Maize Journal (October 2026) 15(2): 101-108

RESEARCH PAPER

Identifying Key Selection Criteria for Grain Yield and Starch Content Improvement in Doubled Haploid lines of maize

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Abstract: The present study assessed genetic variability and trait associations to identify potential selection criteria for improving grain yield and starch content in doubled haploid (DH) maize lines. Ninety DH lines and four replicated checks were evaluated during *kharif,* 2025 using an augmented randomized complete block design at the Maize Research Unit, Agricultural Research Institute, Rajendranagar, Hyderabad, Telangana. Adjusted means were used for genetic variability, correlation and path coefficient analyses. Significant treatment variation was observed for most traits. Grain yield exhibited high broad-sense heritability (96.35%) and genetic advance as a percentage of mean (26.24%), indicating substantial potential for selection. Grain yield showed strong positive correlations with starch content, number of kernels per row, ear diameter and ear length. Before path analysis, variance inflation factor values for the seven selected predictor traits ranged from 1.59 to 4.26, indicating no severe multicollinearity. Starch content exhibited the highest positive direct effect on grain yield (0.644), followed by number of kernels per row (0.253) and ear diameter (0.129). The seven-trait model explained 92.45% of the variation in adjusted mean grain yield. Notably, DH-62 recorded the highest grain yield (108.03 g plant^{-1}) and starch content (63.79%). These findings identify starch content, number of kernels per row and ear diameter as promising selection attributes for improving grain yield, with potential for simultaneous improvement of productivity and starch quality. Further validation across environments and combining-ability studies are required before utilizing promising DH lines in hybrid development.

Keywords: Maize • Doubled haploids • Augmented design • Grain yield • Starch content • Correlation • Path analysis

Introduction

Maize (*Zea mays* L.) is one of the world's major cereal crop, serving as an important source of food, feed and industrial raw material. In addition to grain productivity, grain quality attributes, particularly starch content, are gaining importance because of their applications in starch processing and bioethanol production (Niu *et al.*, 2023). Therefore, simultaneous evaluation of grain yield and starch content is important for identifying maize genotypes with desirable productivity and end-use quality.

Grain yield is a complex quantitative trait influenced by multiple component characters, environmental conditions and genotype × environment (G×E) interactions. Consequently, direct selection for grain yield alone may be less effective than selection supported by a better understanding of its associated traits. Correlation analysis helps quantify the strength and direction of

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Received: 01 October 2026/ Accepted: 22 October 2026

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relationships among traits, while path coefficient analysis partitions these relationships into direct and indirect effects, thereby facilitating the identification of potentially useful selection criteria. (Kumar *et al.,* 2024).

Doubled haploid (DH) technology enables the rapid development of completely homozygous maize lines, providing genetically fixed material for genetic evaluation and selection (Prasanna *et al.,* 2012; Chaikam *et al*., 2019). However, the usefulness of yield-contributing and grain-quality traits as selection criteria depends on the genetic material and traits evaluated. A key research gap is the need to characterize the relationships among grain yield, starch content and associated yield-contributing traits and to distinguish their direct and indirect effects on grain yield in diverse maize DH populations.

Correlation analysis determines the strength and direction of associations between grain yield and its contributing traits, helping identify potential selection criteria (Reddy *et al.,* 2022). However, path coefficient analysis further partitions these associations into direct and indirect effects, providing a better understanding of trait relationships and their usefulness in selection (Pranay *et al*., 2022). The magnitude and direction of these associations may vary depending on the genetic material and population evaluated.

Therefore, the present study evaluated 90 maize DH lines and four checks to assess the associations among grain yield, starch content and yield attributing traits through correlation and path coefficient analyses, with the aim of identifying useful selection criteria for improving grain yield and starch content.

