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Correlation, path coefficient analysis and genetic divergence of morphological and biochemical traits with emphasis on protein-starch relationship in maize (*Zea mays* L.)

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Abstract
Grain yield in maize (*Zea mays* L.) is a complex quantitative trait influenced by multiple morphological, physiological, and biochemical characters. The present study investigated interrelationships among yield components with a special focus on the relationship between protein and starch content. Forty maize inbred lines, along with two checks, were evaluated during Kharif 2024 at R.K. Nagar Farm, Kolhapur, under the All India Coordinated Research Project (AICRP) on Maize in a randomized block design. Correlation analysis revealed that grain yield per plant exhibited highly significant positive associations with cob weight, starch content, 100-kernel weight, number of kernels per row, cob diameter, number of kernel rows per cob, cob length, cob height, and plant height. Protein content, however, showed a strong negative association with both grain yield and starch content, highlighting a trade-off between yield and nutritional quality. Path coefficient analysis confirmed that cob weight exerted the strongest direct positive influence on yield, while protein content and days to silking showed negative direct effects. Genetic divergence analysis grouped the genotypes into five clusters, revealing wide genetic variability, with clusters IV and V showing superior yield and starch performance. This study emphasizes that while starch content can serve as a reliable selection criterion for yield improvement, careful consideration is required to balance the negative association of protein with yield and starch to achieve nutritionally balanced maize.

Keywords: Maize, grain yield, protein, starch, correlation, path analysis, genetic divergence, *Zea mays*

Introduction
Maize (*Zea mays* L.) is one of the most widely cultivated cereal crops globally, playing a critical role in food security, animal feed, and industrial applications. It ranks among the top three cereal crops worldwide in terms of production and area cultivated (Shiferaw *et al*., 2011) [21]. In countries like India, maize has emerged as a vital crop due to its adaptability across diverse agro-climatic conditions and its high yield potential (Zaidi *et al*., 2013) [28].
Grain yield improvement remains a primary goal in maize breeding programs. However, yield is a complex polygenic trait, influenced by multiple morphological, physiological, and biochemical components (Bello *et al*., 2012) [4]. Among biochemical traits, starch and protein content are of particular importance. Starch serves as the primary carbohydrate reserve in maize kernels and contributes directly to grain weight and yield (Zhang *et al*., 2020) [31]. In contrast, protein content contributes to the nutritional quality of the grain, but often shows an inverse relationship with starch accumulation and overall yield due to competitive metabolic pathways (Zarkadas *et al*., 2000) [30]. Understanding these biochemical trade-offs is essential for developing high-yielding and nutritionally improved maize genotypes.
To dissect the complex interrelationships among traits, statistical tools such as correlation and path coefficient analysis are commonly employed. Correlation analysis helps identify associations among traits, while path analysis partitions these into direct and indirect effects, offering deeper insight into trait contributions to yield (Dewey & Lu, 1959; Saleh *et al*., 2002) [7, 20]. Additionally, genetic divergence analysis using Mahalanobis D^2 statistics is effective for identifying genetically diverse parents in breeding programs to exploit heterosis

and broaden the genetic base (Aliu *et al*., 2018) [2].

The present study aimed to assess correlation, path coefficients, and genetic divergence among maize genotypes for morphological, yield, and biochemical traits, with a special focus on the protein-starch-yield relationship to guide future selection and hybrid development.

Materials and Methods

The experiment was conducted at R. K. Nagar Farm, under the All India Coordinated Research Project (AICRP) on Maize, located in Kasba Bawada, Kolhapur. This region falls under the West Coast Plains and Ghat Region (XII) as per the Agricultural Contingency Plan for Kolhapur district (2011). Geographically, the experimental site is situated at 16°42′17.24″N latitude and 74°14′10.74″E longitude, at an elevation of 605 meters above mean sea level. The soil at the site is red, well-drained, and has good moisture-holding capacity, making it suitable for maize cultivation. The plant material used in the present study comprised 40 maize inbred lines along with 2 check varieties. These genotypes were obtained from the All India Coordinated Research Project (AICRP) on Maize, located at Kasba Bawada, Kolhapur. To understand the association among the characters, genotypic and phenotypic correlations coefficient were worked out by adopting method described by Singh and Chaudhary (1977) [23]. Path coefficient analysis was done according to the procedure suggested by Wright (1921) [27] and Dewey and Lu (1959) [7]. The genetic divergence between genotypes was estimated using Mahalanobis D^2 statistic (1936). The grouping of genotypes into different clusters was done using Tocher's method as described by Rao (1952) [19].

