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## EXPLORING TOCOPHEROL DIVERSITY IN MAIZE: UNRAVELING THE LINKS TO AGRO-BIOCHEMICAL TRAITS

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### SUMMARY

Maize (*Zea mays* L.), a major cereal crop, contains a diverse range of minerals, nutrients, and vitamins. Maize genotypes have great diversity in tocopherol (vitamin E) content, which works as an antioxidant and stress tolerant and greatly improves kernel nutritional value. The objective of this study is the screening of maize genotypes for high grain tocopherol content and their association study with other biochemical traits. This study evaluated 27 maize genotypes for grain tocopherol content and other yield- and yield-contributing agrochemical traits. The data underwent the analysis of variance (ANOVA), descriptive statistics, PCA-biplot, and Pearson correlation assessment. The ANOVA showed significant variances for all traits. Mean data indicated G27 and G8 are elite genotypes for both grain yield and tocopherol content. Correlation analysis revealed a significant positive interaction of tocopherol content with oil content and kernel rows and a negative association with days to anthesis. Grain yield per plant has a strong positive interaction with a hundred-seed weight and oil content. In the future, these genotypes can be beneficial to developing biofortified maize hybrids, and these traits' interaction study could help in the indirect selection by improving the selection criteria. Additionally, the screening of indigenous maize germplasm provides elite genetic resources crucial for initiating biofortification breeding programs aimed at enhancing the vitamin E content of maize hybrids.

**Keywords:** Maize (*Z. mays* L.), genotypes, tocopherol, principal component analysis, correlation, biofortification

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**Key findings:** The maize (*Z. mays* L.) germplasm has a great diversity in yield and tocopherol content. Screening of maize genotypes for superior tocopherol genotypes will open new horizons in tocopherol biofortification in maize. This study investigated three high-tocopherol-containing genotypes (G25, G8, and G23) that can be effective in hybrid breeding programs. Additionally, the association study will help in understanding the interaction of tocopherol with other traits, and it can be beneficial to developing an indirect selection method for high-tocopherolic genotypes.

## INTRODUCTION

Maize (*Zea mays* L.) is a cross-pollinated crop from the Poaceae family with a diploid genome of  $2n=2x=20$  chromosomes, and the genome size is about 2.4 Gb, containing over 32,000 genes (Haberer *et al.*, 2005). Its cultivation occurs in over 170 countries, and it is the third most important cereal crop in Pakistan. Maize cultivation in Pakistan covers 1.44 million hectares with a production of 8.24 million tons, where its production decreased lately to 15.4% due to market prices and high input costs (GOP, 2025).

Maize is the staple crop in many countries worldwide, as the grain serves as food, animal and poultry feed, and many industrial uses. Its stalk utilization includes the manufacture of paper, cardboard, and insulating material, while its rachis contributes to the production of pipes, plastics, paint, paste, alcohol for beverages, and automotive biofuels (Popa *et al.*, 2023). With the growth of the poultry and livestock industries, maize consumption has increased manifold. Maize cultivation bears influences from several abiotic and biotic stresses, such as temperature, salt and water stress, nutrient deficiency, insects, diseases, and competition with weeds.

The grain consists of endosperm, germ, pericarp, and tip cap at 82.5%, 10.5%, 5.5%, and 0.9%, respectively. Maize seeds contain macromolecules of carbohydrates (60%–68%), protein (8.5%–10.5%), sugar (3.0%), dietary fiber (12%–16%), and total fat (4.8%) (Serna-Saldivar, 2023). Besides macromolecules, corn grain contains minerals, such as P, K, Ca, Mg, Na, Fe, Cu, Mn, and Zn. It also has vitamins—thiamine (B1), niacin (B2), riboflavin (B3), pantothenic acid (B5), pyridoxine (B6), ascorbic acid (C), tocopherol (E), vitamin K, and folic acid (Berardo *et al.*, 2009).

Tocopherol (vitamin E) functions as a potent antioxidant, inhibits lipid peroxidation, regulates ionic movement, restores fertility, and enhances immunity. The recommended dietary allowance (RDA) for vitamin E for adults is 15 mg/day, while lactating women need 19 mg/day (Horwitt, 2001). Its deficiency causes infection, anemia, stunting of growth, and pregnancy issues. Both developed and developing countries are equally deficient, as an adequate amount of vitamin E comprised only 1/5th of people globally. In America, 90% of the population has an inadequate intake, whereas in Bangladesh, it is 43.5%, and in India, above 90% of pregnant women are severely vitamin E deficient (Shamim *et al.*, 2015). Although large-scale vitamin E deficiency studies have not occurred in Pakistan, vitamin E deficiency is also common due to poor nutrition and deep-cooking practices. A small study conducted in Karachi indicated that apparently 4.9% of healthy persons are tocopherol-deficient (Jilani and Iqbal, 2018).

