

RESEARCH ON THE ANALYSIS OF SOME FACTORS THAT INFLUENCE THE QUANTITY OF POLLEN PRODUCED BY THE INBREED CORN LINES (*Zea Mays* L.)

Nicolae Alexandru ANDREESCU, Doru Ioan MARIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd,
District 1, 011464, Bucharest, Romania

Corresponding author email: andreescu.alexandru26@gmail.com

Abstract

*This study analyzes factors influencing pollen production in inbred maize (*Zea mays* L.) lines, focusing on genetic material and plant density. Trials in Brăila, Romania (2024), tested six lines across three densities (95k, 135k, and 160k plants/ha). Results indicated that density had no statistically significant effect on flowering time ($p = 0.927$) or tassel branching. However, significant genotype-specific differences were found. The Zeta V43 line exhibited the highest number of tassel branches (up to 13.0), while Epsilon V81 achieved the maximum main axis length (42 cm) and the highest pollen dry weight (76.80 g) at 115,000 plants/ha. Tukey HSD tests confirmed a significant genotype $\times$ density interaction, showing that while Zeta V43 branching improves at lower densities, Epsilon V81 excels in pollen output at medium densities. These findings suggest that genetic selection is the primary driver for tassel development, identifying Epsilon V81 and Zeta V43 as superior candidates for breeding programs aimed at optimizing pollen-producing capacity in hybrid seed production.*

Key words: *Zea mays* L., pollen weight, tassel morphology, plant density, inbred lines.

INTRODUCTION

Maize (*Zea mays* L.) is recognized as the most widely cultivated cereal globally, with the United States of America, China, and Brazil leading production rankings (FAO, 2023). In 2023, the global area dedicated to maize cultivation was estimated to be between 200 and 210 million hectares. This crop's widespread cultivation is attributed to its remarkable productivity and versatility. For example, maize serves as a critical component in diverse industries, including animal feed, staple foods such as flours, hominy, oil, and bread, and even in specialized sectors like brewing, pharmaceuticals, and mining (Strazzi, 2015). In Europe, approximately 16 to 18 million hectares were sown with maize during the 2023 season. This figure varied slightly due to factors such as weather conditions, market dynamics, and crop rotation practices. Among the leading European maize producers are France, Ukraine, Romania, and Hungary, with Romania maintaining a dominant position in maize cultivation. Recent data highlights that Romania consistently cultivates around 2.5 million hectares annually, making it the European Union's largest producer

in terms of cultivated area (FAO, 2023). This leadership can be attributed to Romania's favorable climatic and soil conditions, particularly in its southern and eastern regions. From a personal perspective, Romania's strategic emphasis on maize cultivation reflects its broader agricultural strengths and adaptability. These favorable conditions could position the country as a potential hub for innovation in maize-based product development, which could enhance its competitiveness in the global market. The multiple applications of maize in food production, industrial uses, and beyond further underscore its significance in ensuring both economic stability and food security. Maize holds substantial economic significance, and breeding programs are vital for developing genotypes with agronomic traits that align with producer needs and consumer market expectations. Modern maize genotypes have been optimized for traits such as reduced plant and ear heights, more upright leaf angles, smaller tassels (fewer branches and lower mass), shorter intervals between tasselling and silking, decreased grain protein content, and increased yield potential (Duvick, 2005).

One of the most critical stages in maize development is flowering, which directly impacts yield. This phase typically spans 5 to 14 days (Ogden et al., 1969) and begins with pollen release from the tassel, followed shortly by silk emergence. Remarkably, each maize plant produces approximately 25 million pollen grains, ensuring a robust reproductive capacity with around 25,000 pollen grains available per silk (Kiessebach et al., 1949). This interplay between tassel and silk dynamics underscores the complexity of maize reproductive biology. Tassel size plays a pivotal role in yield determination. Larger tassels can reduce grain yield by shading upper leaves, thereby diminishing photosynthetic efficiency, and by acting as sinks for photo assimilates (Edwards, 2011; Souza et al., 2015). However, in environments with stress factors such as limited water availability, larger tassels may become advantageous by ensuring sufficient pollen production for fertilization (Parvez, 2007). Breeding programs have thus focused on selecting smaller tassels to reduce shading effects, enhance photosynthetic performance, and allow higher planting densities. While this strategy improves yield potential, it has inadvertently reduced pollen production (Duvick & Cassmann, 1999). Consequently, understanding factors influencing pollen production and germination has become a priority to support breeding programs and hybrid seed production. Tassel size, defined by traits such as branch number, branch length, spikelet count, and tassel area index, exhibits a complex relationship with yield. Specifically, a negative correlation with grain yield is often observed, alongside a positive correlation with pollen production (Fonseca et al., 2003). This duality poses challenges in breeding programs aimed at optimizing tassel traits.

