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Original Research Article

ESTIMATION OF GENETIC DIVERSITY IN MAIZE INBRED LINES FOR KEY AGRO-PHONOLOGICAL AND MORPHOLOGICAL TRAITS

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Abstract: *Assessment of genetic diversity in cultivated as well as wild germplasm is the prerequisite for improving any crop through breeding and hybridization. The current experimental study was conducted at Maize Research Sub-Station, Charra Pani, Murree, Punjab, Pakistan during the crop season 2023-24 to evaluate the elite maize inbred lines for their genetic variation for key agro-phonological and grain yield associated traits including days to tessling, days to silking, plant height, cob height, cob length, number of grains per row, number of rows per cob, cob girth and grain yield. The experiment was laid out under randomized complete block design with two replications where row-to-row distance was maintained at 75 cm and plant-to-plant distance was maintained at 20 cm. The data was collected for the above given traits which were then subjected to statistical analysis. The results from the analysis of variance revealed that significant genetic variability was present among study key plant traits in maize inbred lines. Furthermore, correlation coefficient analysis was carried out to compute the magnitude and direction of association between different plant traits within and with grain yield. The results unveiled that grain yield had a positive and significant correlation with plant height (r = 0.4966*), number of grains per row (r = 0.5615*) and cob girth (r = 0.5386*) in studied maize inbred lines. To evaluate the variability present among maize inbred lines, principal component analysis (PCA) along with PC1/PC2 biplot was used which categorized these twenty-eight maize inbred lines into nine principal components (PCs). As revealed in PCA, plant height, cob height, cob length, number of grains per row, cob girth and grain yield were the major contributing traits to genetic variability present in the data along with twelve maize inbred lines i.e., YBH-1-1, YBH-1-2, YBH-1-5, YBH-1-6, YBH-1-8, YBH-1-9, YBH-1-10, YBH-2-11, YBH-2-16, YBH-2-17, YBH-3-27 and YBH-3-28.*

Keywords: Principal Component Analysis, Biplot Analysis, Genetic Diversity, Climate Change

Introduction

Maize (*Zea mays* L.) is an immensely important crop worldwide. It is one of the most widely cultivated cereal crops due to its versatility, adaptability, and high productivity (1). Maize serves as a staple food for millions, a key component in animal feed, and a raw material for various industrial products. Its contribution to food security, agricultural development, and the global economy makes it a vital crop for both developed and developing countries (2). Globally, maize is cultivated across diverse agroecological zones, covering vast areas in countries like the United States, China, Brazil, and Mexico. It is a major source of calories and nutrients, particularly in regions of Africa and Latin America, where it forms a significant portion of the daily diet (3). Maize is rich in carbohydrates and provides essential vitamins and minerals, making it a critical food source for populations facing hunger and malnutrition. Beyond its role as a food crop, maize is extensively used in the production of livestock feed, biofuels, and industrial products, such as starch and ethanol (4). This diverse utilization underscores the crop's importance in ensuring food security, supporting livestock industries, and contributing to renewable energy production.

Maize is one of the most widely cultivated crops globally, with the United States, China, and Brazil being the top producers (5). The United States leads with approximately 35.01 million hectares under cultivation, producing around 389.67 million metric tons (MMT) annually, and achieving an average yield of 11.13 tons per hectare due to advanced agricultural practices and hybrid varieties (USDA, 2024). China, the second-largest producer, grows maize on 44.2 million hectares, producing about 288.84 MMT, with an

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average yield of 6.53 tons per hectare, primarily for feed and industrial purposes. Brazil follows, cultivating maize on 21.50 million hectares, with a production of 122 MMT and a yield of 5.67 tons per hectare. Other significant producers include Argentina and India, contributing 50 MMT and 37.67 MMT, respectively, with average yields of 7.14 and 3.35 tons per hectare. Globally, maize is grown on around 203.55 million hectares, yielding approximately 1.225 billion metric tons, with an average global yield of 6.02 tons per hectare. In Pakistan, maize is cultivated on about 1.64 million hectares, mainly in Punjab and Khyber Pakhtunkhwa, producing approximately 9.85 MMT, with an average yield of 6.0 tons per hectare, driven by the adoption of hybrid varieties in recent years (USDA, 2024).