Tocopherol is a ringed (chromanol) long chain with a molecular weight of 402 to 430 g, classified into four types based on the methyl group (CH<sub>3</sub>) on the aromatic ring. Tocopherol has alpha (α), beta (β), gamma (γ), and delta (δ) isomers based on the ring shape. Its synthesis in the plant occurs by the shikimate pathway to construct an aromatic ring and the methylerythritol phosphate (MEP) pathway that synthesizes phytyldiphosphate (PDP) tail in the plastid and cytosol, respectively (Niu *et al.*, 2022). Tocopherol biosynthesis incurs effects from age, organs, regulatory compounds, and hormones. Construction of a tocopherol genetic map and genomic study explores six candidate genes, *HPPD*, *VTE1*, *VTE3*, *VTE4*, *P3VTE5*, and *P4VTE5*, responsible for tocopherol biosynthesis, especially *ZmVTE4*, which

converts  $\delta$ -tocopherol to  $\alpha$ -tocopherol (Li *et al.*, 2012).

It contributes to plant growth regulation, stress tolerance, physiological processes, and defense mechanisms. The  $\alpha$ -tocopherol improves vegetative growth and flowering, carotenoids, plant fresh and dry weights, leaf area, the number of leaves and branches, and plant height. It mitigates oxidative stresses, involves hormonal regulation, senescence, signal transduction, carbohydrate accumulation, nutrient translocation, abiotic stress such as salt stress tolerance and drought stress tolerance, and high- and low-temperature stress tolerance (Maeda *et al.*, 2006; Yusuf *et al.*, 2010; Kumar *et al.*, 2013; Akram *et al.*, 2023; Chishti *et al.*, 2025). It also plays a significant positive role in fighting metal toxicity and UV radiation stresses in crop plants (Yusuf *et al.*, 2010; Hectors *et al.*, 2014).

The maize crop has a great deal of genetic diversity for minerals, vitamins, and amino acids. Several scientific studies identified that maize accessions have a wide range of diversity in kernel tocopherol content that is quantifiable either by RT-PCR or by HPLC/colorimetric methods (Rocheferd *et al.*, 2002). Genomic studies investigated the polymorphism for tocopherol content in corn kernels (Li *et al.*, 2012). Marker-assisted backcrossing (MABC) improved the grain tocopherol content from  $29.11 \mu\text{g g}^{-1}$  to  $63 \mu\text{g g}^{-1}$  in sweetcorn (Feng *et al.*, 2015). It was also evident that ranges of vitamin E compounds in biofortified maize were  $\alpha$ -tocopherol ( $3.4\text{--}34.3 \mu\text{g g}^{-1}$ ) and  $\gamma$ -tocopherol ( $5.9\text{--}54.4 \mu\text{g g}^{-1}$ ) (Muzhingi *et al.*, 2017).

The need for the screening of already present diversity for tocopherol and using the contrasting genotypes as parents of corn hybrids is crucial to fight hidden hunger, food security, and safety issues. Although a few studies on vitamin E in maize have had reports in Pakistan, still a lack of a comprehensive evaluation of indigenous maize germplasm for tocopherol content and its association with morphological-biochemical traits existed (Majeed *et al.*, 2017). In this study, maize indigenous genotypes received evaluation for tocopherol content, with correlation assays

made to plant morphological and biochemical traits. This study will help find good genotypes for tocopherol breeding in maize and indirect selection for tocopherol to develop biofortified maize cultivars.