Materials and methods

The experimental material comprised 90 doubled haploid (DH) lines derived from F_2 population of IC 623880 × KML 109 and IC 627708 × BML 6. The experiment was conducted during the *kharif*, 2025 at the Maize Research Unit, Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, Telangana, India. Four established maize genotypes, BML-100, BML-101, BML-105 and BML-106, were included as replicated checks. The experiment was conducted in an augmented randomized complete block design (augmented RCBD) following the procedure described by Federer (1956). The experimental material was distributed across three blocks, with each block containing 30 DH lines and the four replicated checks. The experimental plots were maintained at 60 cm between rows and 20 cm between plants, with a row length of 4 m and a plot area of 4.8 m². Recommended agronomic practices were followed uniformly throughout the crop season as per the PJTAU, Vyavasaya panchangam. Observations were recorded for 14 quantitative traits: days to 50% anthesis, days to 50% silking, anthesis–silking interval (ASI), days to maturity, plant height (cm), ear height (cm), ear length (cm), ear diameter (cm), number of kernel rows per ear, number of kernels per row, test weight (g), grain yield per plant (g), shelling percentage (%) and starch content (%). Flowering and maturity traits were recorded on a plot basis. Plant and ear measurements were taken from five randomly selected plants per genotype. Grain yield per plant was estimated from the grain weight of the five tagged plants after drying and shelling and expressed as the average in grams per plant. Shelling percentage was calculated as the proportion of grain weight to ear weight, expressed as a percentage.

Starch estimation

Starch content was estimated using the anthrone method as described by Sadasivam and Manickam (1996) and expressed as percentage of grain dry matter. Approximately 100 mg of finely powdered dry grain sample was washed with 80% ethanol to remove free sugars. The residue was hydrolysed using distilled water and 52% perchloric acid, and the pooled extract was made up to a known volume. An aliquot of the extract was mixed with freshly prepared anthrone reagent in concentrated sulphuric acid and heated in a boiling-water bath for 8 minutes. After rapid cooling, absorbance was measured at 620 nm using a spectrophotometer. A glucose standard curve was used to estimate the glucose equivalent, which was multiplied by 0.9 to calculate starch content. Samples, standards and blanks should be processed consistently.

Statistical analysis

The 90 unreplicated DH lines were analysed using the augmented design procedure of Federer (1956), with the four replicated checks used to estimate block effects and experimental error. Analysis of variance (ANOVA) was performed separately for each trait, and adjusted means

were obtained for the DH lines after accounting for block effects. These adjusted means were used for subsequent Variability, correlation and path analyses. Genetic variability parameters, including genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad-sense heritability (H^2), genetic advance (GA) and genetic advance as percentage of mean (GAM), were calculated according to Falconer and Mackay (1996). Broad-sense heritability and genetic advance were estimated following the methods suggested by Johnson *et al.* (1955) and Burton (1952), respectively. Pearson correlation coefficients were estimated among the 14 traits, and their significance was tested at the 5%, 1% and 0.1% probability levels. Prior to path analysis, multicollinearity among candidate predictor traits was assessed using the variance inflation factor (VIF), with values below 5 considered indicative of no severe multicollinearity. Path coefficient analysis was performed following Dewey and Lu (1959), with grain yield as the dependent variable, to partition the correlations of the selected predictor traits into direct and indirect effects. The coefficient of determination (R^2) and residual path coefficient $\sqrt{1-R^2}$ were calculated for the final path model. All statistical analyses were performed using R version 4.6.0 in RStudio version 2026.05.0+218.

Results and discussion

Analysis of variance

The analysis of variance under the augmented randomized complete block design revealed significant variation among treatments for most of the traits evaluated (Table 1). The treatment effect was highly significant ($P \leq 0.001$) for days to 50% anthesis, days to 50% silking, plant height, number of kernels per row, shelling percentage, grain yield and starch content, while significant variation ($P \leq 0.05$) was observed for days to maturity and test weight, and at $P \leq 0.01$ for ear length, ear diameter and number of kernel rows per ear while treatment effect for anthesis–silking interval was non-significant. Significant block effects were detected for most traits, indicating variation among blocks; however, block effects were non-significant for anthesis–silking interval, ear height and test weight. Overall, the significant treatment effects observed for the majority of traits indicating substantial phenotypic differentiation among the evaluated entries. Similar significant variation among maize inbred lines for flowering, plant, ear, kernel and grain yield traits has been reported in recent studies (Sinana *et al.,* 2023; Sharma *et al.,* 2026).