The details of all the genotypes and check varieties used in the study are presented in Table 1

Table 1: List of maize genotypes employed in the present investigation

Sr. No.	Inbreds/Genotypes	Sr. No.	Inbreds/Genotypes
1.	AH23R-005-0063	22.	AH23R-005-0035
2.	AH23R-005-0012	23.	AH23R-005-0005
3.	AH23R-005-0024	24.	AH23R-005-0071
4.	AH23R-005-0041	25.	AH23R-005-0058
5.	AH23R-005-0009	26.	AH23R-005-0049
6.	AH23R-005-0021	27.	AH23R-005-0036
7.	AH23R-005-0007	28.	AH23R-005-0066
8.	AH23R-005-0073	29.	AH23R-005-0032
9.	AH23R-005-0040	30.	AH23R-005-0064
10.	AH23R-005-0018	31.	AH23R-005-0046
11.	AH23R-005-0045	32.	AH23R-005-0054
12.	AH23R-005-0067	33.	AH23R-005-0059
13.	AH23R-005-0016	34.	AH23R-005-0039
14.	AH23R-005-0002	35.	AH23R-005-0074
15.	AH23R-005-0025	36.	AH23R-005-0056
16.	AH23R-005-0031	37.	AH23R-005-0019
17.	AH23R-005-0068	38.	AH23R-005-0055
18.	AH23R-005-0065	39.	AH23R-005-0001
19.	AH23R-005-0057	40.	AH23R-005-0026
20.	AH23R-005-0060	41.	DKC-9144
21.	AH23R-005-0072	42.	PKC-02

Results and Discussion

The analysis of variance (Table 2) showed that the mean sum of squares attributable to genotypic differences was highly significant for all 13 traits, indicating the presence of considerable genetic variability among the 40 inbred lines and 2 check varieties.

Table 2: Analysis of variance for different characters in Maize (*Zea mays* L.)

Sr. No.	Character	Mean sum of square		
		Replication df = 1	Treatment df = 41	Error df = 41
1	Days to 50 percent tasseling	0.43	27.90**	0.53
2	Days to 50 percent silking	3.44	27.69**	1.07
3	Plant height (cm)	457.33	345.30**	116.11
4	Cob height (cm)	65.19	59.74**	19.70
5	Cob length (cm)	0.57	6.58**	0.95
6	Cob diameter (cm)	0.04	0.54**	0.06
7	Number of kernel rows per cob	2.33	8.20**	0.87
8	Number of kernels per row	5.76	19.61**	4.47
9	Cob weight (g)	11.45	2049.14**	79.32
10	100 kernel weight (g)	0.05	17.44**	2.46
11	Protein (%)	0.49	1.63**	0.17
12	Starch (%)	1.94	11.30**	1.11
13	Grain yield per plant (g)	0.98	1760.31**	78.52

*,** significant at 5% and 1% respectively

A) Correlation

Table 3. presents the genotypic correlation coefficients between yield and its related components across all possible comparisons under both environmental conditions.

