



RESEARCH ARTICLE

Agronomic evaluation of maize inbred lines and their potential to produce hybrids adapted to a tropical environment

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Abstract

Maize is the most extensively grown cereal worldwide. It is planted on approximately 197 million hectares and, together with wheat and rice, constitutes a major element of the human diet as well as a key component in global food security. In Malaysia, maize is mainly used for animal feed, although 100 % of it is imported, which affects the nation's food security status. In this sense, developing maize hybrids and varieties could be crucial for the successful cultivation of maize in the country. This study was conducted in Gambang, Pahang, Malaysia, to identify maize inbreds with the best adaptability for the region and the necessary characteristics to be used as inbred parents for developing outstanding hybrids. A total of 17 maize inbreds obtained from Asia and CIMMYT, Mexico were planted in a randomized complete block design (RCBD) with two replications. Each replication was planted on separate planting dates. A full diallel-cross was implemented to determine their ability to produce hybrids in all possible combinations. With the one-way analysis of variance (ANOVA), it was noticed that the seed weight, leaf number and ear aspect of the inbreds had significant differences. The inbreds HPOOL23 and MrChua-OP1 showed the highest seed weight values when used as either males or females. Moreover, the neighbor-joining method helped to identify the inbreds with high yield and good ear quality. This study will serve as a basis for understanding the behaviour of inbreds in hybrid development.

Keywords: correlation analysis; grain yield; maize breeding; phenotypic similarity; phenotypic variability

Introduction

Maize is the cereal crop cultivated globally, covering approximately 197 million hectares in 70 countries across a wide range of latitudes and varying altitudes (1). In addition, together with wheat and rice, maize constitutes a major component of the human diet, accounting for approximately 42 % of the world's food calories and 37 % of the protein intake (1). The FAO foresees that these staple cereals will continue to play a crucial role in food security until 2050 and consequently, it would be fundamental to augment their productivity to effectively address population growth and the triple burden of undernutrition, micronutrient malnutrition and overnutrition (2, 3).

In Malaysia, maize is primarily used for animal feed, although 100 % of it is imported (4, 5). For the 2024-2025 marketing year, the country is projected to import 3.7 Mt of grain to meet livestock feed demand, making the nation extremely dependent on overseas maize supplies (6). This situation has undoubtedly affected Malaysia's food security status (7). Similarly, the New Sources of Wealth (SKB) initiative of the Eleventh Malaysian Plan (11th MP) has recognized that, local maize grain cultivation could be a fundamental approach to augment the food security of Malaysia and reduce maize

imports, ensuring the increments of livestock price could remain steady and therefore affordable for consumers. In this respect, it is essential to continue developing maize hybrids and varieties with high yields that are adapted to Malaysia's conditions, while also implementing new methods to reduce production costs, so that the maize grain industry can become more competitive, attractive and profitable for farmers (7).

Nevertheless, to develop successful maize hybrids, it is crucial to first identify and develop maize inbreds with the potential to produce these hybrids. A detailed characterization of the inbreds is essential, as it can help to understand the genetic diversity available for crop improvement by providing the necessary elements to create heterotic groups. Selection of inbreds with a good combining ability (general combining ability, specific combining ability or both) is needed to develop superior hybrids (8-10). Moreover, the combining ability methods and its effectiveness in developing high-yielding and nutritionally enriched maize hybrids, specifically for Malaysian conditions have been thoroughly discussed in recent studies (11).

Malaysia has been making significant efforts to develop high-yielding hybrids. Initially, the focus was on developing inbred lines, resulting in the creation of over 200 families from

various populations that were generated through self-pollination and subsequently selected based on their performance in subsequent generations (12, 13). By the fifth generation of selfing, the research continued with the evaluation of the inbreds and consequently, with the revelation of many inbred lines that had potential as parentals (14). From their research, the hybrid Putra J-58 was developed and produced a high yield of 6.2 t/ha (15). Subsequently, the private company Green World Genetics (GWG) developed GWG 888, which demonstrated an even higher yield of 9.3 t/ha (4). In addition, positive correlations were found between yield and other traits of interest, such as seed weight, plant height and ear height, after testing 20 maize inbreds in the tropical environment of Malaysia (16).

Despite advancements, further research on maize in Malaysia is needed. This includes testing and developing maize inbreds and hybrids across more regions and states of the country, identifying maize inbreds that could be used as testers, as this is critical for forming heterotic groups and creating superior hybrids (17, 18). Additionally, developing maize inbreds and hybrids with enhanced nutritional content is a proven approach to defeat malnutrition and micronutrient deficiency in the population (19, 11). Consequently, this study was conducted to identify maize inbreds with better adaptability and necessary characteristics to be used as parentals for developing outstanding hybrids.

Materials and Methods

Plant material

A total of 17 maize inbreds consisting of 10 Asian inbreds (8 pure lines and 2 lines derived from Asian populations) and 7 from the International Maize and Wheat Improvement Center (CIMMYT), Mexico (3 CIMMYT maize lines and 4 lines derived from Mexican populations) were used in the experiment (Table 1).

Experimental design

The experiment was conducted from April to August 2023 at the Green World Genetics (GWG) farm in Gambang, Pahang, Malaysia (3.740 °N, 103.051 °E). The average temperature during those months was 28.48 °C, while the average humidity was 79 % and the daily average precipitation was 5.62 mm (20) (Fig. 1). A

