0)	(7.87-19.67)	(12.0-18.0)	(120.0-461.34)	(65.80-79.60)	18.16-28.29	11.58-29.89	135.33-153.66	11.20-13.13
PV	171.08	49.46	21.19	24.93	0.028	5.47	2.89	6157.18	9.69	7.9447	16.59	31.12	0.25
GV	144.58	39.48	19.18	23.17	0.026	0.59	1.67	5654.04	9.26	7.3977	14.40	28.92	0.19
PCV	9.56	10.15	5.91	6.08	32.56	15.48	12.08	26.30	4.21	12.8655	19.41	3.80	4.08
GCV	8.79	9.07	5.62	5.86	31.31	11.91	9.18	25.20	4.12	12.4147	18.08	3.66	3.54
H^2(b)	0.90	0.93	0.84	0.80	0.92	0.59	0.59	0.91	0.96	0.93	0.87	0.93	0.75
GAM	11.00	11.64	16.64	16.69	62.02	18.88	14.39	49.75	08.29	24.68	34.69	7.28	6.34

cm, varying from 7.87 cm (KDQPM38) to 19.66 cm (KDQPM2). The mean number of cobs per plant was 1.35, with a range of 1.10 to 1.60. The number of kernel rows per cob varied from 12.00 to 18.00, with KDQPM8 recording the highest and KDQPM24, KDQPM26 and KDQPM27 the lowest values. The mean value for the number of kernels cob^{-1} was 298.33 with a wide range of 120.00 (KDQPM37) to 461.33 (KDQPM33). The mean value for shelling percentage was 73.95 %, with a range from 65.80 (KDQPM38) to 79.60 % (KDQPM19). The mean value for 100-seed weight was 21.90 g, with a range from 8.16 (KDQPM36) to 28.29 g (KDQPM16). Days to maturity averaged 146.74 days, with KDQPM35 (135.33 days) being the earliest to mature and VQL17 (153.66 days) being the latest. Grain yield varied significantly from 11.58 q/ha (KDQPM4) to 29.89 q/ha (VQL1), with a mean yield of 20.99 q/ha. The quality trait, i.e., protein content, varied from 11.20 (KDQPM38) to 13.13 % (KDQPM8), with an average value of 12.37 %. These results confirm significant genetic variation that can be utilized for yield and quality improvement.

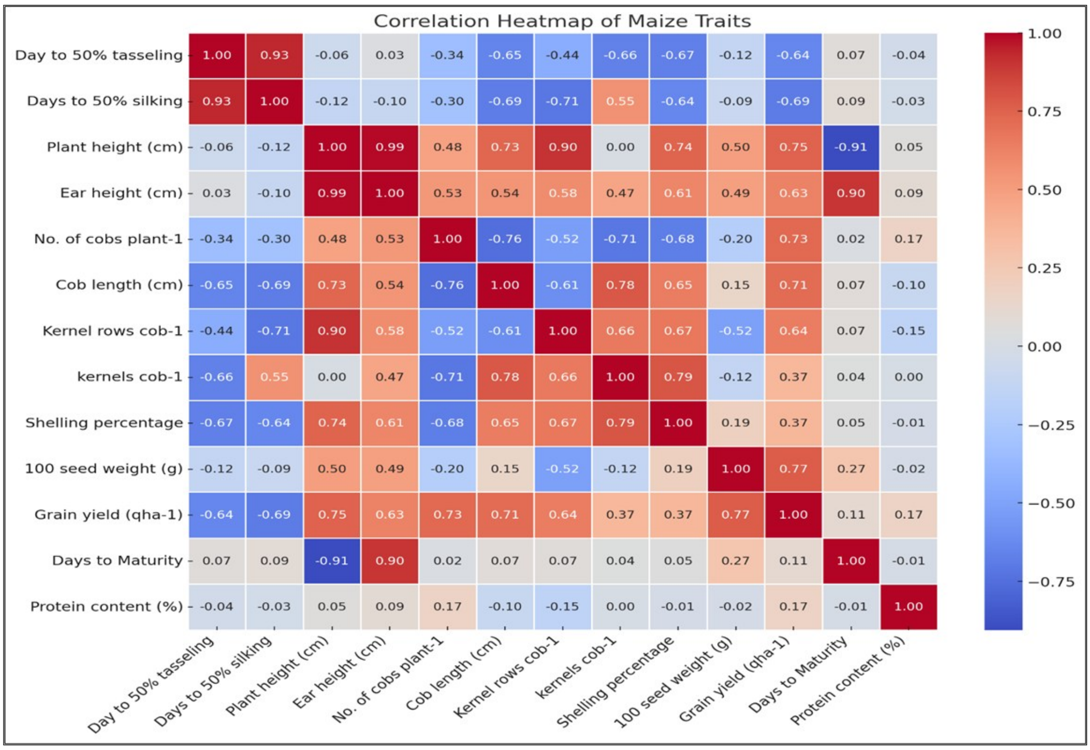
Genotypic coefficient of variation, PCV, heritability (broad sense) and genetic gain