Table 1. Analysis of variance for fourteen quantitative traits in doubled haploid maize lines evaluated under augmented randomized complete block design

Trait	Blocks (ignoring treatments) Df:2	Adjusted treatments (eliminating blocks) Df:93	Checks Df:3	Blocks (eliminating checks and DH lines) Df:2	Entries (DH lines + checks) Df:93	DH lines (unreplicated test entries) Df:89	Checks vs DH lines Df:1	Error Df:6
Days to 50% anthesis	55.186***	11.005**	5.667*	1.083	12.168***	12.153***	33.047***	0.750
Days to 50% silking	47.245***	10.801**	6.000*	1.750	11.779**	11.588**	46.201***	0.750
Anthesis–silking interval	0.275	0.582	0.111	0.083	0.586	0.597	1.025	0.528
Days to maturity	49.618***	10.827*	2.000	4.083	11.807*	11.578*	61.554**	1.750
Plant height	68.701*	400.919***	356.750***	31.000	401.729***	405.246***	223.722**	11.667
Ear height	37.850	107.186**	145.676**	29.010	107.376**	106.102**	105.879**	7.472
Ear length	2.142	4.848**	7.814**	1.561	4.861**	4.797**	1.708	0.418
Ear diameter	1.487*	1.500**	2.543**	0.401	1.524**	1.458**	4.269**	0.196
No. of kernel rows per ear	4.605**	1.943**	6.271***	0.173	2.038**	1.886**	2.839**	0.191
No. of kernels per row	14.157**	33.616***	5.332	2.521	33.866***	32.070***	279.314***	1.152
Test weight	2.186	28.519*	39.132*	17.688	28.186*	26.660*	131.151**	6.132
Shelling percentage	6.633**	10.483***	4.728**	6.580**	10.484***	10.789***	0.692	0.428
Grain yield	217.890***	262.920***	84.416***	4.563	267.507***	233.243***	3866.290***	3.278
Starch content	7.604***	8.956***	2.320***	0.031	9.119***	8.266***	105.358***	0.044

Genetic variability

The variability among the inbred lines was measured using mean, GCV and PCV for all 13 traits presented in Table 2. A wide range of variability was observed for all the traits, of which grain yield/plant ranges from 38.60 g to 108.03 g/plant with an overall mean of 71.7 g/plant and starch content varied from 49.49% to 63.79% with a mean value of 56.25%. DH-62 recoded an yield of 108.03 g/plant with starch content of 63.79%. The estimates of genetic variability revealed that grain yield exhibited high genotypic and phenotypic coefficients of variation (GCV: 12.97%; PCV: 13.22%), coupled with high heritability (96.35%) and genetic advance as per cent of mean (GAM: 26.24%), indicating substantial genetic variability and good potential for improvement through selection. Similar findings for maize grain yield, with high heritability and genetic advance, have been reported by Kumar *et al.* (2024). Starch content recorded very high heritability (98.54%) but low GCV (3.06%) and GAM (6.27%), indicating limited variability and a comparatively lower expected response to selection for this trait in the evaluated doubled haploid lines. Thus, selection for grain yield can be prioritized, while starch content should be considered alongside yield and other quality attributes in maize improvement programmes.

Correlation analysis

Pearson correlation coefficients were estimated among the adjusted means of the 90 doubled haploid (DH) maize lines to assess the associations among grain yield and related traits (Table 3). Grain yield (GY) exhibited significant positive associations with several yield-related traits. The strongest positive association was observed with starch content (SC; r = 0.927***), followed by number of kernels per row (NKPR; r = 0.816***), ear diameter (ED; r = 0.771***), ear length (EL; r = 0.719***), plant height (PH; r = 0.553***), test weight (TW; r = 0.386***), number of kernel rows per ear (NKRE; r = 0.379***), ear height (EH; r = 0.364***), and anthesis–silking interval (ASI; r = 0.267*). In contrast, shelling percentage (SP) showed a negligible and non-significant association with GY (r = 0.005). The strong positive associations of GY with SC, NKPR, ED and EL indicate that variation in grain yield was closely associated with variation in starch content and major ear and kernel attributes in the evaluated DH population. Plant and ear traits showed several significant positive interrelationships. PH was strongly correlated with EH (r = 0.785***), and was also positively associated with EL (r = 0.515***), ED (r = 0.596***), NKRE (r = 0.433***), NKPR (r = 0.457***), TW (r = 0.326**), GY (r = 0.553***), and

Table 2. Estimates of genetic variability parameters, heritability and genetic advance for fourteen traits in ninety doubled haploid maize line