The reduction of tassel size has been instrumental in improving planting efficiency and yield under optimal conditions, there is a growing need to develop adaptive strategies for stress-prone environments. Incorporating genetic diversity and exploring innovative breeding techniques could balance the trade-offs between pollen production and yield. This balance is critical to sustaining productivity as environmental conditions continue to fluctuate due to climate change. The main objective of

this study was to evaluate the influence of genetic material and plant density on tassel morphology and flowering synchrony in inbred maize (*Zea mays* L.) lines. By analysing traits such as branch count, main axis length, and pollen dry weight, this research aims to identify optimal genotypes and agronomic conditions for maximizing pollen production in hybrid seed production programs.

MATERIALS AND METHODS

The institutional and administrative framework was provided by the Doctoral School for Plant and Animal Resources Engineering and Management, organized within the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Field trials were conducted on the farm II Olteanu Ștefan SRL, located in the village of Viziru, Brăila County, in the southeastern part of Romania, within the historical region of Muntenia. Geographically, it is situated in the western part of Brăila County, near the border with Galați County, at approximately 45°01' North latitude and 27°43' East longitude. The area has a temperate-continental climate, typical of the lowland regions in eastern Romania, with influences from the Bărăgan Plain. The average annual temperature is 11°C, while the average annual precipitation ranges between 450–500 mm, evenly distributed throughout the year. The Viziru area is highly suitable for intensive agriculture due to its fertile soils (chernozems), hot summers, and the presence of a well-developed irrigation infrastructure in the region. However, the management of climate-related risks (such as drought and strong winds) and soil issues (including salinization and compaction) is essential for ensuring sustainability.

The experiment was designed as a two-factor trial. Factor A consisted of six maize (*Zea mays* L.) inbred lines with varying relative maturities (RM): Alfa R26 and Beta T00 (RM 80), Gamma T13 and Delta A39 (RM 100), and Epsilon V81 and Zeta V43 (RM 120). Factor B involved three plant densities: 95,000, 135,000, and 160,000 plants per hectare, respectively. **Factor A - Genetic material (*Zea mays* L.):**

a1 - Alfa R26 (relative maturity 80);

a2 - Beta T00 (relative maturity 80);

a3 - Gamma T13 (relative maturity 100);

a4 - Delta A39 (relative maturity 100);
a5 - Epsilon V81 (relative maturity 120);
a6 - Zeta V43 (relative maturity 120).
and **Factor B - Plant density:**
b1 - 95.000 plants per hectare;
b2 - 135.000 plants per hectare;
b3 - 160.000 plants per hectare.

The experimental plots were sown in the optimal period characteristic for this region, April 9, 2024, at a sowing depth of 6 centimetres and a row spacing of 60 centimetres. The design used was the completely randomized block design (Table 1), with 3 replications, each plot measuring 5 meters in length and consisting of 4 rows.

Table 1. Completely randomized block design

Plant density 160.000 plants / hectare	Replication 3	a4b3	a5b3	a6b3	Buffer strip
		a1b3	a2b3	a3b3	
	Replication 2	Buffer strip			
		a4b3	a5b3	a6b3	
		a1b3	a2b3	a3b3	
		Buffer strip			
Replication 1	a4b3	a5b3	a6b3		
	a1b3	a2b3	a3b3		
Buffer strip					
Plant density 135.000 plants / hectare	Replication 3	a4b2	a5b2	a6b2	
		a1b2	a2b2	a3b2	
	Replication 2	Buffer strip			
		a4b2	a5b2	a6b2	
		a1b2	a2b2	a3b2	
		Buffer strip			
Replication 1	a4b2	a5b2	a6b2		
	a1b2	a2b2	a3b2		
Buffer strip					
Plant density 95.000 plants / hectare	Replication 3	a4b1	a5b1	a6b1	
		a1b1	a2b1	a3b1	
	Replication 2	Buffer strip			
		a4b1	a5b1	a6b1	
		a1b1	a2b1	a3b1	
		Buffer strip			
Replication 1	a4b1	a5b1	a6b1		
	a1b1	a2b1	a3b1		
Buffer strip					