The results revealed for all the traits studied, phenotypic variance exceeded genotypic variance, indicating that environmental factors exert a considerable influence on the expression of these traits. Some characters like plant height, days to 50 % tasseling, days to 50 % silking, days to maturity and shelling percentage along with protein content, showed low GCV and PCV (<10 %). In contrast, characters like number of kernels cob^{-1}, number of cobs plant^{-1}, grain yield, cob length, 100-seed weight, number of kernel rows cob^{-1} and ear height showed moderate variability ranging from 10 to 40 %. Among these, the highest levels of genetic variability were seen in number of kernels cob^{-1}, number of cobs plant^{-1}, grain yield, 100-seed weight and cob length, highlighting these traits as strong candidates for improvement through direct selection, while characters like shelling percentage, protein content and days to maturity showed the lowest levels of genetic variability; these were in conformity with the findings of earlier studies, suggesting a predominance of non-additive gene action (29-31).

Heritability measures varied from 57 to 93 %, with very high values (over 90 %) for 100-seed weight, silking days, maturity, number of kernels cob^{-1} and tasseling days, indicating that additive gene effects are more influential for governing these traits. Characters like grain yield and plant height also exhibited high heritability (86 and 84 % respectively), but moderate levels of heritability were reported for protein content, shelling percentage and number of cobs plant^{-1}. The largest genetic advance (as a percentage of mean) was reported for the number of kernels cob^{-1}, the number of cobs plant^{-1} and grain yield, further strengthening the evidence for the potential role of additive gene action for governing these traits, which is consistent with earlier reports (32-34). High heritability together with high genetic advance in these traits suggests that selection would be very effective. However, high heritability with low genetic advance as reported in traits like flowering and maturity points toward strong environmental influence, which should be carefully considered when planning selection strategies.

Estimates of genotypic correlation coefficients

A critical analysis of the correlation results revealed several key associations between yield and yield-attributing traits (Table 5, Fig. 1). A detailed examination of genotypic correlations among 13 traits highlighted several key relationships that impact grain yield. Grain yield per ha showed a strong positive correlation with 100-seed weight, followed by days to maturity, number of cobs plant^{1}, cob length, number of kernel rows cob^{-1}, ear height and kernels cob^{-1}. This result suggests that these traits are crucial targets for breeding programs aiming to enhance yield. Similar findings have been reported earlier for cob length (32), plant height (35), ear height (36) and 100-seed weight (37). Days to 50 % tasseling was significantly positively correlated with days to 50 % silking, days to maturity, plant height and ear height but showed a significant negative correlation with cob length, grain yield, kernels cob^{-1} and kernel rows cob^{-1}.