Trait	Mean	GCV	PCV	ECV	Heritability	GA	GAM
Days to 50% anthesis	61.142	3.024	3.339	1.416	82.007	3.449	5.641
Days to 50% silking	64	2.86	3.164	1.353	81.709	3.408	5.326
ASI	2.869	4.673	25.751	25.324	3.293	0.05	1.747
Days to maturity	104.025	1.672	2.101	1.272	63.357	2.852	2.742
Plant height	103.849	10.969	11.451	3.289	91.75	22.476	21.643
Ear height	44.528	12.948	14.329	6.139	81.645	10.731	24.1
Ear length	11.674	10.411	11.791	5.535	77.962	2.211	18.936
Ear diameter	11.516	5.725	6.898	3.848	68.878	1.127	9.787
No. of kernel rows per ear	12.329	6.197	7.14	3.546	75.338	1.366	11.081
No. of kernels per row	22.876	14.38	15.126	4.692	90.379	6.442	28.162
Test weight	24.755	11.035	14.894	10.003	54.894	4.169	16.842
Shelling percentage	72.922	2.511	2.666	0.897	88.683	3.552	4.87
Yield	71.704	12.974	13.218	2.525	96.351	18.811	26.235
Starch	56.246	3.064	3.087	0.374	98.535	3.524	6.266

Table 3. Pearson correlation coefficients among adjusted means of 90 doubled haploid maize lines for grain yield and associated traits

Trait	DFT	DFS	ASI	DTM	PH	EH	EL	ED	NKRE	NKPR	TW	SP	GY	SC
DFT	—													
DFS	0.977***	—												
ASI	–0.153	0.058	—											
DTM	0.966***	0.991***	0.068	—										
PH	–0.354***	–0.348***	0.045	–0.364***	—									
EH	–0.355***	–0.342***	0.087	–0.338**	0.785***	—								
EL	–0.050	–0.005	0.235*	–0.003	0.515***	0.344***	—							
ED	–0.196	–0.165	0.169	–0.169	0.596***	0.420***	0.786***	—						
NKRE	–0.086	–0.080	0.032	–0.101	0.433***	0.156	0.456***	0.540***	—					
NKPR	–0.115	–0.086	0.137	–0.106	0.457***	0.257*	0.773***	0.703***	0.440***	—				
TW	–0.008	0.016	0.135	0.005	0.326**	0.178	0.566***	0.544***	0.115	0.291**	—			
SP	–0.039	0.008	0.213*	0.037	–0.107	–0.054	–0.266*	–0.245*	–0.064	–0.124	–0.304**	—		
GY	–0.237*	–0.185	0.267*	–0.210*	0.553***	0.364***	0.719***	0.771***	0.379***	0.816***	0.386***	0.005	—	
SC	–0.207*	–0.161	0.234*	–0.182	0.527***	0.338**	0.607***	0.690***	0.385***	0.716***	0.280**	0.053	0.927***	—

SC (r = 0.527***). Similarly, EH showed positive associations with EL (r = 0.344***), ED (r = 0.420***), NKPR (r = 0.257*), GY (r = 0.364***), and SC (r = 0.338**). These relationships indicate that variation in plant and ear height was associated with variation in several ear, kernel and yield-related traits.

Strong positive relationships were also observed among the major ear and kernel traits. EL was positively correlated with ED (r = 0.786***), NKRE (r = 0.456***), NKPR (r = 0.773***), TW (r = 0.566***), GY (r = 0.719***), and SC (r = 0.607***). Similarly, ED showed significant positive associations with NKRE (r = 0.540***), NKPR (r = 0.703***), TW (r = 0.544***), GY (r = 0.771***), and SC (r = 0.690***). NKRE was positively correlated with NKPR (r = 0.440***), GY (r = 0.379***), and SC (r = 0.385***), whereas its association with TW was non-significant (r = 0.115). NKPR showed positive associations with TW (r = 0.291**), GY (r = 0.816***), and SC (r = 0.716***). Collectively, these relationships indicate that ear size and kernel number traits were closely interrelated and were also associated with grain yield.

ASI showed generally weak associations with the other traits. Significant positive correlations were observed with EL (r = 0.235*), SP (r = 0.213*), GY (r = 0.267*), and SC (r = 0.234*), whereas its associations with the remaining traits were non-significant. The generally low magnitudes of these correlations indicate relatively weak associations of ASI with the major yield-related traits in the evaluated population.