The grain yield per plant showed highly significant and positive association with cob weight (0.982) followed by starch (0.8041), 100 kernel weight (0.7423), number of kernels per row (0.7387), cob diameter (0.6044), number of row per cob (0.6016), cob length (0.582), cob height (0.4567) and plant height (0.4212). The character protein (-0.6048) was negatively associated and significant at the genotypic level. The character days to 50 percent tasselling (-0.0292) and days 50 percent silking (-0.0686) was negatively associated but non-significant at the genotypic level. These findings are in agreement with the reports of Lad *et al*. (2018) [13], who observed significant and positive correlations of plant height, cob height, cob length, number of kernel per cob, 100-kernel weight. Similarly, Ali *et al*. (2015) [1] also reported strong positive genotypic associations of yield with 100-kernel weight, cob diameter, number of kernels per row, and plant height. Similarly, Kumar *et al*. (2025) [11] reported a positive association for traits such as cob girth, number of kernel rows per cob, cob weight, and 100-kernel weight. Kumar and Kumar (2000) [12] reported positive correlations of grain yield with plant height, cob weight, and the number of kernels per row. Likewise, Umakanth and Khan (2001) [25] found similar associations for cob width, cob length, and plant height. Studies by Tang Hua *et al*. (2004) [24] and Mohan *et al*. (2002) [16] also demonstrated that traits like plant height, cob dimensions, and kernel rows per cob have significant positive relationships with grain yield. These findings are consistent with Wannows *et al*. (2010) [26], who identified a strong positive correlation between grain yield and both the number of kernels per row and ear length. This indicates that selecting for these traits can be an effective strategy to enhance maize grain yield. Furthermore, Beiragi *et al*. (2011) [3] emphasized that kernel number per row and ear length exhibit significant positive genotypic correlations with total yield, reinforcing their value as selection criteria in breeding programs focused on yield improvement. Furthermore, days to 50 percent tasseling (-0.0292) and days to 50 percent silking (-0.0686) showed negative but non-significant genotypic associations with grain yield, which is consistent with the results of Noor *et al*. (2018) [18], who found weak and non-significant negative correlations of these phenological traits with yield.

The results of the study indicate that the wide variability among traits provides a strong foundation for effective selection in crop improvement programs. Correlation analysis helps in understanding the interrelationships among different quantitative traits, which is important for selecting appropriate breeding methods. The genetic correlation coefficients offer insight into the degree of association between traits, making them valuable tools for achieving overall genetic improvement in maize.

In the present study, Grain yield per plant in maize exhibited strong and highly significant positive genotypic associations with several key yield-contributing traits, notably cob weight, starch content, 100-kernel weight, number of kernels per row, cob diameter, number of rows per cob, cob length, cob height, and plant height. These positive correlations suggest that improvement in these traits could directly enhance grain yield. Conversely, protein content showed a significant negative correlation with yield, indicating a possible trade-off between yield and protein concentration. Days to 50 percent tasseling and silking were negatively correlated with yield but were non-significant, implying limited influence on yield performance. Overall, traits with strong positive correlations can serve as reliable selection criteria in yield improvement programs.

Table 3: Genotypic correlation among grain yield and its attributing characters in maize (*Zea mays* L.)

Observations	Days to 50 percent tasseling	Days to 50 percent silking	Plant height (cm)	Cob height (cm)	Cob length (cm)	Cob diameter (cm)	Number of kernel rows per cob	Number of kernels per row	Cob weight (g)	100 kernel weight (g)	Protein (%)	Starch (%)	Yield per Plant (g)
Days to 50 percent tasseling	1	0.9739**	0.1394	0.1761	-0.2778*	-0.2511*	-0.2776*	-0.3367**	0.0223	-0.0118	0.2265*	-0.1594	-0.0292
Days to 50 percent silking		1	0.1807	0.2128	-0.3496**	-0.3195**	-0.3563**	-0.4467**	-0.0277	-0.0123	0.2893**	-0.1935	-0.0686
Plant height (cm)			1	0.9989**	0.3164**	0.2587*	0.2207*	0.3445**	0.4186**	0.2919**	-0.3478**	0.5419**	0.4212**
Cob height (cm)				1	0.3247**	0.2636*	0.2358*	0.3628**	0.4598**	0.3033**	-0.3075**	0.545**	0.4567**
Cob length (cm)					1	1.0377**	0.9737**	0.8651**	0.5773**	0.0553	-0.4405**	0.5601**	0.582**
Cob diameter (cm)						1	0.9636**	0.8655**	0.5964**	0.1049	-0.4725**	0.5604**	0.6044**
Number of kernel rows per cob							1	0.855**	0.607**	0.0667	-0.4477**	0.5715**	0.6016**
Number of kernels per row								1	0.7429**	0.1604	-0.5917**	0.6901**	0.7387**
Cob weight (g)									1	0.7088**	-0.5932**	0.788**	0.982**
100 kernel weight (g)										1	-0.3507**	0.4575**	0.7423**
Protein (%)											1	-0.8436**	-0.6048**
Starch (%)												1	0.8041**
Yield per Plant (g)													1