In Pakistan, maize is the third most important cereal crop after wheat and rice. It plays a significant role in the agricultural economy, providing income to farmers and serving as a key ingredient in poultry feed and other livestock industries. The primary maize-growing regions in Pakistan include Punjab and Khyber Pakhtunkhwa, where both spring and autumn maize varieties are cultivated. Maize contributes to the food and nutritional security of the country, especially in rural areas, where it is consumed in various traditional forms, such as bread (1) and porridge (dalia). Maize production in Pakistan has steadily increased due to the introduction of hybrid varieties, improved agronomic practices, and government initiatives aimed at enhancing crop productivity. The crop's importance extends beyond human consumption and animal feed; maize is also a source of raw material for numerous industries, including the production of starch, corn oil, and various processed foods (6). This wide range of uses makes maize a critical component of Pakistan's agricultural sector and a driver of economic development.

Although the grain yield of maize in Pakistan is near the global average yield of maize, yet Pakistan is far behind from top producing countries due to high temperatures, less irrigation water availability, disease infestation (stalk rot and leaf blight), insect-pest attack (shoot fly and maize borers), high input rates, poor management of crop, unstable governmental policies related to prices of inputs and crop, use of substandard, low-quality seed. Therefore, there is an urgent need for improved maize hybrids that could yield twice the existing traditional maize hybrids under changing environmental conditions. To achieve this, the evaluation of existing germplasm is the first step two words developing the high-yielding, climate-smart maize hybrids. Genetic diversity serves as the foundation for breeding programs, as it enables the identification of genotypes with desirable traits such as higher yield potential, disease resistance, drought tolerance, and nutrient efficiency. By evaluating the genetic variation within maize germplasm, breeders can select parent lines with complementary traits to create hybrid varieties that exhibit superior agro-physiological characteristics. Additionally, genetic diversity assessment allows breeders to address challenges posed by climate change, evolving pest pressures, and soil nutrient depletion, ensuring that maize varieties remain resilient and adaptable to various environmental conditions. Therefore, the current study was designed to evaluate the existing maize inbred lines of temperate to temperate-tropical nature at the Maize Research Sub-Station, Charra Pani, Murree, and Punjab, Pakistan.

Methodology

The current experimental study was conducted at the Maize Research Sub-Station, Charra Pani, Murree, Punjab, Pakistan during the crop season 2022-23. The experimental material consisted of twenty-eight maize inbred lines of diverse origin (Table 1). The experiment was laid out under randomized complete block design with two replications, where the line-to-line distance was maintained at 75 cm while the plant-to-plant distance was maintained at 18 cm. At sowing, seeds were sown with the help of a dibbler at the rate of two seeds per hell which were then thinned to single, strong seedling during the early seedling stage. Standard agronomic and plant protection measures were carried out for all the treatments across all the replications.

The data regarding plant phonological and morphological traits including days to tasseling, days to Silking, plant height, cob height, cob length, number of grains per row, number of rows per cob, cob girth and grain yield were measured. The data was recorded from 10 guarded plants per genotype per replication.

The obtained data were then subjected to statistical data analysis including analysis of variance, correlation coefficients analysis and principal component analysis to estimate the genetic divergence between inbred lines for the given traits (7, 8). To compute the results, three statistical packages i.e. Statistixs 8.1, XLSTAT, and OriginPro were used.

Table 1: Inbred lines used in the study

Sr. No	Inbred Line	Sr. No	Inbred Line	Sr. No	Inbred Line
1	YBH-1-1	11	YBH-1-10	21	YBH-3-21
2	YBH-1-2	12	YBH-2-11	22	YBH-3-22
3	YBH-1-3	13	YBH-2-12	23	YBH-3-23
4	YBH-1-4	14	YBH-2-13	24	YBH-3-24
5	YBH-1-5	15	YBH-2-14	25	YBH-3-25
6	YBH-1-6	16	YBH-2-15	26	YBH-3-26
7	YBH-1-7	17	YBH-2-16	27	YBH-3-27
8	YBH-1-8	18	YBH-2-17	28	YBH-3-28
9	YBH-1-9	19	YBH-2-18		
10	YBH-1-1	20	YBH-2-19		