For the observation and determination of the studies, the following factors were considered:
(1) Sowing date;
(2) The number of days from sowing when 90% of the plants in the plot have visible panicles and anthers releasing pollen;
(3) Number of tassel branches: the number of branches is counted for 5 representative plants/panicles, and the average is calculated (Photo 1);

(4) Size of the main tassel axis: The main panicle axis is measured for 5 representative plants/panicles, and the average is calculated (Photo 1. A);

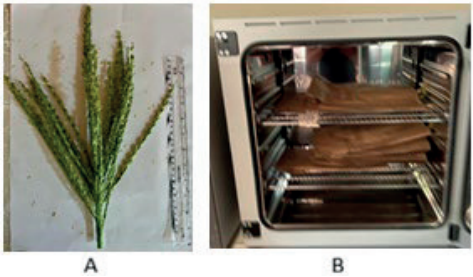


Photo 1. A. Measuring tassel axis; B. Placing the samples in the Binder oven

(5) Tassel weight: 12 panicles are cut at the flowering 90% stage, approximately 5 centimetres below the last branch of the panicle. The sample is weighed and then placed to dry for 72 hours at 55°C in a Binder oven (Foto 1. B). The sample is weighed immediately after being removed from the oven following the 72-hour drying period. This will allow us to determine the amount of pollen released through drying.

RESULTS AND DISCUSSIONS

Flowering

Flowering observations were determined when 90% of the plants in the plot exhibited anthers releasing pollen. Analysing all the genetic material together, we can observe that the average flowering rate decreases slightly as the density increases, but the differences are quite small (Table 2).

Table 2: ANOVA test result (density - P90%)

Density (plants/ha)	Mean P90 (Days to Flowering)	Min	Max	Standard Deviation
75,000	90.5	82	103	~7.26
115,000	89.43	81	102	~7.66
160,000	88.83	82	99	~5.38

Running an Anova analysis results in p-value = 0.927, where is much higher than 0.05, means that there are no statistically significant differences between the 3 densities in terms of flowering day (P90%) (Table 3).

Table 3: ANOVA analysis to determine the influence of density on flowering

Sursa	Sum of Squares	DF	F	p-value (PR(>F))
Densitate	8.78	2	0.077	0.927
Rezidual (eroare)	859.67	15	—	—

After comparing genetic material and density, we observe that P90 values vary more between genetic materials than between densities. Density has a slightly visible effect in some cases (e.g. Delta A39 or Epsilon V81), but it is not a consistent or statistically significant effect (confirmed by ANOVA) according with Figure 1.

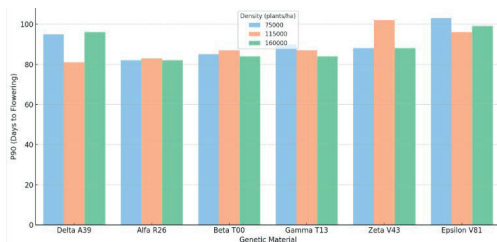


Figure 1. Comparative flowering time (P90%) by material and density

Number of branches per tassel

The number of branches per tassel is determined by counting the number of branches of 5 representative plants in the plot, after which the average of the 5 tassels was calculated. Analysing the graph compared to all genetic materials, we observe that all values are relatively close between densities (Figure 2)

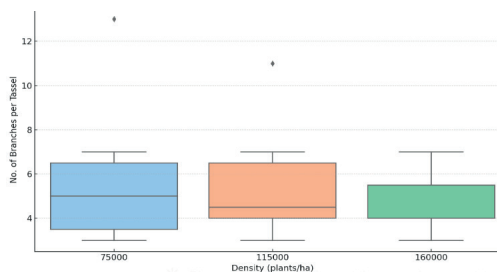


Figure 2. Effect of density on number of branches per tassel

In the comparative graph showing the number of branches per panicle for each genetic material, at the 3 densities tested (Figure 3) density does not seem to significantly influence branching within each material (also confirmed by the previous ANOVA). But it is observed that there

are clear differences between genetic materials – for example, Zeta V43 has many more branches than the others genetics, while some materials (e.g. Gamma T13 or Epsilon V81) are quite constant regardless of density.