## MATERIALS AND METHODS

The maize (*Z. mays* L.) research proceeded in the Department of Plant Breeding and Genetics (PBG), University of Agriculture, Faisalabad (UAF), Punjab, Pakistan (latitude =  $31^{\circ} 30' \text{ N}$ , longitude =  $73^{\circ} 10' \text{ E}$ , and altitude =  $184.4 \text{ m}$ ). Twenty-seven tropical maize genotypes, indigenously developed, came from the Department of PBG, UAF. The experiment had a randomized complete block design (RCBD) with three replicates. Each treatment's sowing was on four rows of 6-meter length, where  $P \times P = 20 \text{ cm}$  and  $R \times R = 75 \text{ cm}$ . Sowing two seeds in each hole ensured a good plant population, with extra plants removed at the seedling stage. Data recorded took place for plant height (PH, cm), ear height (EH, cm), internodal distance (ID, cm), stem diameter (SD, cm), the number of leaves per plant (NLP), days to silking (DS), and days to tasseling (DT). Other recorded traits included anthesis silking interval (ASI), the number of rows per cob (NRC), the number of seeds per row (NSR), the number of seeds per cob (NSC), cob weight (CW, g), and cob diameter (CD, cm). Macronutrients comprised ash (ash content, %), oil c (oil content, %), Prot (protein content, %), GTC (grain tocopherol content,  $\mu\text{g g}^{-1}$ ), HSW (100-seed weight), and GTP (grain yield per plant, g). Grain ash, protein, and oil content quantification utilized the NIR spectrophotometer using 75 g of maize flour for each sample (Zhang *et al.*, 2024). Quantifying the grain's total tocopherol content employed the HPLC method, where subjecting ground samples (20 g) to Soxhlet extraction used 130 mL of n-hexane at  $60^{\circ} \text{ C}$  for 8 h. The solvent removal under vacuum was at  $45^{\circ} \text{ C}$  with a rotary evaporator, with the residue dissolved in 1 mL of 2-propanol. A 20- $\mu\text{L}$  aliquot preceded its injection into an HPLC system equipped with a C18 column (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ). Detection continued using a

fluorescence detector at 295 nm excitation and 325 nm emission. The column had a maintenance at 30 °C, with a mobile phase of acetonitrile:methanol (1:1, v/v) at a flow rate of 1 mL/min (Majeed *et al.*, 2017).

The recorded data underwent analysis of variance to test the significance of the treatment's difference (Steel *et al.*, 1997). Descriptive statistics analysis engaged the software Statistic 10.0v. Correlation among traits' computation employed the Pearson Correlation (Pearson, 1901), with the *corrplot* package on R providing graphical presentations. Variance analysis continued by PCA (principal component analysis); the biplot construction used the mean values (Ammar *et al.*, 2024).

## RESULTS AND DISCUSSION

### Analysis of variance

The recorded data of all studied traits, subjected to analysis of variance, tested the significant differences among the maize (*Z. mays* L.) genotypes at 1% and 5% significance levels. The ANOVA of the traits appears in Table 1. By ANOVA, it occurred that all studied genotypes had significant variability among them for all the traits. Several studies conducted on the maize gene pool evaluated the diversity for tocopherol content, yield, and yield-contributing parameters. The evaluated 103 commercial maize hybrids for tocopherol ranged from 19.24 to 54.44 µg/g (Gunjević *et al.*, 2023). This means that genotypes are different from each other significantly in all studied traits. The mean values of each genotype for all traits of investigation are available in a supplementary Table S1. For the anthesis silking interval, genotypes G14 and G3 showed maximum values, whereas G19, G27, G15, and G16 gave the least values. Days to tasseling and days to silking fell in the same clade, and G12 had the highest mean value for DS and DT, followed by G10, G24, and G17, respectively. Traits of stem diameter and plant height were in the same group, followed by

internodal distance. The genotypes, G24, G11, and G17, have the highest mean values for the above clade. Another most important clade has the grain tocopherol content and grain oil content, followed by the subclade line of grain yield per plant. It means these three characters have close mean values due to a close association of genes that needs further investigation. The highest value for this group is present in genotype G8, followed by G27, G11, and G16. These genotypes could be the good source for the pooling of the rest of the vital traits in these genotypes, as they already possess three crucial traits.

### Descriptive analysis

The minimum and maximum values represented by genotypes, their standard deviation, and coefficient of variance occur in Table 2. The highest grain tocopherol (µg g<sup>-1</sup>) resulted in G26 (37.57), G23 (35.77), G16 (35.67), and G8 (35.47), making them the high-yielding genotypes. It is clear from the mean data that genotypic performance is not perpetually superior or inferior for all the traits. Tocopherol has a polygenic inheritance with great variability and complex interaction with the environment in different crops (Kassem *et al.*, 2024). A genotype is superior in some traits and lagging in some other traits, which helps us in the selection of genotypes with contrasting traits for the development of desired hybrids.