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Result

The results obtained from the analysis of variance unveiled the presence of highly significant variations among 28 maize inbred lines on the basis of studied plant traits i.e., days to tasseling (DT), days to silking (2), plant height (PH), cob height (CH), cob length (CL), number of grains per row (Gr/R), number of rows per cob (R/Cob), cob girth (CG) and grain yield (GY) (Table 2). The variations were highly significant at 1% confidence level which shows that the inbred lines belong to different groups based on their genetic makeup and structure and thus could be used in different breeding programs aimed at improving maize yield and quality. Similar results have been reported by several scientists who showed that the genetic diversity present among maize genotypes /inbred lines is one of the most important sources of variation for plant breeders to improve existing crop varieties or to develop new cultivars that could perform sustainably under the changing climatic scenario (9-14).

Table 2: Mean Square (MS) of key plant traits in maize inbred lines

SOV	df	GY	DT	DS	PH	CH	CG	CL	Gr/R	R/Cob
Replications	1	2138	2.9^{E-29}	0.28	48.2	6.4	0.37	2.16	0.45	4.4^{E-31}
Treatments	27	5850139^{**}	23.2^{**}	25.48^{**}	1881.6^{**}	676.9^{**}	57.33^{**}	15.57^{**}	127.7^{**}	8.65^{**}
Error	27	46835	2.6	3.69	29.7	13.7	0.32	0.64	1.41	0.45

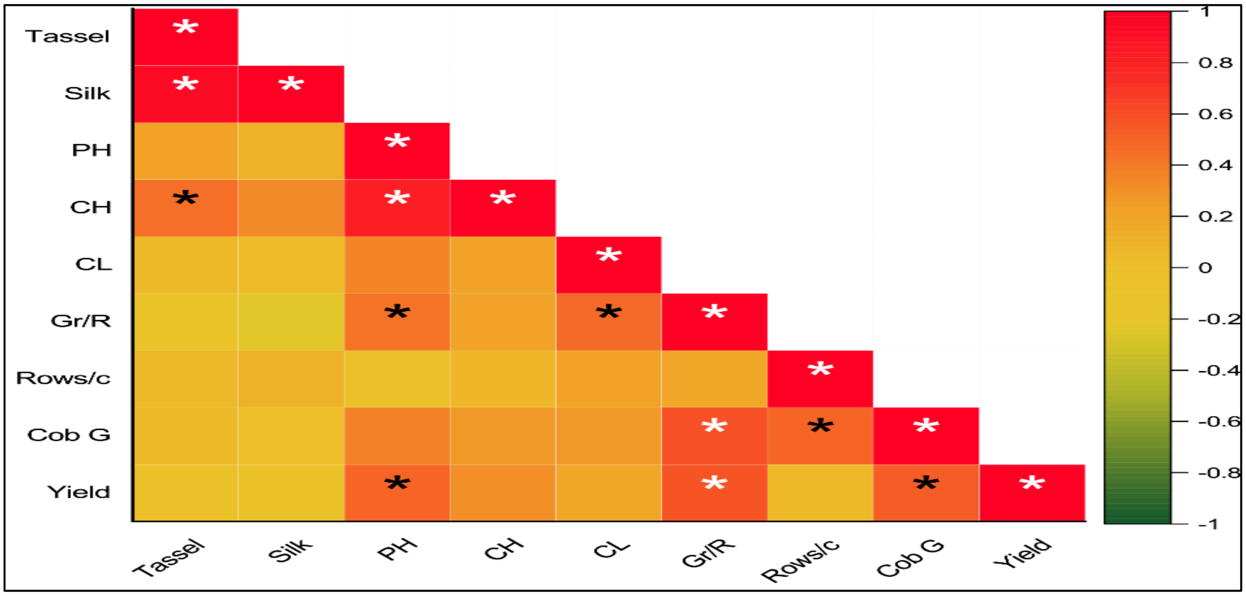
The correlation coefficient analysis is one of the most widely used statistical methods to compute the strength and direction of relationships among different variables under given conditions. In the current study, correlation coefficient analysis was carried out to estimate the magnitude and direction of relationships among different maize inbred lines used in the study (Table 3 & Figure 1). The results revealed that grain yield had a significantly positive correlation with plant height (r = 0.4966^{*}), number of grains per row (r = 0.5615^{*}) and cob girth (r = 0.5386^{*}) while negatively associated with days to tessling (r = -0.0248^{NS}) and days to silking (r = -0.0495^{NS}), however, these negative relationships were not significant. Furthermore, grain yield had a positive correlation with cob height (r = 0.3091^{NS}), cob length (r = 0.1803^{NS}), and number of rows per cob (r = 0.0550^{NS}) but these associations were also non-significant. Similarly, cob girth had a positive and significant correlation with the number of rows per cop (r = 0.4974^{*}) and the number of grains per row (r = 0.5979^{NS}) in maize inbred lines. Similar findings had already been reported by several researchers who showed that grain yield has a significant association with days to tasseling, days to Silking, plant height, cob height, cob length, number of grains per row, number of rows per cob and cob girth under different growth conditions (15-22).