	Day to 50% tasseling	Days to 50% silking	Plant height (cm)	Ear height (cm)	No. of cobs plant-1	Cob length (cm)	Kernel rows cob-1	kernels cob-1	Shelling percentage	100 seed weight (g)	Grain yield (qha-1)	Days to Maturity	Protein content (%)
Day to 50% tasseling	1.00	0.93	-0.06	0.03	-0.34	-0.65	-0.44	-0.66	-0.67	-0.12	-0.64	0.07	-0.04
Days to 50% silking	0.93	1.00	-0.12	-0.10	-0.30	-0.69	-0.71	0.55	-0.64	-0.09	-0.69	0.09	-0.03
Plant height (cm)	-0.06	-0.12	1.00	0.99	0.48	0.73	0.90	0.00	0.74	0.50	0.75	-0.91	0.05
Ear height (cm)	0.03	-0.10	0.99	1.00	0.53	0.54	0.58	0.47	0.61	0.49	0.63	0.90	0.09
No. of cobs plant-1	-0.34	-0.30	0.48	0.53	1.00	-0.76	-0.52	-0.71	-0.68	-0.20	0.73	0.02	0.17
Cob length (cm)	-0.65	-0.69	0.73	0.54	-0.76	1.00	-0.61	0.78	0.65	0.15	0.71	0.07	-0.10
Kernel rows cob-1	-0.44	-0.71	0.90	0.58	-0.52	-0.61	1.00	0.66	0.67	-0.52	0.64	0.07	-0.15
kernels cob-1	-0.66	0.55	0.00	0.47	-0.71	0.78	0.66	1.00	0.79	-0.12	0.37	0.04	0.00
Shelling percentage	-0.67	-0.64	0.74	0.61	-0.68	0.65	0.67	0.79	1.00	0.19	0.37	0.05	-0.01
100 seed weight (g)	-0.12	-0.09	0.50	0.49	-0.20	0.15	-0.52	-0.12	0.19	1.00	0.77	0.27	-0.02
Grain yield (qha-1)	-0.64	-0.69	0.75	0.63	0.73	0.71	0.64	0.37	0.37	0.77	1.00	0.11	0.17
Days to Maturity	0.07	0.09	-0.91	0.90	0.02	0.07	0.07	0.04	0.05	0.27	0.11	1.00	-0.01
Protein content (%)	-0.04	-0.03	0.05	0.09	0.17	-0.10	-0.15	0.00	-0.01	-0.02	0.17	-0.01	1.00

Fig. 1. Genotypic correlation coefficients for morphological and quality component traits in maize (*Zea mays* L.). Strong positive correlations appear in deep red, while strong negative correlations are shown in deep blue.

Table 5. Genotypic correlation coefficients for morphological and quality component traits in maize (*Zea mays* L.)

Parameters	Day to 50 % tasseling	Days to 50 % silking	Plant height (cm)	Ear height (cm)	No. of cobs plant^{-1}	Cob length (cm)	Kernel rows cob^{-1}	kernels cob^{-1}	Shelling percentage	100 seed weight (g)	Grain yield (qha^{-1})	Days to maturity	Protein content (%)
Day to 50 % tasseling	-------	0.9349**	-0.0629	0.0318**	-0.3373	-0.6523**	-0.4431**	-0.6609**	-0.6748**	-0.1193	-0.6388**	0.0690**	-0.0351
Day to 50 % silking		-------	-0.1177	-0.1046	-0.3033	-0.6866**	-0.7093**	0.5544	-0.6449**	-0.0873**	-0.6919**	0.0912**	-0.0292
Plant height (cm)			-------	0.9903**	0.4813	0.7341**	0.9037**	0.0043	0.7449**	0.4969*	0.7530**	-0.9056**	0.0548
Ear height (cm)				-------	0.5269*	0.5440*	0.576*	0.469*	0.610	0.4928*	0.6277**	0.9029**	0.0933
No. of cobs plant^{-1}					-------	-0.7558	-0.5245	-0.7109	-0.6802	-0.2048	0.7314**	0.0219	0.1672
Cob length (cm)						-------	-0.6051*	0.7848**	0.6464**	0.1468	0.7112**	0.0729	-0.0976
Kernel rows cob^{-1}							-------	0.6587**	0.6664**	-0.5166*	0.6434**	0.0676	-0.1520
kernels cob^{-1}								-------	0.7924	-0.1210*	0.3653**	0.0412	0.0020
Shelling percentage									-------	0.1894	0.3706	0.0510**	-0.0129
100 seed weight (g)										-------	0.7736**	0.2719	-0.0173
Grain yield (qha^{-1})											-------	0.1135	0.1676
Days to maturity												-------	-0.0066
Protein content (%)												---	-- -------

* Significant at 5 % level; ** significant at 1 % level

Similarly, days to 50 % silking correlated positively with maturity days but negatively with grain yield, cob length and kernel rows cob^{-1}. Ear height exhibited strong positive correlations with important yield components, such as cob length, kernels cob^{-1}, kernel rows cob^{-1} and 100-seed weight. Interestingly, shelling percentage and protein content generally did not show correlations with most traits. However, grain yield was negatively correlated with protein content, which aligns with earlier findings (38). Overall, these correlations indicate that traits like cob length, kernels cob^{-1} and seed weight directly enhance yield potential and should be emphasized in maize breeding efforts.