Table 1. Different inbreds used in the experiment

S.No	Inbreds	Origin	Type of material
1	NS12	Asia	Asian Line
2	TESTER-A	Asia	Asian Line
3	TESTER-B	Asia	Asian Line
4	PH6W	Asia	Asian Line
5	5241	Asia	Asian Line
6	43009	Asia	Asian Line
7	CR36	Asia	Asian Line
8	26211	Asia	Asian Line
9	MrChua-OP1	Asia	Line derived from Asian populations
10	MrChua-OP8	Asia	Line derived from Asian populations
11	CML481	CIMMYT-Mexico	CIMMYT maize line
12	CML310	CIMMYT-Mexico	CIMMYT maize line
13	CML264	CIMMYT-Mexico	CIMMYT maize line
14	HPOOL23	CIMMYT-Mexico	Line derived from CIMMYT - Mexican population
15	HPOOL33	CIMMYT-Mexico	Line derived from CIMMYT - Mexican population
16	HPOOL26	CIMMYT-Mexico	Line derived from CIMMYT - Mexican population
17	HPOOL22	CIMMYT-Mexico	Line derived from CIMMYT - Mexican population

randomized complete block design (RCBD) with two replications was used in this experiment (21). Each replication was planted on separate planting dates, as in addition of evaluating the maize inbreds, the intention was also to do all the possible crosses between the different inbreds to get preliminary data on how they function best (as male parent or as female parent); and to so, it was essential to plant two sets of inbreds at two different planting dates, to make sure all of them got crossed (synchronize) with the others, despite the differences in the flowering days. The first planting date was April 11, 2023 and the second one was April 19, 2023. In both planting dates, four rows were sown per variety and each row consisted of 26 plants. The

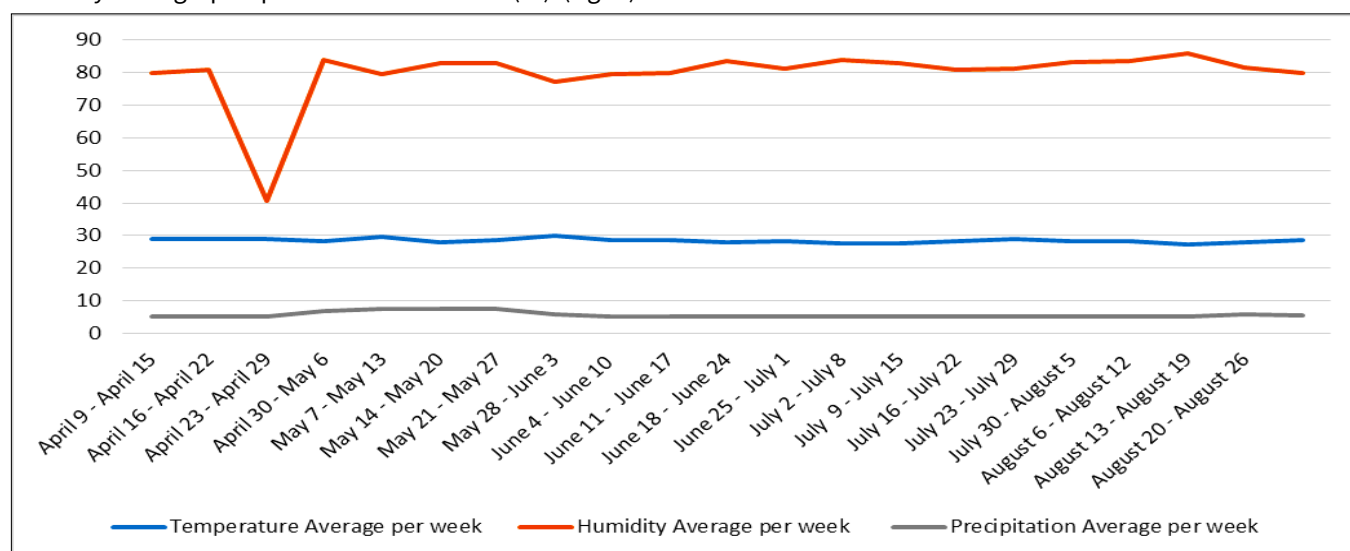


Fig. 1. Weather information for the entire period of the experiment (20).

distance between plants within a row was 25 cm and the distance between rows was 75 cm. The cultivation technique and agronomic practices were performed according to the parameters established by the Malaysian Agricultural Research and Development Institute (MARDI) (22). The data collected during flowering time included plant height, plant aspect, disease tolerance (*Bipolaris maydis* and *Exserohilum turcicum*) and leaf number. In contrast, the data collected during the harvesting time included seed weight (seed weight-female and seed weight-male) and ear aspect (Table 2). Correspondingly, a full cross-diallel was implemented to determine their ability to produce hybrids in all possible combinations (31, 32).

Data analysis

The one-way analysis of variance (ANOVA) was performed to elucidate the genotype effect on each trait. The Tukey-Kramer test at a 0.05 level of significance was conducted to understand the difference between genotype means. The Pearson correlation coefficient analysis was performed using genotype means to elucidate the linear relationship between traits with the following formula (33):

$$r = \frac{n\sum XY - \sum X \sum Y}{\sqrt{(n\sum x^2 - (\sum x)^2) \cdot (n\sum y^2 - (\sum y)^2)}} \quad (\text{Eqn. 1})$$

Where, n = the number of data points, i.e., (x, y pairs), in the data set, $\sum XY$ = the sum of the product of the x -value and y -value for each point in the data set, $\sum X$ = the sum of the x -values in the data set, $\sum Y$ = the sum of the y -values in the data set, $\sum x^2$ = the sum of the squares of the x -values in the data set and $\sum y^2$ = the sum of the squares of the y -values in the data set.

Furthermore, a cluster analysis using the neighbour-joining method, based on the Gower dissimilarity matrix, was performed to understand the grouping of genotypes using the genotype means of eight traits. The cluster analysis was performed to group the genotypes into clusters based on their phenotypic similarity. The Neighbor-joining method is one of the clustering techniques for phylogenetic tree reconstruction. The use of this method is crucial in identifying the different branch lengths between the study subjects, based on their similarities and dissimilarities (34). The statistical analyses were performed using SAS OnDemand for Academics (welcome.sas.oda.com) and the cluster analysis was conducted using PBSTAT-CL (35, 36).