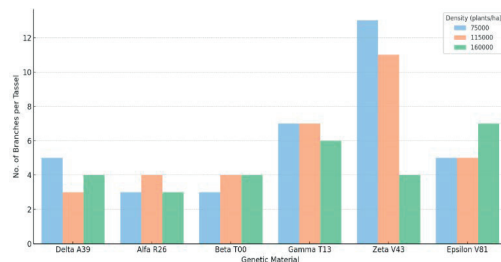


Figure 3. No. of branches per tassel by genetic material and density

Using the Tukey HSD test (Statistical grouping, same letter = not significantly different) to determine if there is a statistical difference between the genetic materials on the number of branches on the tassel, it is found that the Zeta V43 material is statistically significantly different from all other materials, having the highest average number of branches (9,333), according with Table 4. It can be deduced that Zeta V43 is a valuable candidate for selection and improvement, having a significantly higher number of branches than the other materials.

Table 4. Tukey Table with P-Values

	Material	Mean No. of Branches	Group	p-value vs Zeta V43
1	Zeta V43	9.333	a	-
2	Gamma T13	6.667	b	0.0309
3	Epsilon V81	5.667	b	0.001
4	Delta A39	4.0	c	0.0
5	Beta T00	3.667	c	0.0
6	Alfa R26	3.333	c	0.0

Using Tukey HSD test for interpretation of differences between genetic materials and densities (Table 5) show that Zeta V43 have the highest average number of tassel branches (13.0) among all combinations, is statistically significantly different from all other material × density combinations (assigned to group “a”, indicating its superior performance for “b”, and “b” superior performance for “c” etc.). Zeta V43 (115000 density) also performs very well, with an average of 11.0 branches, falls into group “b”, meaning it is significantly different from group “a”, but still better than the rest.

Table 5. Interpretation of differences between genetic materials and densities (Tukey HSD)

Material (Density)	Mean No. of Branches	Group * Significant Diff.
Zeta V43 (75000)	13	a
Zeta V43 (115000)	11	b
Gamma T13 (75000)	7	c
Gamma T13 (115000)	7	c
Epsilon V81 (160000)	7	c
Gamma T13 (160000)	6	d
Delta A39 (75000)	5	e
Epsilon V81 (75000)	5	e
Epsilon V81 (115000)	5	e
Delta A39 (160000)	4	f
Beta T00 (160000)	4	f
Beta T00 (115000)	4	f
Zeta V43 (160000)	4	f
Alfa R26 (115000)	4	f
Alfa R26 (160000)	3	g
Delta A39 (115000)	3	g
Beta T00 (75000)	3	g
Alfa R26 (75000)	3	g

The genetic material Zeta V43 at 75.000 plants/ha shows the best tassel development, with the highest and most statistically distinct number of branches, by reducing the sowing density appears to enhance tassel branching for this genotype. These findings strongly support the use of Zeta V43 at lower densities in breeding programs or agronomic trials where panicle development is a selection criterion.

Size of the main tassel axis

The size of the main axis per tassel is determined by measuring the main axis (in centimetres) of 5 representative plants in the plot, after which the average of the 5 tassels was calculated. In the comparative chart (Figure 4) is observable that Epsilon V81 at high density (160,000 plants/ha) stands out with the highest main axis length (42 cm), other combinations, such as Beta T00 (160000) and Delta A39 / Gamma T13 at 75000, show similarly high values, around 39-40 cm.

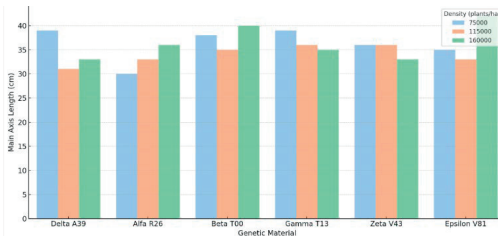


Figure 4. Main axis length (cm) by genetic material and density

There are clear variations between combinations, and an apparent influence of planting density on main axis length for certain genotypes.

Tukey HSD test (Statistical grouping, same letter = not significantly different), indicate that Epsilon V81 (160000) shows the highest axis length (42.0 cm) and is statistically superior to all other combinations (Group a), Beta T00 (160000), with 40.0 cm, falls into group b, significantly different from Epsilon V81 ($p = 0.0$), Gamma T13 (75000) and Delta A39 (75000) both recorded 39.0 cm, forming group c, also significantly lower than the top performer, while Beta T00 (75000) is in group d, with 38.0 cm, and is significantly lower than all of the above (Table 6).