Estimation of genetic divergence

Grouping of genotypes into different clusters (D^2 analysis)

Based on the performance of the genotypes, fifty genotypes (including checks) were grouped into 8 clusters (Table 6, Fig. 2) as per the Mahalanobis D^2 analysis (25). Cluster I was the largest, having 23 genotypes indicating genetic similarity among them, followed by cluster II with 16 genotypes and cluster IV with 6 genotypes. Custer III, V, VII and VIII each contained 1 genotype.

The 45 maize inbred lines were classified into eight clusters. The data indicates that Mahalanobis D^2 values serve as a strong indicator of genetic diversity among the experimental plant material. The genotypes were distributed randomly across different clusters, suggesting that the genetic variation is not solely associated with the geographical origin of the plants. Instead, factors such as genetic drift and various forms of selection, both natural and human-driven, as well as the exchange of genetic material, have likely played significant roles in creating this diversity. Prior research has produced similar conclusions (39).

Mean intra- and inter-cluster distance

The distances between genotypes within the same cluster (intra-cluster distances) were smaller than the distances between genotypes from different clusters (inter-cluster distances). This depicts that genotypes grouped in the same cluster were more similar to each other and had less diversity. Table 7 shows that cluster IV had the largest intra-cluster distance value of 73.15, indicating higher diversity within that group. This was followed by cluster II with 59.26 and cluster I with 43.47 respectively. The inter-cluster distance (D^2) value was highest between cluster IV and cluster VIII with a distance of 341.59, followed by cluster VII and cluster VIII with 321.49, cluster III and cluster VIII with 270.51, cluster I and cluster VIII with 245.63, cluster IV and cluster V with 226.51 and cluster IV and cluster VI with 191.40, suggesting more diversity in the genetic makeup of the genotypes included in these clusters. The minimum inter-cluster distance was observed between cluster V and cluster VI with a distance of 45.60.

Statistical distance measures how genetically different clusters are from one another. The smallest distance between clusters was found between clusters V and VI, suggesting that these groups of genotypes are genetically quite similar and share many common traits. On the other hand, the greatest genetic distance was recorded between clusters IV and VIII, indicating that these clusters are the most genetically distinct. Selecting parents from such diverse clusters for hybridization programs can facilitate the development of novel recombinants. Previous findings also followed a similar trend (40). The highest genetic diversity within clusters, as indicated by the maximum intra-cluster distance, was found in cluster IV, followed by clusters II and cluster I. This pattern may be due to limited gene flow

Table 6. Distribution of different QPM genotypes into clusters based on D^2 statistics

Cluster	Number of genotypes in the cluster	Variety/accession number of the genotypes
1	23	KDQPM 17, KDQPM 37, KDQPM 42, VQL1, KD QPM 41, KDQPM 12, KDQPM 21, KDQPM 16, CML193, KDQPM 43, KDQPM 9, KDQPM 24, KDQPM 28, KDQPM 11, KDQPM 7, KDQPM 6, KDQPM 31, KDQPM 18, KDQPM 2, KDQPM 44, VQL3, KDQPM 47, KDQPM 48
2	16	KDQPM 3, KDQPM 5, KDQPM 23, KDQPM 32, KDQPM 34, KDQPM 36, VQL2, CML163, KDQPM 46, KDQPM 30, KDQPM 39, KDQPM 40, KDQPM 25, KDQPM 29, KDQPM 1, KDQPM 27
3	1	KDQPM 8
4	6	KDQPM 35, KDQPM 45, KDQPM 15, KDQPM 19, KDQPM 10, KDQPM 33
5	1	KDQPM 26
6	1	KDQPM 4
7	1	KDQPM 22
8	1	KDQPM 38