Table 2. List of the evaluated traits and their descriptions

Trait	Evaluation time	Trait description and importance	How the trait was measured
Plant height	Flowering time	This trait evaluates the size of the plant, which can be used to assess plant vigour and predict biomass (23).	Using a long measuring rod divided into centimetres, the plant height was measured from the base of the stem (at soil level) to the top of the canopy or the highest point.
Plant aspect	Flowering time	This trait evaluates the general appearance of the plant (evaluating how vigor, healthy and nice it looks).	The parameters used to evaluate this trait were on a qualitative scale from 1 to 5, where 1 represented the best performances (very vigorous, with no diseases) and 5 the worst ones (full of diseases and terrible appearance) (24).
<i>Bipolaris maydis</i> tolerance	Flowering time	This trait specifically evaluates whether the plant has the disease at a small, moderate, or null level. <i>B. maydis</i> is a worldwide serious foliar disease of maize that can cause a 40 % loss in yields (25).	The parameters used to evaluate this trait were on a qualitative scale from 1 to 5, where 1 represented the best performance (zero presence of the disease) and 5 the worst (full of the disease) (24).
<i>Exserohilum turcicum</i> tolerance	Flowering time	This trait specifically evaluates whether the plant has the disease at a small, moderate, or null level. <i>E. turcicum</i> can cause extensive defoliation and could result a 50 % loss of the yield (26).	The parameters used to evaluate this trait were on a qualitative scale from 1 to 5, where 1 represented the best performance (zero presence of the disease) and 5 the worst (full of the disease) (24).
Leaf number	Flowering time	As the number of leaves, as well as their distribution in maize plants, are crucial aspects in determining the plant architecture, population structure and the plant's yield potential (27), it is meaningful to evaluate them.	It was determined by visual count of the leaves (27).
Seed weight-female	Harvesting time	As the raw seed weight is a component that directly affects the yield, it is fundamental to determine this trait (28). The seed weight-female helps measure the ability of the inbred to produce ears and seeds when used as females, regardless of whether it received pollen from other inbreds (29).	The seed weight of the female refers to sum of the seed weights of all the ears that used the same inbred as female if they came out from crosses or from selfs if they came from that same specific inbred (29). Moreover, before doing this, we need to ensure that each ear is well identified (i.e., which male and female parents were used to produce it), so we can then group the ears based on the female parent and subsequently weigh them all together. The weights were measured with a balance.
Seed weight-male	Harvesting time	As the raw seed weight is a component that directly affects the yield, it is fundamental to determine this trait (28). The seed weight-male helps to measure the ability of the inbred to produce ears and seeds when these are used as males, regardless of which other inbred its pollen is going to be fertilized. (29).	The seed weight of the male refers to sum of the seed weights of all the ears that used the same inbred as male if they came out from crosses or from selfs if they came from that same specific inbred (29). Moreover, before doing this, we need to ensure that each ear is well identified (i.e., which male and female parents were used to produce it), so we can then group the ears based on the male and subsequently weigh them all together. The weights were measured with a balance.
Ear aspect	Harvesting time	The ear aspect is an important trait reflecting the visual quality of the ear, including kernel uniformity, size and overall appearance. In this sense, the ear aspect is a crucial factor in selecting varieties and hybrids (30).	The parameters used to evaluate this trait were inside a qualitative scale from 1 to 5; where the 1 represented the best performances (very good looking ears, filled of kernels, disease free) and 5 the worst ones (full of diseases, totally rotten and terrible appearance) (24).

Results and Discussion

Phenotypic variability among genotypes

Table 3 displays average values for various morphological traits of different maize genotypes. The traits include seed weight-female (SW-female), when the genotype was used as female, seed weight-male (SW-male), when the genotype was used as male, plant height (PH), plant aspect (PA), leaf number (LN), ear aspect (EA) and the incidence of *Bipolaris maydis* (*B. maydis*) and *Exserohilum tursicum* (*E. tursicum*) (Table 2). The results of the ANOVA (Table 4) showed that the genotype had a significant effect only in SW-female, SW-male, LN and EA. The coefficient of variations (CV) oscillated between 7.79 % (LN) and 17.97 % (SW-female and SW-male). Likewise, the three genotypes used as females with the best seed weights were HPOOL23 (335.97 g), MrChua-OP1 (322.76 g) and CR36 (191.48 g); whereas the three genotypes used as males with the best seed weights were HPOOL23 (22.63 g), MrChua-OP1 (207.51 g) and Tester B (207.51 g). On the other hand, the genotypes MrChua-OP8 (77.26 g), HPOOL26 (55.26 g) and 5241 (52.52 g) demonstrated the lowest seed weights as female parents and CML310 (44.60 g) had the lowest seed weight as a male parent. Complementarily, as it was noticed in the Table 3, for genotypes 26211 and CML481 no data were collected for seed weight used as male or female parent; and for HPOOL33 no data was gotten for seed weight used as female, which means that no ears were obtained out of these materials due to a very poor performance. Moreover, this information is essential, as the seed weight of the ears is an effective trait that contributes to grain yield, which is the most desirable trait in maize (37, 38).

In addition, for the evaluation of both diseases, as well as for the plant aspect, most genotypes showed a score of 2 (adequately resistant), indicating no significant differences. On the other hand, the LN ranged from 8.66 (TESTER-A) to 12.85 (MrChua-OP8), suggesting that MrChua-OP8 exhibited better vegetative growth compared to the others (Table 3). Correspondingly, a higher number of leaves can indicate a more vigorous plant, which may be more competitive for resources such as light and nutrients (39). Nonetheless, another characteristic that revealed significant differences among the genotypes was the EA. Good ear aspect often correlates with better grain quality, which is crucial for both marketability and processing (40). Likewise, good EA can also indicate resistance to ear diseases. Poor ear quality can result from fungal infections or pest damage; thus, genotypes with better ear quality are likely more resistant to these issues (41). Furthermore, the EA also functions as fundamental reservoir for photosynthetic products in maize and is directly related to the yield (42). Additionally, based on the results obtained, the genotype 26211 has the highest EA rating (3.82), indicating better ear quality, while CML481 has the lowest EA rating (1.90), indicating poor ear quality.