TW was positively correlated with EL (r = 0.566***), ED (r = 0.544***), NKPR (r = 0.291**), PH (r = 0.326**), GY (r = 0.386***), and SC (r = 0.280**), while showing a significant negative association with SP (r = –0.304**). SP also exhibited significant negative correlations with EL (r = –0.266*) and ED (r = –0.245*), but its association with GY was negligible and non-significant. SC showed significant positive associations with PH, EH, EL, ED, NKRE, NKPR, TW and GY, while its correlations with DT (r = –0.207*) and DFS (r = –0.161) were negative, with only the association with DT being significant. The strong association of SC with GY, together with its positive relationships with several ear and kernel traits, indicates that starch content varied closely with yield and yield-component traits in the evaluated DH population.

Overall, GY was most strongly associated with SC, NKPR, ED and EL, whereas SP showed virtually no

association with GY. The substantial interrelationships among several predictor traits, particularly the very high correlations among DT, DS and DTM and the strong associations among several ear traits, indicated the need to consider redundancy and multicollinearity before conducting path analysis. Therefore, based on their association with GY, biological relevance and multicollinearity assessment, SC, NKPR, ED, EL, PH, NKRE and TW were selected as predictor variables for path coefficient analysis. Importantly, correlation coefficients describe associations and do not by themselves establish direct or causal effects; hence, path analysis was subsequently used to partition the associations of the selected traits with GY into direct and indirect effects. Gogoi *et al.* (2025) and Praveen Kumar *et al.* (2022) also obtained similar results.

Selection and assessment of predictor traits for path analysis

The correlation analysis revealed substantial interrelationships among several yield-associated traits, indicating the possibility of redundancy among predictor variables in a multiple regression framework. Therefore, multicollinearity was assessed before conducting path coefficient analysis using the variance inflation factor (VIF). Seven traits, namely starch content (SC), number of kernels per row (NKPR), ear diameter (ED), ear length (EL), plant height (PH), number of kernel rows per ear (NKRE) and test weight (TW), were retained as predictor variables for the final path model based on their association with grain yield, biological relevance to yield formation and the assessment of predictor redundancy.

The Variation inflation factor values ranged from 1.59 for NKRE to 4.26 for EL (Table 4). The VIFs for SC, NKPR, ED, EL, PH, NKRE and TW were 2.54, 3.59, 4.17, 4.26, 1.68, 1.59 and 1.88, respectively. All values were below the commonly used threshold of 5, indicating that severe multicollinearity was not evident among the retained predictors. Thus, the seven-trait model provided a statistically interpretable set of predictors for partitioning the observed association of these traits with grain yield. The exclusion of other measured traits from the final path model should not be interpreted as indicating that they are biologically unimportant; rather, the analysis was restricted to a non-redundant set of yield-associated predictors for interpretation of direct and indirect effects.

Table 4. Variance inflation factor (VIF) of predictor variables included in the final path coefficient analysis

Predictor trait	VIF
Starch content (SC)	2.54
Number of kernels per row (NKPR)	3.59
Ear diameter (ED)	4.17
Ear Length (EL)	4.26
Plant height (PH)	1.68
Number of kernel rows per ear (NKRE)	1.59
Test weight	1.88

Path coefficient analysis

Path coefficient analysis revealed that starch content (SC) exerted the highest positive direct effect on grain yield (0.644), followed by number of kernels per row (NKPR; 0.253) and ear diameter (ED; 0.129). Grain yield exhibited strong positive correlations with SC (r=0.927), NKPR (r=0.816), ED (r=0.771) and ear length (EL) (r=0.719). The strong association of SC with grain yield reflected both its substantial direct effect and positive indirect

Table 5. Direct and indirect effects of predictor traits on grain yield in 90 doubled haploid lines of maize

Trait	Direct effect	Via SC	Via NKPR	Via ED	Via EL	Via PH	Via NKRE	Via TW	Total indirect effect	Correlation with GY
SC	0.644	—	0.181	0.089	0.017	0.013	–0.030	0.013	0.283	0.927
NKPR	0.253	0.461	—	0.091	0.022	0.012	–0.035	0.014	0.564	0.816
ED	0.129	0.444	0.178	—	0.022	0.015	–0.043	0.026	0.641	0.771
EL	0.028	0.391	0.195	0.101	—	0.013	–0.036	0.027	0.691	0.719
PH	0.025	0.340	0.115	0.077	0.014	—	–0.034	0.015	0.527	0.553
NKRE	–0.079	0.248	0.111	0.070	0.013	0.011	—	0.005	0.458	0.379
TW	0.047	0.181	0.073	0.070	0.016	0.008	–0.009	—	0.339	0.386

Note: Diagonal values represent direct effects. Off-diagonal values represent indirect effects through the corresponding predictor trait. Total indirect effect is the sum of all indirect effects for the respective trait. Correlation with GY represents the observed Pearson correlation between the predictor trait and grain yield.