*,** significant at 5% and 1% respectively

B) Path Coefficient Analysis

Path coefficient analysis is a valuable statistical tool used to understand the direct and indirect effects of yield-contributing traits on grain yield in maize. In the current study, this analysis was conducted following the method proposed by Dewey and Lu (1959) [7] to determine the nature and extent of these effects. While correlation analysis helps identify associations among traits, combining it with path analysis offers a more dependable approach for selection in breeding programs. Since grain yield is a complex trait influenced by many components, it is essential to distinguish which traits influence it directly and which do so indirectly. Therefore, understanding the direct and indirect contributions of each trait to yield is important for effective crop improvement.

The detailed results of the path analysis, including the direct and indirect effects of individual traits on grain yield, are presented in Table 4.

In the present investigation, traits showing the highest positive direct effects on grain yield included cob weight (1.2498), followed by cob height (0.4072), cob length (0.3657), days to 50 percent tasseling (0.2367), cob diameter (0.0552), and starch content (0.0306). These results suggest that selecting these traits directly can be effective in improving yield. On the other hand, traits such as protein content (-0.019), 100-kernel weight (-0.1204), number of kernel rows per cob (-0.3241), plant height (-0.3614), number of kernels per row (-0.4287), and days to 50 percent silking (-0.4375) showed negative direct effects on grain yield. Additionally, the residual effect of 0.1861 indicates the presence of other contributing factors not included in the analysis, suggesting further study is needed to fully understand the variation in grain yield.

The present findings align well with earlier reports by Kumar *et al*. (2004) [10], who demonstrated that traits such as cob width, cob length, and days to 50 percent tasseling had a positive direct influence on grain yield, indicating their potential as key selection criteria in maize breeding. Similarly, Brar *et al*. (2008) [6] also observed positive direct effects of cob width, cob length, and cob height on grain yield, further supporting the idea that selection for these cob-related traits can enhance productivity. The results are also consistent with Zarie *et al*. (2012) [29], who reported a negative direct effect of the number of kernels per row on grain yield, reinforcing the observation in the current study that this trait may not be ideal for direct selection in yield improvement programs. Additionally, Nataraj *et al*. (2014) [17] found positive direct effects of plant height, cob height, cob width, days to 50 percent tasseling, and cob weight on grain yield, which closely mirrors the present investigation. Grace *et al.* (2018) [8] further confirmed that traits like days to 50 percent silking, plant height, cob height, and cob length had notable effects on yield, in agreement with both the positive and negative contributions observed in this study.

However, it exerted a negative indirect effect via days to 50 percent tasseling (-0.0049), days to 50 percent silking (-0.0059), and protein content (-0.0258).

Based on the path coefficient analysis, it can be concluded that cob weight, cob height, cob length, days to 50 percent tasseling, cob diameter, and starch content had the strongest positive direct effects on grain yield.

These traits can be considered key components for direct selection in breeding programs aimed at improving maize yield. Prioritizing these traits may enhance the efficiency of selection and contribute significantly to overall yield improvement.

Table 4: Direct (diagonal) and Indirect (above and below diagonal) path effects of different characters towards grain yield at genotypic level in maize.