Phenotypic correlations among traits

The phenotypic correlation analysis exhibited positive and negative correlations between the traits of interest under study (Table 5). Nonetheless, the strongest positive correlation was observed among plant height and leaf number ($r = 0.877$; $p < 0.01$), which aligns with the idea that taller plants have more leaves. Consequently, this correlation was followed by the one between PA and *B. maydis* ($r = 0.845$; $p < 0.01$), indicating that

Table 3. Mean performance of maize genotypes for different morphological traits

Inbreds	SW-female (g)	SW-male (g)	PH (cm)	PA	<i>B. maydis</i>	<i>E. tursicum</i>	LN	EA
HPOOL23	335.97 ^a	222.63 ^a	116.81 ^{ab}	2.23 ^a	2.26 ^a	1.00 ^a	9.64 ^{ab}	2.91 ^{ab}
MrChua-OP1	322.76 ^a	207.51 ^a	121.22 ^{ab}	2.16 ^a	2.02 ^a	1.00 ^a	10.50 ^{ab}	2.71 ^{ab}
CR36	191.48 ^{ab}	110.05 ^{ab}	120.30 ^{ab}	2.46 ^a	2.42 ^a	1.00 ^a	10.06 ^{ab}	2.84 ^{ab}
PH6W	150.41 ^{ab}	104.64 ^{ab}	118.63 ^{ab}	2.20 ^a	2.06 ^a	1.00 ^a	10.08 ^{ab}	3.20 ^{ab}
CML310	144.47 ^{ab}	44.60 ^b	122.05 ^b	2.40 ^a	2.14 ^a	1.03 ^a	10.63 ^{ab}	2.95 ^{ab}
TESTER-B	143.03 ^{ab}	207.51 ^a	121.17 ^{ab}	2.33 ^a	2.19 ^a	1.02 ^a	10.18 ^{ab}	3.14 ^{ab}
HPOOL22	138.77 ^{ab}	168.02 ^{ab}	120.32 ^{ab}	2.10 ^a	2.02 ^a	1.05 ^a	11.10 ^{ab}	2.64 ^{ab}
NS12	128.02 ^{ab}	113.43 ^{ab}	120.43 ^{ab}	2.33 ^a	2.05 ^a	1.00 ^a	11.09 ^{ab}	2.71 ^{ab}
43009	126.74 ^{ab}	96.51 ^{ab}	128.38 ^{ab}	2.68 ^a	2.61 ^a	1.55 ^a	10.39 ^{ab}	2.90 ^{ab}
CML264	124.21 ^{ab}	155.02 ^{ab}	117.70 ^{ab}	2.06 ^a	2.02 ^a	1.00 ^a	10.69 ^{ab}	3.19 ^{ab}
TESTER-A	119.30 ^{ab}	150.41 ^{ab}	98.34 ^b	2.42 ^a	2.10 ^a	1.12 ^a	8.66 ^b	2.55 ^{ab}
MrChua-OP8	77.26 ^b	137.38 ^{ab}	155.98 ^a	2.23 ^a	2.06 ^a	1.00 ^a	12.85 ^a	3.19 ^{ab}
HPOOL26	55.26 ^b	136.00 ^{ab}	135.96 ^{ab}	2.00 ^a	2.00 ^a	1.00 ^a	12.03 ^{ab}	3.22 ^{ab}
5241	52.52 ^b	126.74 ^{ab}	119.85 ^{ab}	2.59 ^a	2.33 ^a	1.03 ^a	10.04 ^{ab}	2.94 ^{ab}
26211	.	.	126.30 ^{ab}	2.53 ^a	2.32 ^a	0.96 ^a	11.78 ^{ab}	3.82 ^a
CML481	.	.	127.20 ^{ab}	2.20 ^a	2.10 ^a	1.00 ^a	10.50 ^{ab}	1.90 ^b
HPOOL33	.	128.02 ^{ab}	116.67 ^{ab}	1.96 ^a	2.00 ^a	1.03 ^a	10.11 ^{ab}	2.92 ^{ab}
F-test	** (≤ 0.01)	* (0.02)	ns (0.12)	ns (0.90)	ns (.89)	ns (0.21)	* (0.04)	* (0.04)
Mean	150.73	140.56	122.78	2.29	2.16	1.05	10.61	2.93
Range	52.52-335.97	44.6-222.63	98.34-155.98	1.96-2.68	2.00-2.61	0.96-1.55	8.66-12.85	1.90-3.82
CV (%)	17.97	17.97	9.34	15.77	14.84	14.6	7.79	12.07

SW-female: average seed weight when the line was used as female parent; SW-male: average seed weight when the line was used as male parent; PH: plant height; PA: plant aspect; *B. maydis*: *Bipolaris maydis*; *E. tursicum*: *Exserohilum tursicum*; LN: leaf number; EA: ear aspect; CV: coefficient of variation; *: Significant at $\alpha = 0.05$; **: Significant at $\alpha = 0.01$; ns: not significant. Means followed by the same letter in the same column are not significantly different at $\alpha = 0.05$ based on the Tukey-Kramer test. The ratings of PA, *B. maydis*, *E. tursicum* and EA oscillated between oscillated from 1 = highly resistant or great performance, to 5 = highly susceptible or most undesired performance

Table 4. Summary of ANOVA mean squares for quantitative agronomic traits of maize inbred lines

Trait	Genotype	Replication	CV (%)
SW-female	2.24 **	8.66 ^a	17.98
SW-male	1.44 *	8.66 ^a	17.98
PH	256.52	114.90	9.34
PA	0.07	0.04	15.78
<i>B. maydis</i>	0.06	0.00	14.85
<i>E. tursicum</i>	0.04	0.04	14.60
LN	1.75 *	1.27	7.79
EA	0.26 +	0.88 *	12.07

SW-female: average seed weight when the line was used as female parent; SW-male: average seed weight when the line was used as male parent; PH: plant height; PA: plant aspect; *B. maydis*: *Bipolaris maydis*; *E. tursicum*: *Exserohilum tursicum*; LN: leaf number; EA: ear aspect; For SW-female and SW-male, replication represents type (self or cross); +, *, **: significant at 0.1, 0.05 and 0.01 level, respectively

plants with better visual aspects are more resistant to *B. maydis*, as resistant plants did not show any symptoms of the disease (Ovoid injuries with a dark brown border, extended russet injuries and /or orange stippled injuries) and in consequence, their aspect was better (43). In addition, a moderate positive correlation with *E. tursicum*, although not statistically significant ($r = 0.488$; $p > 0.05$), suggests a similar trend, where better plant aspect is found to be associated with disease resistance. Specifically, the absence of disease symptoms is associated with a better plant aspect. Complementarily, it was observed that *E. tursicum* displayed a positive correlation with *B. maydis* ($r = 0.610$; $p < 0.01$), indicating that genotypes resistant to one disease are likely to be resistant to the other. This could be due to general disease resistance mechanisms or genetic factors (28). Moreover, there is a moderate positive correlation between SW-female and SW-male ($r = 0.526$; $p < 0.05$), suggesting that some genotypes produce similar seed weights when used as female or male parents, which indicates the lack of significance in maternal effects for this specific trait (44). The remaining correlation coefficients fall within the range of -0.5 to 0.5.