Table 3: Correlation coefficient between different plant traits in maize inbred lines

	Tassel	Silk	PH	CH	CL	Gr/R	Rows/c	Cob G	Yield
Tassel	1	0.9239	0.2015	0.4446	0.0560	-0.1270	0.0493	0.0525	-0.0248
Silk	0.9239	1	0.1175	0.3334	0.0205	-0.2259	0.1051	0.0114	-0.0495
PH	0.2015	0.1175	1	0.8018	0.3582	0.4228	-0.0052	0.3708	0.4966
CH	0.4446	0.3334	0.8018	1	0.2152	0.2073	0.0655	0.2613	0.3091
CL	0.0560	0.0205	0.3582	0.2152	1	0.4744	0.2150	0.2744	0.1803
Gr/R	-0.1270	-0.2259	0.4228	0.2073	0.4744	1	0.1958	0.5979	0.5615
Rows/c	0.0493	0.1051	-0.0052	0.0655	0.2150	0.1958	1	0.4974	0.0550
Cob G	0.0525	0.0114	0.3708	0.2613	0.2744	0.5979	0.4974	1	0.5386
Yield	-0.0248	-0.0495	0.4966	0.3091	0.1803	0.5615	0.0550	0.5386	1

Assessment of genetic diversity in crop plants has always been important to plant scientists. In the current study, two different multivariate approaches i.e., principal component analysis and cluster analysis were used to evaluate the genetic diversity present among maize inbred lines under the temperate to tropical-temperate conditions of Murree, Pakistan. The principal component analysis categorized nine plant traits into nine principal components, ranging the eigenvalue from 3.168 in PC-1 to 0.0575 in PC-9, while their individual variance ranged from 35.2% in PC-1 to 0.6% in PC-9 (Table 4 and Figure 2). The cumulative PC-1 and PC-2 variance was 59.6%, which could perfectly be used to draw the PC-1/PC-2 combined biplot (Figure 3). The PC-1/PC-2 biplot showed that days to tasseling, days to silking, cob height, plant height, grain yield, cob girth and number of grains per row were the most distinctive traits as they were at maximum distance from their point of origin, wherever the number of rows per cob and cob length showed minimum variations among the plant traits. The principal component analysis depicted that the maximum variations in PC-1 were mainly due to plant height (0.44846), cob height (0.39252), cob length (0.30503), number of grains per row (0.39624), cob girth (0.41154) and grain yield (0.38534) while the variations in PC-2 were due to days to tasseling (0.60421) and days to silking (0.60760) while in PC-3 the major reason for variation in data was due to number of rows per cob (0.72695) as depicted by their factor loading values (Table 5).