Observations	Days to 50 percent tasseling	Days to 50 percent silking	Plant height (cm)	Cob height (cm)	Cob length (cm)	Cob diameter (cm)	Number of kernel rows per cob	Number of kernels per row	Cob weight (g)	100 kernel weight (g)	Protein (%)	Starch (%)
Days to 50 percent tasseling	0.2367	0.2305	0.033	0.0417	-0.0658	-0.0594	-0.0657	-0.0797	0.0053	-0.0028	0.0536	-0.0377
Days to 50 percent silking	-0.4261	-0.4375	-0.079	-0.0931	0.153	0.1398	0.1559	0.1954	0.0121	0.0054	-0.1266	0.0847
Plant height (cm)	-0.0504	-0.0653	-0.3614	-0.361	-0.1143	-0.0935	-0.0798	-0.1245	-0.1513	-0.1055	0.1257	-0.1958
Cob height (cm)	0.0717	0.0866	0.4067	0.4072	0.1322	0.1073	0.096	0.1477	0.1872	0.1235	-0.1252	0.2219
Cob length (cm)	-0.1016	-0.1279	0.1157	0.1188	0.3657	0.3795	0.3561	0.3164	0.2111	0.0202	-0.1611	0.2048
Cob diameter (cm)	-0.0139	-0.0176	0.0143	0.0145	0.0573	0.0552	0.0532	0.0478	0.0329	0.0058	-0.0261	0.0309
Number of kernel rows per cob	0.0899	0.1155	-0.0715	-0.0764	-0.3155	-0.3122	-0.3241	-0.2771	-0.1967	-0.0216	0.1451	-0.1852
Number of kernels per row	0.1444	0.1915	-0.1477	-0.1555	-0.3709	-0.3711	-0.3666	-0.4287	-0.3185	-0.0688	0.2537	-0.2959
Cob weight (g)	0.0278	-0.0346	0.5232	0.5746	0.7215	0.7454	0.7586	0.9284	1.2498	0.8858	-0.7413	0.9849
100 kernel weight (g)	0.0014	0.0015	-0.0351	-0.0365	-0.0067	-0.0126	-0.008	-0.0193	-0.0853	-0.1204	0.0422	-0.0551
Protein (%)	-0.0043	-0.0055	0.0066	0.0058	0.0084	0.009	0.0085	0.0112	0.0112	0.0067	-0.019	0.016
Starch (%)	-0.0049	-0.0059	0.0166	0.0167	0.0171	0.0171	0.0175	0.0211	0.0241	0.014	-0.0258	0.0306
Grain yield per Plant (g)	-0.0292	-0.0686	0.4212	0.4567	0.582	0.6044	0.6016	0.7387	0.982	0.7423	-0.6048	0.8041

R^2 = 0.9654, Residual effect = 0.1861

C) Genetic divergence

Estimates of genetic divergence in 40 inbreds/genotypes and 2 checks for 13 characters was obtained by following Mahalanobis D^2 statistics.

I) Cluster formation

The cluster formation was obtained (Tocher's method Fig.1), as described by Rao (1952). The 42 genotypes (40 inbreds and 2 checks) under investigation were grouped into five clusters, out of which cluster I emerged as the largest cluster with 20 inbreds followed by cluster II with 16 genotypes (15 inbreds and 1 checks), cluster III with 3 inbreds and cluster IV with 2 inbreds. The clusters V is solitary. Details of genotypes belonging to different clusters are presented in table 5

Table 5: Distribution of 42 genotypes (40 inbreds and 2 checks) of maize into different cluster

Cluster	Number of Genotypes Included	Names of Genotypes	Common traits
I	20	AH23R-005-0068, AH23R-005-0064, AH23R-005-0001, AH23R-005-0025, AH23R-005-0054, AH23R-005-0035, AH23R-005-0058, AH23R-005-0021, AH23R-005-0036, AH23R-005-0032, AH23R-005-0031, AH23R-005-0007, AH23R-005-0019, AH23R-005-0057, AH23R-005-0072, AH23R-005-0055, AH23R-005-0039, AH23R-005-0066, AH23R-005-0049, AH23R-005-0071	Moderate days to 50 percent tasseling (49.33) and silking (53.00), Average protein content (8.33%), Low grain yield per plant
II	16	AH23R-005-0012, AH23R-005-0046, AH23R-005-0040, AH23R-005-0060, AH23R-005-0018, AH23R-005-0059, AH23R-005-0009, AH23R-005-0041, AH23R-005-0005, AH23R-005-0016, AH23R-005-0063, AH23R-005-0067, AH23R-005-0002, AH23R-005-0065, AH23R-005-0024, DKC-9144	Early maturity
III	3	AH23R-005-0074, AH23R-005-0026, AH23R-005-0056	Late maturity
IV	2	AH23R-005-0073, AH23R-005-0045	Superior performance in cob traits
V	1	PKC-02	Plant height, cob height; starch with good protein.