Although plant height (PH) can be positively correlated with the yield potential in maize, taller plants often have more biomass and greater photosynthetic capacity, which can contribute to higher grain yields. However, excessively tall plants may be prone to lodging, which can negatively affect

yield (45). In fact, according to a study in China in 2023, the yield components (kernel number and thousand-kernel weight) were negatively correlated with lodging resistance (more lodging less yields); and that this resistance was primarily due to a good stem and root allocation, as well as to a lower plant and ear height (46). Moreover, this situation is evident in this study, as the genotype MrChua-OP8, which had the highest plant height (155.98 cm), yielded the lowest amount when used as a female parent (77.26 g) due to lodging. However, when used as a male parent, it produced significantly higher yields (137.38 g), as in this case, only the pollen of MrChua-OP8 was used to pollinate other genotypes that were not affected by lodging. Nonetheless, significant genetic variability in PH allows breeders to select for optimal plant height that balances biomass production and resistance to lodging (47).

Cluster analysis of maize inbred lines

In this study, a dendrogram with three clusters was constructed using Neighbor-joining method (Fig. 2 & Table 6). The cluster one was formed of two sub-clusters, in which the first of this sub-clusters was conformed, at the same time with two groups. One group integrating only NS12 and the other group including Tester A and HPOOL 22. On the other hand, the sub-cluster two of the first cluster, was formed also with two groups. The first group formed with CML 310 and 43009; and the second group, comprised with two sub-groups. The first sub-group including HPOOL 33 and 5241 and the second sub-group containing PH6W and CR36. Likewise, the cluster two, consisted also with two sub-clusters. The first sub-cluster involving CML 264 and the second sub-cluster composed with 26211 and HPOOL 26. Finally, the cluster three, entailing, just as previous cases, two sub-clusters. The first sub-cluster formed only with Tester B and the second sub-cluster formed with two groups. In the first group, integrating CML 481 and in the second group, integrating HPOOL 23 and Mr Chua-OP1.

In addition, by comparing the relationships presented in the dendrogram (Fig. 2) with the means of maize morphological traits (Table 3), it can be seen that the clusters, the sub-clusters, groups and sub-groups were formed according to the similarity of the inbreds for ear aspect, seed weight-female, seed weight- male and leaf number, as these were the only traits in which significant differences were exhibited among genotypes and clusters. Moreover, in relation to seed weight-female and seed weight- male, cluster three was the one with the highest means compared to the other two clusters (267.25 g for seed weight-female and 212.55 g for seed weight-male) even though CML 481 did not present any data

Table 5. Correlation between seven traits of maize inbred lines

	SW-female	SW-male	PH	PA	<i>B. Maydis</i>	<i>E. Tursicum</i>	LN	EA
SW-female	1							
SW-male	.526 *	1						
PH	-0.293	-0.127	1					
PA	-0.142	-0.342	-0.087	1				
<i>B. maydis</i>	0.049	-0.202	0.0002	0.845**	1			
<i>E. Tursicum</i>	-0.13	-0.258	-0.018	0.488	0.610 **	1		
LN	-0.371	-0.137	0.877 **	-0.212	-0.212	-0.199	1	
EA	-0.322	-0.096	0.266	0.105	0.141	-0.121	0.41	1

SW-female: average seed weight when the line was used as female parent; SW-male: average seed weight when the line was used as male parent; PH: plant height; PA: plant aspect, *B. maydis*: *Bipolaris maydis*; *E. tursicum*: *Exserohilum tursicum*; LN: leaf number; EA: ear aspect; *: significant at $\alpha = 0.05$; **: significant at $\alpha = 0.01$