Similarly, with respect to inbred lines, significant variations were observed in maize inbred lines in three important



principal components i.e., PC-1, PC-2 and PC-3. In PC-1, twelve maize inbred lines i.e., YBH-1-1, YBH-1-2, YBH-1-5, YBH-1-6, YBH-1-8, YBH-1-9, YBH-1-10, YBH-2-11, YBH-2-16, YBH-2-17, YBH-3-27 and YBH-3-28 contributed significantly to the variation in the data. In PC-2, seven maize inbred lines i.e., YBH-1-3, YBH-1-4, YBH-2-14, YBH-2-18, YBH-2-19, YBH-3-21 and YBH-3-24 shared most of the variations in the data. In the third and last principal component, PC-3, eight inbred lines i.e., YBH-1-7, YBH-2-12, YBH-2-13, YBH-2-15, YBH-3-22. YBH-3-23, YBH-3-25 and YBH-3-26 contributed to variation in the data (Table 6). Principal component analysis and biplots are frequently used by plant scientists who found them very effective in assessing the genetic divergence present among crop genotypes under different experimental conditions (22-28)

Figure 2: Scree plot of Principal Component Analysis in Maize Inbred lines

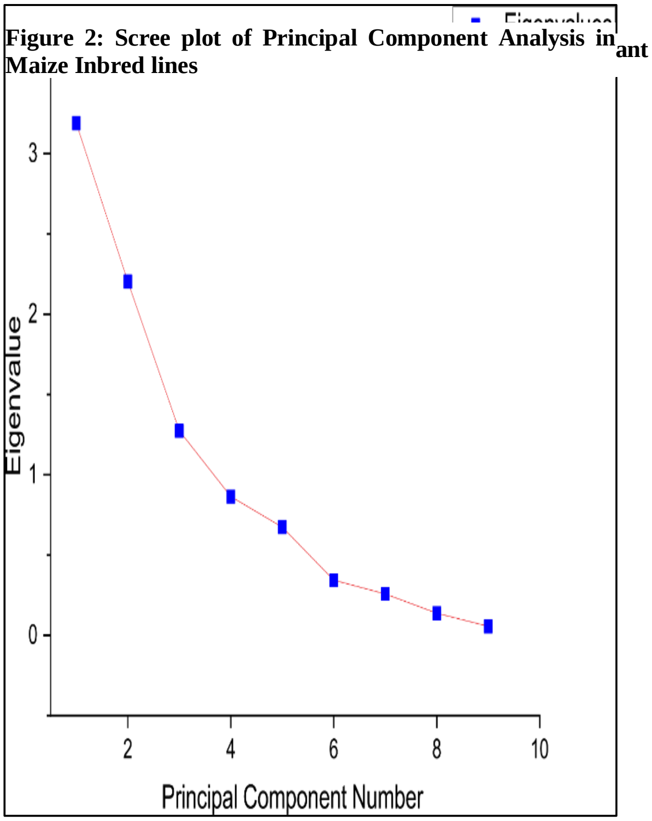


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Figure 3: Biplot of studied plant traits and maize inbred Lines

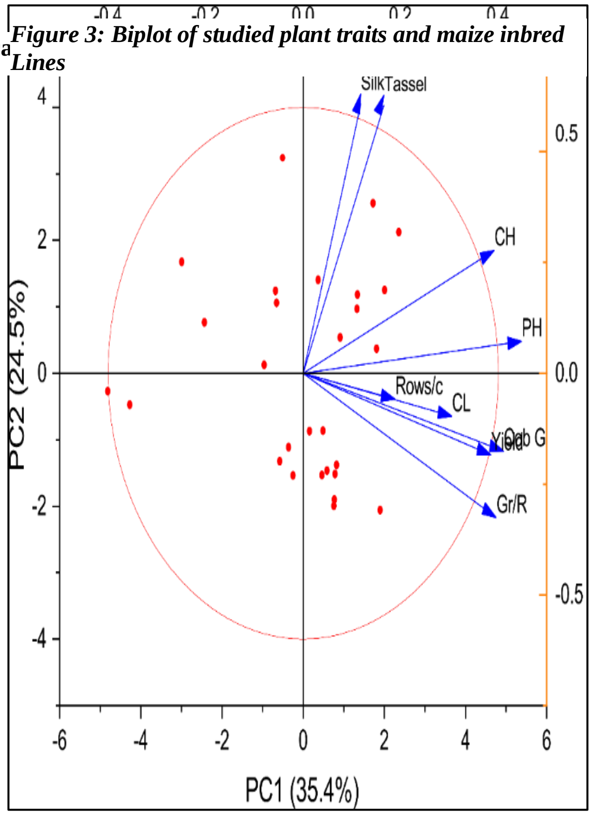

Table 4: Eigenvalues, variance and cumulative variance percentage in twenty-eight maize inbred lines through Principal Component Analysis