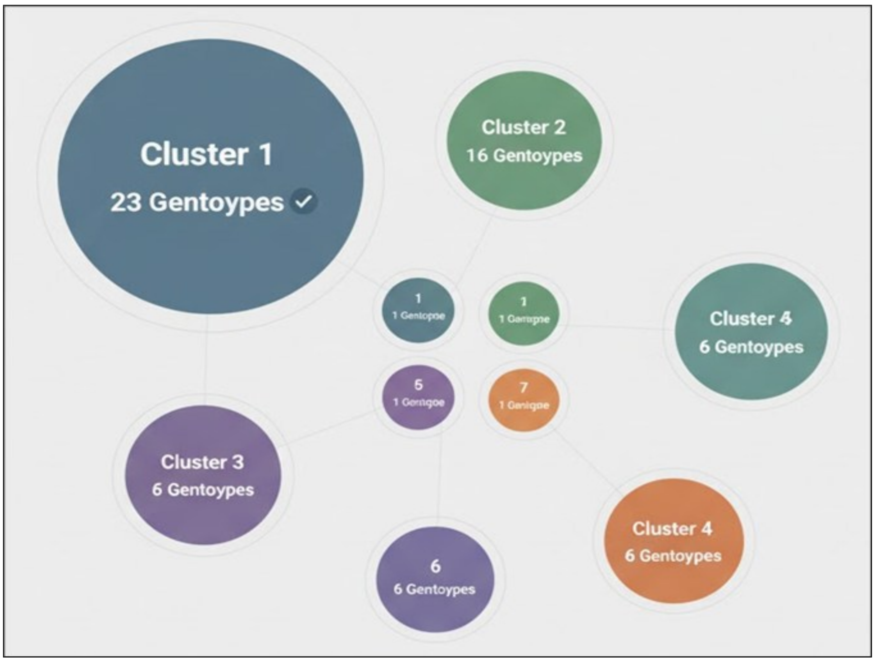


Fig. 2. Distribution of different QPM genotypes into clusters.

Table 7. Average inter-cluster (above diagonal) and intra-cluster (diagonal) D^2 values among different maize genotypes

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
1	43.47	95.06	58.08	78.35	127.42	117.67	66.71	245.63
2		59.26	111.28	122.90	102.00	91.93	142.05	148.97
3			0.00	74.41	171.15	132.57	70.31	270.51
4				73.15	226.51	191.40	109.09	341.59
5					0.00	45.60	165.93	81.00
6						0.00	162.71	82.25
7							0.00	321.49
8								0.00

or selective breeding practices focusing on diverse traits within these groups of genotypes.

Cluster means for morphological and quality component traits in different clusters

The evaluation of mean values for different morphological and agronomic traits showed significant variation among the eight identified clusters, as shown in Table 8. Most genotypes were included in cluster I, with moderate values for most of the traits, including grain yield (20.91 q/ha), plant height (135.68 cm) and 100-seed weight (23.32 g). Only sixteen genotypes were included in cluster II and recorded the highest grain yield (22.50 q/ha) and the highest number of cobs plant^{-1} (1.56). Only a single genotype was included in cluster III, which excelled in cob length (16.95 cm), kernel rows cob^{-1} (18.00), kernels cob^{-1} (450) and protein content (13.13 %), making it a strong candidate for enhancing yield and quality traits. Cluster IV, comprising six genotypes, was notable for early flowering and maturity, a high shelling percentage (77.27 %) and a significant kernel number (375.22). Despite having the lowest kernel count and grain yield (15.17q/ha), cluster V recorded the highest 100-seed weight (25.20 g).

Only a single genotype was included in each of the remaining clusters VI, VII and VIII and they showed distinct extremes; the poorest results for grain yield (11.58 q/ha) and ear traits were shown by cluster VI, whereas the shortest plants (92.33 cm) and the lowest shelling percentage (65.80 %) were presented by cluster VIII, suggesting poor yield potential. Cluster VII showed a favorable mix of grain yield (20.01 q/ha), seed weight (24.25 g) and plant height (145.67 cm). It is pivotal to recognize that cluster averages may obscure outstanding individual genotype performance. Hence, parent selection for crossing programs should be based on both

Table 8. Cluster means for morphological and quality component traits in different clusters of maize genotypes