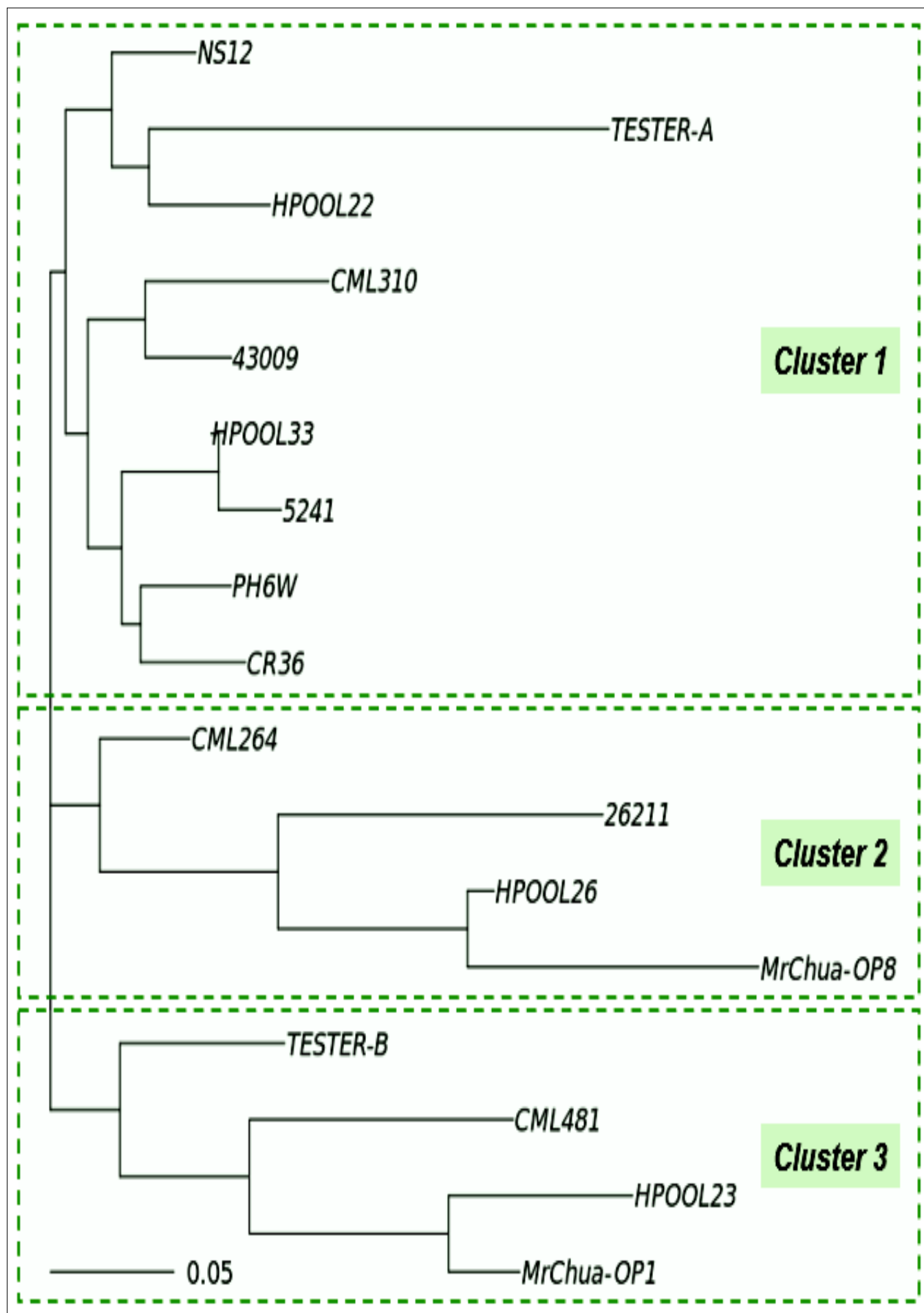


Fig. 2. Neighbor-joining tree of maize lines based on Gower dissimilarity matrix.

Table 6. The Gower dissimilarity coefficients among maize inbred lines

	MrChua- OP8	HPOOL26	43009	26211	CML481	CML310	MrChua- OP1	TESTER-B	NS12	HPOOL22	CR36	PH6W	5241	CML264	HPOOL23	HPOOL33	TESTER-A
MrChua-OP8																	
HPOOL26	0.128																
43009	0.324	0.232															
26211	0.366	0.179	0.282														
CML481	0.577	0.401	0.189	0.44													
CML310	0.4	0.308	0.109	0.267	0.222												
MrChua-OP1	0.534	0.446	0.312	0.323	0.175	0.342											
TESTER-B	0.378	0.29	0.196	0.275	0.275	0.228	0.187										
NS12	0.32	0.228	0.1	0.281	0.226	0.141	0.273	0.207									
HPOOL22	0.342	0.253	0.177	0.293	0.215	0.203	0.213	0.146	0.077								
CR36	0.404	0.313	0.11	0.341	0.238	0.151	0.239	0.183	0.111	0.172							
PH6W	0.351	0.257	0.105	0.287	0.308	0.135	0.317	0.14	0.131	0.192	0.079						
5241	0.314	0.192	0.136	0.328	0.259	0.193	0.332	0.186	0.144	0.19	0.129	0.127					
CML264	0.288	0.2	0.149	0.245	0.294	0.181	0.27	0.113	0.127	0.11	0.173	0.108	0.146				
HPOOL23	0.596	0.508	0.366	0.383	0.303	0.404	0.103	0.217	0.372	0.31	0.267	0.321	0.34	0.307			
HPOOL33	0.382	0.248	0.114	0.344	0.269	0.175	0.181	0.163	0.122	0.167	0.054	0.079	0.022	0.112	0.162		
TESTER-A	0.51	0.422	0.289	0.63	0.426	0.354	0.391	0.294	0.257	0.235	0.269	0.279	0.254	0.239	0.382	0.245	

for seed weight-female and seed weight-male. However, for leaf number and ear aspect, cluster three was not the best one. For leaf number its values ranged between 9.64 and 10.50, while in cluster two, the values oscillated between 10.69 and 12.03. Likewise, for the ear aspect, cluster two had the highest values, with a mean of 3.35 and a range of 3.19 to 3.82. In contrast, the means for ear aspect in clusters one and three were very similar, at 2.85 for cluster one and 2.66 for cluster three.

Likewise, by creating these categorisations based on phenotypical expressions, such as ear aspect or seed weight, together with those based on how well they combine with already proven specific testers, is how we should create heterotic groups. In addition, by using this strategy is less costly to evaluate the relative performance of a large number of inbreds, in terms of their ability to produce hybrids with high level of heterosis, which is the augmented performance of a hybrid compared to their inbred parents and it mostly gets expressed, when two inbred parents of different heterotic groups get crossed (48-50).

Conclusion

The inbreds had significant effects on seed weight, as well as on leaf number and ear aspect. HPOOL23 and MrChua-OP1 proved to be the best, with the highest seed weight values when used as both males and females. Correspondingly, the traits that exhibited the highest correlations were plant height and leaf number with $r = 0.877$; $p < 0.01$. Moreover, the neighbour-joining method helped identify the clusters with the highest mean for seed weight (SW-female and SW-male), which was cluster three and to identify the cluster with the best ear aspect mean, which was cluster two. This study provides a valuable information for understanding the behavior of inbreds in developing hybrids.