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Principal Components	Eigenvalues	Variance	Cumulative Variance
Principal Component-1	3.16816	35.2	35.2
Principal Component-2	2.19562	24.4	59.6
Principal Component-3	1.29609	14.4	74
Principal Component-4	0.87337	9.7	83.7
Principal Component-5	0.66432	7.4	91.1
Principal Component-6	0.34166	3.8	94.9
Principal Component-7	0.26581	3	97.8
Principal Component-8	0.13741	1.5	99.4
Principal Component-9	0.05755	0.6	100

Table 5: Factor loading/ Correlation between plant traits and principal components in maize inbred lines

Inbred Lines/ Principal Components	PC1	PC2	PC3
Day to tasseling	0.16542	0.60421	0.12805
Days to silking	0.11812	0.6076	0.20489
Plant Height	0.44846	0.07212	-0.39894
Cob height	0.39252	0.27735	-0.30478
Cob length	0.30503	-0.09691	0.11548
Number of grains per row	0.39624	-0.32552	0.02328
Number of rows per cob	0.18865	-0.0587	0.72695
Cob girth	0.41154	-0.17582	0.34018
Grain Yield	0.38534	-0.18414	-0.17757

Table 6: Percentage contribution of twenty maize inbred lines in significant principal components

Inbred Lines/Principal Components	PC1	PC2	PC3
YBH-1-1	0.81857	-1.37644	-0.20986
YBH-1-2	1.8942	-2.0542	0.59382
YBH-1-3	-2.99222	1.68005	0.56383
YBH-1-4	-0.25226	-1.53296	-1.00677
YBH-1-5	2.00536	1.25445	-0.70682
YBH-1-6	0.78198	-1.5112	-1.26919
YBH-1-7	0.48591	-0.85709	0.89565
YBH-1-8	0.75536	-1.99364	-0.64004
YBH-1-9	-4.81835	-0.26617	-0.11768
YBH-1-10	0.90959	0.54579	0.37387
YBH-2-11	1.33587	1.18724	-0.30994
YBH-2-12	0.15409	-0.86706	1.87697
YBH-2-13	0.58437	-1.4575	1.4167
YBH-2-14	0.36874	1.40946	0.14311
YBH-2-15	0.46058	-1.52459	0.59447
YBH-2-16	1.80635	0.37299	1.75424
YBH-2-17	1.32404	0.97344	-0.25436
YBH-2-18	1.71943	2.56347	0.50964
YBH-2-19	-0.68341	1.24646	0.23012
YBH-2-20	-0.58088	-1.31645	0.39999
YBH-3-21	-2.43649	0.77137	0.47531
YBH-3-22	-0.96293	0.13245	-2.59327
YBH-3-23	-0.6549	1.06146	2.06082
YBH-3-24	-0.51018	2.90781	-1.30885
YBH-3-25	-4.273	-0.46919	0.3015
YBH-3-26	-0.35995	-1.10655	-2.33831
YBH-3-27	0.76548	-1.89735	-0.53557
YBH-3-28	2.35465	2.12395	-0.89937

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Conclusion

The analyzed data revealed the presence of significant genetic variations among twenty maize inbred lines for studied plant traits. The correlation coefficient analysis, which was executed to compute the magnitude and direction of association between different plant traits within and with grain yield, unveiled that grain yield had a positive and significant correlation with plant height (r = 0.4966*), number of grains per row (r = 0.5615*) and cob girth (r = 0.5386*) in studied maize inbred lines. The principal component analysis (PCA) along with PC1/PC2 biplot categorized twenty-eight maize inbred lines into nine principal components (PCs) based on their variability. PCA further revealed that plant height, cob height, cob length, number of grains per row, cob girth and grain yield were the major contributing traits to genetic variability present in the data along with twelve maize inbred lines i.e., YBH-1-1, YBH-1-2, YBH-1-5, YBH-1-6, YBH-1-8, YBH-1-9, YBH-1-10, YBH-2-11, YBH-2-16, YBH-2-17, YBH-3-27 and YBH-3-28. Therefore, these inbred lines must be used in maize hybridization programs aimed at improving maize yield.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department Concerned.

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

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