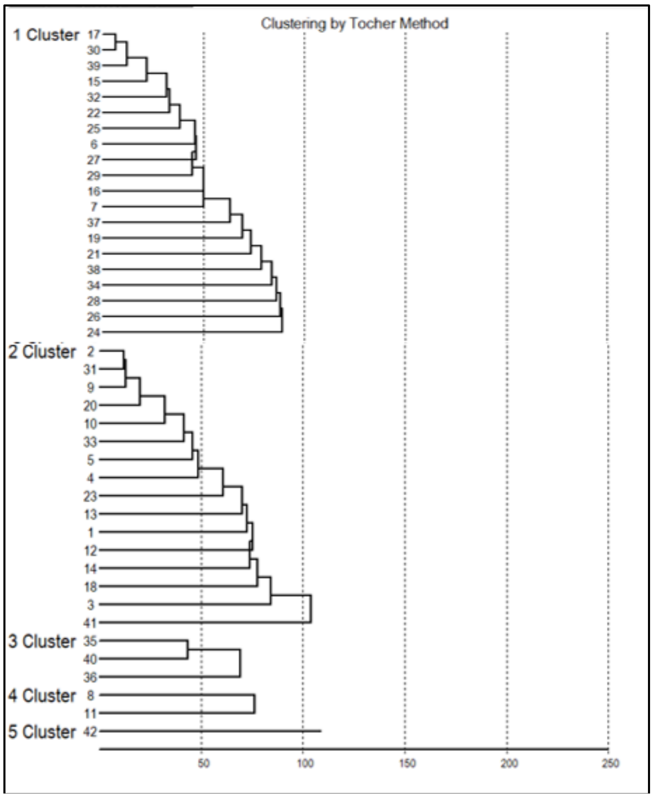

Fig 1: Dendrogram (cluster diagram) showing relationship among 42 maize (*Zea mays* L.) genotypes in five clusters based on Mahalnobis D2 values

II) Intra and inter cluster distance

The intra and inter-cluster D² and D values were calculated using D² statistics. The average D² values of the cluster members served as indicators of intra and inter-cluster distances. These results are shown in Table 6.

The highest intra-cluster distance was observed for cluster III (D^2 = 9.147) followed by cluster II (D^2 = 8.756) and cluster IV (D^2 = 8.739) indicates the presence of wide genetic diversity among the genotypes present within these cluster. Whereas, lowest intra-cluster distance observed in cluster I (D^2 = 8.501). The clusters V showed no intra cluster distances because mono-genotypic in nature. The maximum inter-cluster distance observed between clusters II and III (D^2 = 23.36) followed by clusters III and IV (D^2 = 22.955), clusters II and V (D^2 = 21.657) indicates that greater the distance between two clusters, the greater will be the divergence. The minimum inter-cluster distances between cluster I and II (D^2 = 13.79) followed by cluster I and III (D^2 = 13.98) indicating proximity with each other. These results are in accordance with Singh *et al*., (1999) [22], Khumkar and Singh (2002) [9], Marker and Krupakar (2009) [15] and Bhadru *et al*., (2020) [5]

Table 6: Average intra and inter cluster D^2 and D values of five clusters formed from 42 genotypes (40 inbreds and 2 checks)

Cluster	I	II	III	IV	V
I	8.501 (2.92)	13.79 (3.71)	13.98 (3.74)	16.886 (4.11)	15.713 (3.96)
II		8.756 (2.96)	23.36 (4.83)	14.477 (3.80)	21.657 (4.65)
III			9.147 (3.02)	22.955 (4.79)	14.383 (3.79)
IV				8.739 (2.96)	16.519 (4.06)
V					0

Conclusion

The present study highlights the importance of cob weight, cob architecture, and starch content as reliable selection criteria for enhancing maize grain yield. Starch content showed strong positive association with yield, making it a key trait for yield improvement. Protein content, however, showed strong negative associations with both starch and yield, reflecting a trade-off between nutritional quality and productivity. Breeding strategies should therefore aim at achieving a balance—developing maize hybrids that combine high yield with acceptable protein levels. Genetic divergence analysis revealed wide variability, and genotypes such as AH23R-005-0067, AH23R-005-0045, AH23R-0018, AH23R-005-0049, and AH23R-005-0073 were identified as promising parents. Overall, these results provide a roadmap for maize improvement programs targeting yield enhancement while addressing the protein-starch trade-off.

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