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Authors' contributions

MG carried out the breeding experiments, participated in the data analysis and drafted the manuscript. NF conceived of the study, participated in its design and revised the manuscript. MK and KMM participated in the design of the study and contributed to the development of the discussion. CK participated in collecting plant materials. SW helped in the statistical analysis. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

References

- FAOSTAT. FAO Stat [Internet]. Rome: FAO; 2021 [cited 2020 Dec 06].
- FAO. The Future of Food and Agriculture: Trends and challenges [Internet]. Rome: FAO; 2017 [cited 2020 Dec 06]. Available from: <http://www.fao.org/3/a-i6583e.pdf>
- Poole N, Donovan J, Erenstein O. Agri-nutrition research: Revisiting the contribution of maize and wheat to human nutrition and health. Food Policy. 2020;101:101976. <https://doi.org/10.1016/j.foodpol.2020.101976>
- Nor NA, Rabu MR, Adnan MA, Rosali MH. An overview of the grain corn industry in Malaysia. FFTC Agri Policy Platform (FFTC-AP). 2019. [cited 2020 Mar]. Issue 10:2020.
- USDA. Malaysia: Grain and feed annual [Internet]. Washington, D.C.: United States Department of Agriculture; 2020 [cited 2020 Dec 6].
- FAO. Global Information and Early Warning System (GIEWS) [Internet]. Rome: FAO; 2025 [cited 2025 Mar 26]. Available from: <https://www.fao.org/giews/countrybrief/country.jsp?code=MYS&lang=en>
- Mohammad N, Mohd Pakri MA, Nik Omar NR, Nazm MS, Zainol Abidin AZ, Rabu MR, et al. Quantifying farmers' willingness and perception towards grain corn cultivation. Eco Tech Manag Rev. 2022;18:1–9.
- Flint-Garcia SA, Buckler ES, Tiffin P, Ersoz E, Springer NM. Heterosis is prevalent for multiple traits in diverse maize germplasm. PLoS One. 2009;4(10):4:e7433. <https://doi.org/10.1371/journal.pone.0007433>
- Barrett BA, Kidwell KK. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. 1998;38:1261–71. <https://doi.org/10.2135/cropsci1998.0011183X003800050025x>
- Deitos A, Arnhold E, Mora F, Miranda GV. Yield and combining ability of maize cultivars under different ecogeographic conditions. Crop Breed Appl Biotechnol. 2006;6(3):222–27. <https://doi.org/10.12702/1984-7033.v06n03a06>
- Mingramm GY, Mohammed KS, Khandaker MM, Chua KA, Fatimah HNN. Combining ability for grain yield and nutritional quality of maize grown in Malaysia: a review. Sabrao J Breed Genet. 2024;56(2):534–46. <https://doi.org/10.54910/sabrao2024.56.2.7>
- Saleh G, Yap TC. Development of inbred lines in grain maize. In: Yap TC, Saad MS, editors. Progress Report 1988. Improvement of food crops: rice, corn and sweet potato. Serdang: Universiti Pertanian Malaysia. 1988.
- Saleh G, Sujiprihati S. Heterosis with respect to diversity among locally-developed maize inbred lines in Malaysia. In: CIMMYT, editor.. Book of abstracts. The genetics and exploitation of heterosis in crops. an international symposium; 1997 Aug 17–22; Mexico City: Mexico. Mexico D.F.: CIMMYT; 1997. p. 154–55.
- Saleh G, Sujiprihati S, Ali ES. Characterization of selected tropical maize inbred lines developed in Malaysia. J Trop Agric Fd Sc. 2001;29(1):29–38.
- Saleh GB, Abdullah D, Anuar AR. Performance, heterosis and heritability in selected tropical maize single, double and three-way cross hybrids. J Agric Sci. 2002;138:21–28. <https://doi.org/10.1017/S0021859601001757>
- Faruq G, Farhana N, Zain MF, Majid NA, Rahman MM, Rahman M, et al. Grain yield and associated traits of maize (*Zea mays* L.) genotypes in the Malaysian tropical environment. Afr J Res. 2011;6(28):6147–54. <https://doi.org/10.5897/AJAR11.1331>
- USDA- Foreign Agricultural Service [Internet]. 2021 [cited 2020 Dec 06].
- Moreira Guimarães LJ, Miranda GV, Oliveira de Lima R, Maia C, Rodrigues de Oliveira L, Vagno de Souza L. Performance of testers with different genetic structure for evaluation of maize inbred lines. Cienc Rural. 2012;42(5):770–76. <https://doi.org/10.1590/S0103-84782012000500002>
- Prasanna BM, Palacios-Rojas N, Hossain F, Muthusamy V, Menkir A, Dhlwayo T, et al. Molecular breeding for nutritionally enriched maize: Status and prospects. Front Genet. 2020;10:1392. <https://doi.org/10.3389/fgene.2019.01392>
- Weather Underground, Inc [Internet]. 2025 [cited 2025 Apr 22]. Available from: <https://www.wunderground.com/history/weekly/my/kuantan/WMKD/date/2023-8-20>
- Ogunsanya BG, Sulaimon MO. Application of randomized completely Block design to the Yield of Maize. Int J Sci Res. 2016;3–12.
- Khusairy MK, Shah ES, Ahmad MH, Abdul RR, Fikri AAS. Evaluation of planting spacing and effects on the yield for Malaysian grain corn production. Adv Agri Food Res J. 2021;2(1):a0000211. <https://doi.org/10.36877/aafjr.a0000211>
- Ruicheng Q, Man Z, Yong H. Field estimation of maize plant at jointing stage using an RGB-D camera. Crop J. 2022;10(5):1274–83. <https://doi.org/10.1016/j.cj.2022.07.010>
- Chintamanani S, Hulbert SH, Johal GS, Balint-Kurti PJ. Identification of a maize locus that modulates the hypersensitive defense response, using mutant-assisted gene identification and characterization. Genetics. 2009;184(3):813–25. <https://doi.org/10.1534/genetics.109.111880>
- Kumar B, Choudhary M, Kumar K, Kumar P, Kumar S, Bagaria PK, et al. Maydis leaf blight of maize: update on status, sustainable management and genetic architecture of its resistance. Physiol Mol.Plant Pathol. 2022;121:101889. <https://doi.org/10.1016/j.pmp.2022.101889>
- Jakhar DS, Kumari R, Kumar P, Singh R, Kumar A. *Exserohilum turcicum* [Pass.] resistance in maize: A sustainable agricultural approach for studying plant-microbe interactions. In: Singh HB, Sarma BK, editors. Emerging trends in plant-microbe interaction: Current advances and future challenges. Volume 1. Amsterdam: Elsevier; 2023;1:363–73. <https://doi.org/10.1016/B978-0-323-91875-6.00016-5>
- Li D, Wang X, Zhang X, Chen Q, Xu G, Xu D, et al. The genetic architecture of leaf number and its genetic relationship to flowering time in maize. New Phytol. 2016;210(1):256–68. <https://doi.org/10.1111/nph.13765>
- Sah RP, Chakraborty M, Prasad K, Pandit M, Tudu VK, Chakravarty MK, et al. Impact of water deficit stress in maize: Phenology and yield components. Sci Rep. 2020;10:2944. <https://doi.org/10.1038/s41598-020-59689-7>
- Bonipas AJ, Rajashekhar MK, Gopalakrishna N, Sidramappa CT, Zerka R, Bindiganavile SV, et al. Maternal effects, reciprocal differences and combining ability study for yield and its component traits in maize (*Zea mays* L.) through modified diallel analysis. Peer J. 2024;12:e17600. <https://doi.org/10.7717/peerj.17600>
- Wang J, Zhao S, Zhang Y, Lu X, Du J, Wang C, et al. Investigating the genetic basis of maize ear characteristics: A comprehensive genome-wide study utilizing high-throughput phenotypic measurement method and system. Front Plant Sci. 2023;14:1248446. <https://doi.org/10.3389/fpls.2023.1248446>
- Sadalla HA, Barznji MO, Kakarash SA. Full diallel crosses for estimation of genetic parameters in maize. Iraqi J Agric Sci. 2017;48(SI):30–40. <https://doi.org/10.36103/ijas.v48iSpecial.243>
- Fan XM, Zhang YD, Jeffers DP, Bi YQ, Kang MS, Yin XF. Combining ability of yellow lines derived from CIMMYT populations for use in subtropical and tropical mid-altitude maize production environments. Crop Sci. 2018;58:169–79. <https://doi.org/10.2135/cropsci2017.05.0291>
- Freedman D, Pisani R, Purves R. Statistics (international student edition). 4th ed. New York: WW Norton & Company; 2007.
- Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406–25.