It is conclusive that Epsilon V81 benefits from higher density (160k) in terms of main axis development, this response highlights its potential under denser planting systems or as a target for breeding selection. The results show clear genotype $\times$ density interaction, influencing a key morphological trait for pollen production.

Table 6. Tukey HSD results - main axis length by genetic material $\times$ density

Material (Density)	Mean Main Axis Length (cm)	Group * Significant Diff.
Epsilon V81 (160000)	42	a
Beta T00 (160000)	40	b
Gamma T13 (75000)	39	c
Delta A39 (75000)	39	c
Beta T00 (75000)	38	d
Zeta V43 (75000)	36	e
Zeta V43 (115000)	36	e
Alfa R26 (160000)	36	e
Gamma T13 (115000)	36	e
Gamma T13 (160000)	35	f
Beta T00 (115000)	35	f
Epsilon V81 (75000)	35	f
Zeta V43 (160000)	33	g
Alfa R26 (115000)	33	g
Delta A39 (160000)	33	g
Epsilon V81 (115000)	33	g
Delta A39 (115000)	31	h
Alfa R26 (75000)	30	i

Tassel weight

The method used for determined the quantity of pollen is the picking and cutting of 12 consecutive tassels at the flowering 90% stage from each plot, approximately 5 centimetres below the last branch of the tassel. The sample

is weighed and then placed to dry for 72 hours at 55°C in a Binder oven (Photo 1.B). The sample is weighed immediately after being removed from the oven following the 72-hour drying period. This will allow us to determine the amount of pollen released through drying. In the data observation table (Figure 5) it is visible that Epsilon V81 consistently shows the highest pollen dry weight across all densities, with a notable peak at 115,000 plants/ha (76.80 grams), Zeta V43 performs well at 115,000 as well, but its production drops sharply at 160,000 density, while Beta T00 and Gamma T13 exhibit a gradual decline in pollen production as density increases, Alfa R26 and Delta A39 remain among the lowest producers regardless of density.

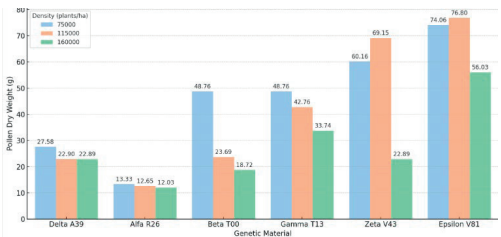


Figure 5. Pollen dry weight by genetic material and density

Putting all pairwise comparisons between every combination of genetic material $\times$ density, Tukey HSD test table for Pollen Dry Weight (Table 7), showing that the combination Epsilon V81 at 115,000 plants/ha yielded the highest pollen dry weight (76.80 g) and belongs to group "a", making it significantly superior to all other combinations ($p < 0.001$). Even Epsilon V81 at 75,000 and Zeta V43 at 115,000, although close in absolute values are statistically distinct, being placed in groups "b" and "c", if we move down the list, Zeta V43 at 75000 and Epsilon V81 at 160000 show clearly lower performances and are grouped separately, indicating a strong statistical drop-off in pollen production. The results confirm a highly significant genotype $\times$ density interaction affecting pollen productivity. Epsilon V81 responds extremely well to medium density (115k), making it an ideal candidate for environments or purposes where maximizing pollen output is critical. The rapid decline in pollen weight at higher densities (160k), especially for genotypes like Zeta V43,

suggests that optimal density management is essential for maintaining reproductive potential.

Table 7. Tukey HSD results - Pollen Dry Weight (g)

Material (Density)	Mean Pollen Dry Weight (g)	Group * Significant Diff.
Epsilon V81 (115000)	76.8	a
Epsilon V81 (75000)	74.06	b
Zeta V43 (115000)	69.15	c
Zeta V43 (75000)	60.16	d
Epsilon V81 (160000)	56.03	e
Beta T00 (75000)	48.76	f
Gamma T13 (75000)	48.76	f
Gamma T13 (115000)	42.76	g
Gamma T13 (160000)	33.74	h
Delta A39 (75000)	27.58	i
Beta T00 (115000)	23.69	j
Delta A39 (115000)	22.9	k
Delta A39 (160000)	22.89	l
Zeta V43 (160000)	22.89	l
Beta T00 (160000)	18.72	m
Alfa R26 (75000)	13.33	n
Alfa R26 (115000)	12.65	o
Alfa R26 (160000)	12.03	p

To correlate tassel characteristics a scatter diagram is used (scatterplot), is a graph that uses dots to represent the relationship between two numerical variables.