Clusters	Day to 50 % tasseling	Days to 50 % silking	Plant height (cm)	Ear height (cm)	No. of cobs plant^{-1}	Cob length (cm)	Kernel rows cob^{-1}	kernels cob^{-1}	Shelling percentage	Test weight (g)	Grain yield (qha^{-1})	Maturity days	Protein content
1	79.88	84.10	135.68	68.75	0.43	15.78	14.03	314.72	75.02	23.32	20.91	148.10	12.46
2	76.06	80.17	138.60	70.33	0.66	14.23	13.79	261.79	72.31	20.51	22.50	145.58	12.33
3	79.33	84.33	149.00	74.67	0.40	16.95	18.00	450.00	75.25	18.26	21.88	147.67	13.13
4	73.22	77.17	142.94	73.89	0.40	16.38	15.44	375.22	77.27	20.91	20.76	141.94	12.14
5	82.67	86.67	139.00	72.00	0.73	13.05	12.00	124.00	66.77	25.20	15.17	152.67	12.37
6	79.33	84.00	119.00	59.33	0.40	9.30	12.67	113.60	68.55	18.53	11.58	151.33	12.43
7	78.33	83.67	145.67	61.67	0.40	15.59	13.33	309.33	75.42	24.25	20.01	145.33	11.83
8	82.33	86.67	92.33	46.67	1.00	7.87	12.00	120.00	65.80	19.30	15.43	153.00	12.50

inter-cluster distances and divergence in specific traits to identify promising combinations for yield and quality enhancement in maize.

Contribution of different characters towards divergence

The trait-wise percentage involvement to overall genetic divergence (Table 9) depicted that grain yield (q/ha) was the major factor for variation (26.21 %), followed by kernel rows cob^{-1} (16.40 %), cob length (11.35 %) and 100-seed weight (9.39 %). Moderate contributions were noted for days to 50 % tasseling (7.1 %), days to maturity (6.45 %) and number of cobs plant^{-1}(6.29 %). Minimal contributions were reported from shelling percentage (4.49 %), days to 50 % silking (3.59 %), plant height (2.78 %), kernels cob^{-1}(2.17 %), protein content (1.96 %) and ear height (1.82 %).

Priority should be given to traits that contribute the most to Mahalanobis D^2values when selecting clusters for further breeding and choosing parents for hybridization. In this study, the traits with the highest contribution to genetic divergence were the grain yield (q/ha), kernel rows cob^{-1}, cob length, 100-grain weight, days to 50 % tasseling, days to maturity and number of cobs plant^{-1}. Earlier reports also support these findings (41, 42).

Table 9. Percentage contribution of individual traits towards total divergence in maize (*Zea mays* L.)

Traits	Number of times appearing first in ranking	Percent contribution towards total divergence
Day to 50 % tasseling	7	0.52
Days to 50 % silking	177	13.09
Plant height (cm)	154	11.39
Ear height (cm)	58	4.28
No. of cobs plant^{-1}	37	1.99
Cob length (cm)	267	19.75
Kernel rows cob^{-1}	135	9.99
Kernels cob^{-1}	74	5.47
Shelling percentage	51	3.78
Test weight (g)	43	3.18
Grain yield (qha^{-1})	27	22.12
Days to maturity	299	2.74
Protein content (%)	23	1.70
	1352	**100.00**

Conclusion

More than 70 % of the total genetic divergence was accounted to yield-contributing traits, highlighting their significance in breeding strategies. After evaluating maturity, yield and protein trait performance, genotypes KDQPM35, KDQPM48, VQL17, KDQPM46 and KDQPM33 were distinguished as promising candidates for further genetic enhancement. The results thus provide a useful genetic base for developing high-yielding, nutritionally improved QPM hybrids suited to temperate climates. Future studies should focus on multi-location evaluation of these promising lines, validation through molecular markers and their use in hybridization programs to combine yield and quality traits. In addition, integrating climate-resilient and biofortification traits will help develop stable QPM hybrids adaptable to changing environmental conditions.

Authors' contributions

AAL and MWA carried out the experiment, collected observations and analysed the data. AAL guided the research by formulating the research concept; ZAD and AAL approved the final manuscript. AAL participated in the design of the study and performed the statistical analysis. BD, S, VMD, AT, AL, DP, SJ[1], VDC and SJ[2] contributed by conducting the experiment and helped to edit, summarise and revise the manuscript. All authors read and approved the final manuscript (SJ[1] stands for Shourabh Joshi and SJ[2] stands for Sapna Jarial).

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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