35. SAS Institute Inc. SAS/STAT® 15.3 User's Guide. Cary, NC: SAS Institute Inc; 2023 [cited 2025 Jul 06]. Available from: <https://welcome.sas.oda.com>
36. Suwarno WB, Sobir, Sitaresmi T. PBSTAT-CL: Cluster analysis for plant genetic diversity studies (Version 2.2.1) [computer software]. Bogor: IPB University; 2025. <https://www.pbstat.com>
37. Mousavi SMN, Nagy J. Evaluation of plant characteristics related to grain yield of FAO410 and FAO340 hybrids using regression models. Cereal Res Commun. 2020;49:161–69. <https://doi.org/10.1007/s42976-020-00076-3>
38. Abakemal D, Hussein S, Derera J, Laing M. Farmers' perceptions of maize production systems and breeding priorities and their implications for the adoption of new varieties in selected areas of the highland agro-ecology of Ethiopia. J Agric Sci. 2013;5(11):159–72. <https://doi.org/10.5539/jas.v5n11p159>
39. Hammer GL, Sinclair TR, Chapman SC, Van Oosterom E. On systems thinking, systems biology and the in silico plant. Plant Physiol. 2009;149(3):1164–72.
40. Pixley KV, Bjarnason MS. Stability of grain yield, endosperm modification and protein quality of hybrid and open-pollinated quality protein maize (QPM) cultivars. Crop Sci. 2002;42(6):1880–87. <https://doi.org/10.2135/cropsci2002.1882>
41. Smith DR, White DG. Diseases of Corn. In: Sprague G F, Dudley J W, editors. Corn and corn improvement. 3rd ed. Madison, WI: ASA, CSSA and SSSA. 1998. p. 687–766. <https://doi.org/10.2134/agronmonogr18.3ed.c12>
42. Wang J, Zhao S, Zhang Y, Lu X, Du J, Wang C, et al. Investigating the genetic basis of maize ear characteristics: a comprehensive genome-wide study utilizing high-throughput phenotypic measurement method and system. Front Plant Sci. 2023;14. <https://doi.org/10.3389/fpls.2023.1248446>
43. Ferreira CM, Saluci JCG, Vivas M, Santos JS, de Andrade Junior MS, Vivas JMS, et al. Characterization of the *Bipolaris maydis*: symptoms and pathogenicity in popcorn genotypes (*Zea mays* L.). Braz J Biol. 2021;84:e256799. <https://doi.org/10.1590/1519-6984.256799>
44. Sánchez-Ramírez FJ, Mendoza-Castillo MC, Mendoza-Rodríguez M, Castillo González F, Cruz-Izquierdo S, Castro-Nava S, et al. Combining ability of inbred lines for maize (*Zea mays* L.) single cross hybrid production under irrigation. Agrociencia. 2017;51(4):393–407.
45. Sibale EM, Darrah LL, Zuber MS. Comparison of two cycles of recurrent selection in maize for stalk quality and grain yield. Crop Sci. 1992;32(6):1274–79.
46. Zhang P, Gu S, Wang Y, Xu C, Zhao Y, Liu X, et al. The relationships between maize (*Zea mays* L.) lodging resistance and yield formation on dry matter allocation to ear and stem. Crop J. 2023;11:258–68. <https://doi.org/10.1016/j.cj.2022.04.020>
47. Li X, Guo T, Mu Q, Li X, Yu J. Genomic and environmental determinants and their interplay underlying phenotypic plasticity. Proc Natl Acad Sci USA. 2018;115(24):6679–84. <https://doi.org/10.1073/pnas.1718326115>
48. Melchinger AE. Genetic diversity and heterosis in crops. In: Coors JG, Pandey S, editors. Genetics and exploitation of heterosis in crops. Madison (WI): ASA, CSSA; 1999. p. 1999;99–118. <https://doi.org/10.2134/1999.geneticsandexploitation.c10>
49. Shull GH. The composition of a field of maize. Am Breeders Assoc Rep. 1908;4:296–301. <https://doi.org/10.1093/jhered/os-4.1.296>
50. Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA. Use of SSRs for establishing heterotic groups in subtropical maize. Theor Appl Genet. 2003;107:947–57. <https://doi.org/10.1007/s00122-003-1333-x>

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