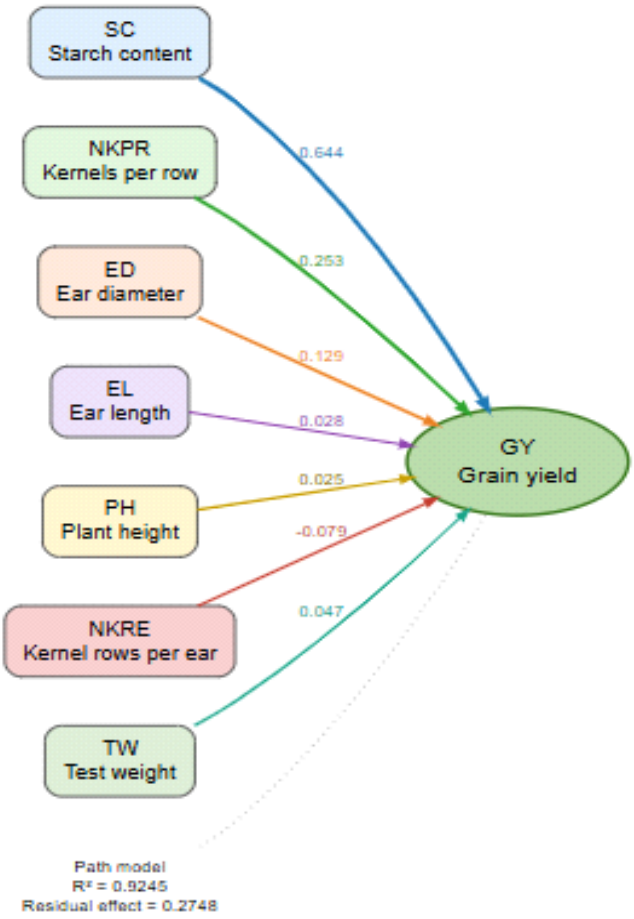


Figure 1. Path diagram showing direct effects of selected predictor traits on grain yield in 90 doubled haploid lines of maize

contributions through NKPR and ED. Similarly, the association of NKPR with grain yield was supported by its positive direct effect and indirect contribution through SC. Although EL, plant height, number of kernel rows per ear and test weight exhibited comparatively small direct effects, their associations with grain yield were largely mediated through other predictor traits. The seven-trait model explained 92.45% of the variation in grain yield (R^2=0.9245), indicating strong explanatory power within the evaluated doubled haploid population. Comparable studies in maize have also identified kernel number and ear-related traits as important selection criteria for grain yield improvement (Praveen Kumar *et al.,* 2024; Gogoi *et al.,* 2025). Collectively, these findings highlight SC, NKPR and ED as promising selection attributes for improving grain yield, with the strong positive association between SC and grain yield offering potential for simultaneous improvement of productivity and starch quality.

Conclusion

The evaluated doubled haploid maize lines exhibited significant genetic variability for most of the studied traits, indicating considerable scope for improvement through selection. Grain yield showed high GCV, PCV, heritability and genetic advance as per cent of mean, suggesting substantial genetic variability and good selection potential. Correlation and path analyses identified starch content, number of kernels per row and ear diameter as important traits associated with grain yield. DH-62 recorded the highest grain yield (108.03 g plant^{-1}) and starch content (63.79%), highlighting its potential for further evaluation. These findings provide a basis for selecting superior doubled haploid lines for developing high-yielding, starch-rich maize hybrids, subject to multi-environment validation and combining-ability assessment.

Acknowledgment

Authors of the Manuscript are most grateful to PJTAU & ICAR-Indian Institute of maize Research for granting them the opportunity to conduct their research and for providing necessary facilities

Author's contribution

SD has conceptualized the theme of this research work. VK analysis the data.SD, VK, SK and BD have written the draft of the manuscript. YKR, SN, LPK, MB and ACH edited the manuscript. All authors finally read and approved the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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