Each dot represents one observation, in our case, a specific genetic material at a certain planting density. The X-axis shows the value of one trait. The Y-axis shows the value of another trait. In the diagram which correlate main axis length and number of branches per tassel (Figure 6), no strong linear correlation is visible, some materials (e.g., Zeta V43) combine high branching with moderate axis length.

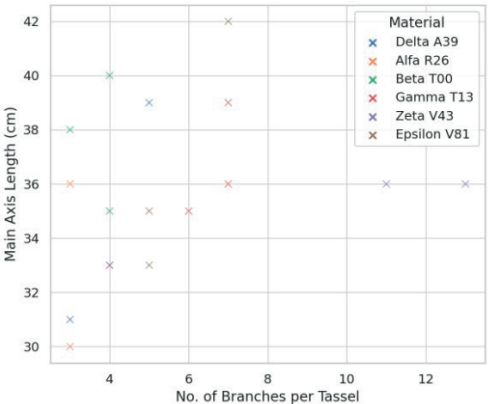


Figure 6. No. of branches vs Main axis length

In the diagram which correlate number of branches per tassel with pollen dry weight (Figure 7) show us a weak positive trend: more branches may slightly associate with higher pollen weight. However, outliers exist (e.g., Epsilon V81) achieves high pollen weight with moderate branching.

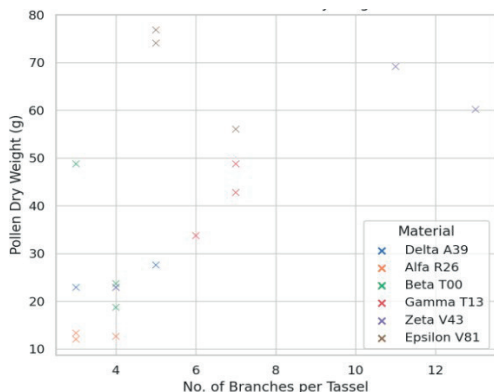


Figure 7. No. of branches vs Pollen dry weight

In the diagram which correlate main axis length with pollen dry weight (Figure 8), this pair shows a stronger positive trend, genetic materials with longer main axes (e.g., Epsilon V81, Gamma T13) tend to have higher pollen productivity.

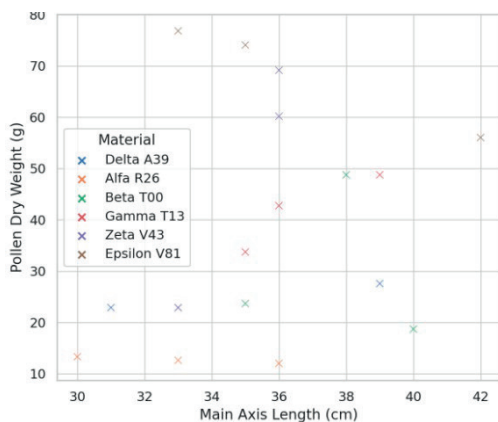


Figure 8. Main axis length vs Pollen dry weight

CONCLUSIONS

Genetic material is the primary driver of trait expression, with significant differences ($p < 0.05$) observed across all studied parameters. Specifically, Zeta V43 demonstrated superior

tassel branching, while Epsilon V81 recorded the highest pollen dry weight and main axis length, particularly at medium or high densities. Planting density modulates trait expression through significant genotype-specific responses. While genotypes like Epsilon V81 benefit from higher densities for pollen productivity, others, such as Zeta V43, optimize branching and reproductive traits at lower densities. This highlights a strong genotype $\times$ density interaction, confirmed by ANOVA and Tukey HSD tests.

Positive correlations between main axis length and pollen dry weight suggest high potential for indirect selection. Conversely, the weak correlation between branch number and other traits indicates that branching is primarily a structural rather than a functional attribute.

A medium density of 115,000 plants/ha appears to optimize both structural and reproductive performance for the top-performing genotypes. However, no universal optimal density was identified, confirming that agronomic management must be tailored to specific genotypes.

Planting density had no statistically significant effect on the time to flowering (P90%), indicating that flowering phenology in these lines is relatively stable across the tested populations.

Future programs should prioritize trait-by-trait optimization, focusing on pollen traits and axis development in density-responsive genotypes. Further multi-environmental testing is recommended to validate these trends.

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