### PCA biplot analysis

The PCA biplot is the visualization tool for analyzing patterns in multivariate data. In the PCA biplot, reducing the dimensionality of the dataset helped identify the patterns among variables. The PCA biplot (Figure 1) indicates that Dim1 and Dim2 cumulatively contribute 34.2% of the interaction. The distance from the origin in a PCA biplot indicates the contribution or weight of a variable to the principal components. Variables that are farther away from the origin have a higher impact on the principal components, while variables closer to the origin have a lower

**Table 1.** Analysis of variance for different agronomic, physiological, and yield-contributing traits of maize genotypes.

S.O.V.	DF	PH	EH	ID	SD	NLP	DS	DT	ASI	NRC	NSR	NSC	CW	CD	ASH	Prot	Oil C	GTC	HSW	GYP
Geno	26	4787.32**	749.73**	23.79**	5.28**	5.10**	32.56**	22.81**	11.13**	12.61**	51.13**	21112**	4133.93**	127.05**	1.445**	4.095**	0.11**	65.31**	4.82**	3846.26**
Rep	2	62.02	211.54	0.24	0.03	1.81	0.30	0.79	0.11	5.48	4.70	4299.30	219.70	4.52	1.18	0.38	0.02	0.14	61.29	465.58
Error	52	142.13	176.73	2.75	0.43	0.72	2.05	2.52	2.26	1.48	4.39	1792.40	78.57	1.759	0.79	0.18	0.04	0.32	2.06	1439.46

\*: significant at 5%, \*\*: significant at 1%, PH: Plant Height (cm), EH: Ear Height (cm), ID: Internodal Distance (cm), SD: Stem Diameter (cm), NLP: Number of Leaves per Plant, DS: Days to Silking, DT: Days to Tasseling, ASI: Anthesis Silking Interval, NRC: Number of Rows per Cob, NSR: Number of Seeds per Row, NSC: Number of Seeds per Cob, CW: Cob Weight (g), CD: Cob Diameter (cm), Ash: Ash Content (%), Oil c: Oil Content (%), Prot: Protein Content (%), GTC: Grain Tocopherol Content ( $\mu\text{g g}^{-1}$ ), HSW: 100-Seed Weight, and GTP: Grain Yield per Plant (g).

**Table 2.** Descriptive analysis for different agronomic, physiological, and yield-contributing traits of maize genotypes.

Parameters\ Traits	PH	EH	ID	SD	NLP	DS	DT	ASI	NRC	NSR	NSC	CW	CD	ASH	Prot	Oil C	GTC	HSW	GYP
Minimum	135.67	53.67	12.53	4	9	48	44.33	0	10.67	20.67	268.67	108.33	15.5	3.47	10.6	2.37	21	20.81	95.2
Mean	202.14	81.89	16.59	6.2	12.22	51.94	49.02	2.92	14.445	29.07	419.58	174.86	39.44	4.87	12.85	2.75	29.19	26.94	144.89
Maximum	270.67	120.67	22.5	8.87	14.33	62	56.67	6	19.33	39.67	581.33	252.17	47.77	7.13	15.87	3.13	37.57	35.79	218.05
Variance	970.8	162.93	7.0318	0.84	1.69	10.85	7.6	0.21	4.2	17.04	4037.1	678	37.35	0.48	1.36	0.03	21.77	18.43	452
SD	31.15	12.76	2.65	0.92	1.3	3.29	2.75	0.46	2.05	4.12	63.53	26.03	6.11	0.69	1.16	0.19	4.66	4.29	21.26
C.V.	15.41	15.58	15.98	14.84	10.66	6.34	5.62	15.74	14.19	14.2	15.14	14.89	15.49	14.23	9.08	6.9	15.98	15.93	14.67

PH: Plant Height (cm), EH: Ear Height (cm), ID: Internodal Distance (cm), SD: Stem Diameter (cm), NLP: Number of Leaves per Plant, DS: Days to Silking, DT: Days to Tasseling, ASI: Anthesis Silking Interval, NRC: Number of Rows per Cob, NSR: Number of Seeds per Row, NSC: Number of Seeds per Cob, CW: Cob Weight (g), CD: Cob Diameter (cm), Ash: Ash Content (%), Oil c: Oil Content (%), Prot: Protein Content (%), GTC: Grain Tocopherol Content ( $\mu\text{g g}^{-1}$ ), HSW: 100-Seed Weight, and GTP: Grain Yield per Plant (g).

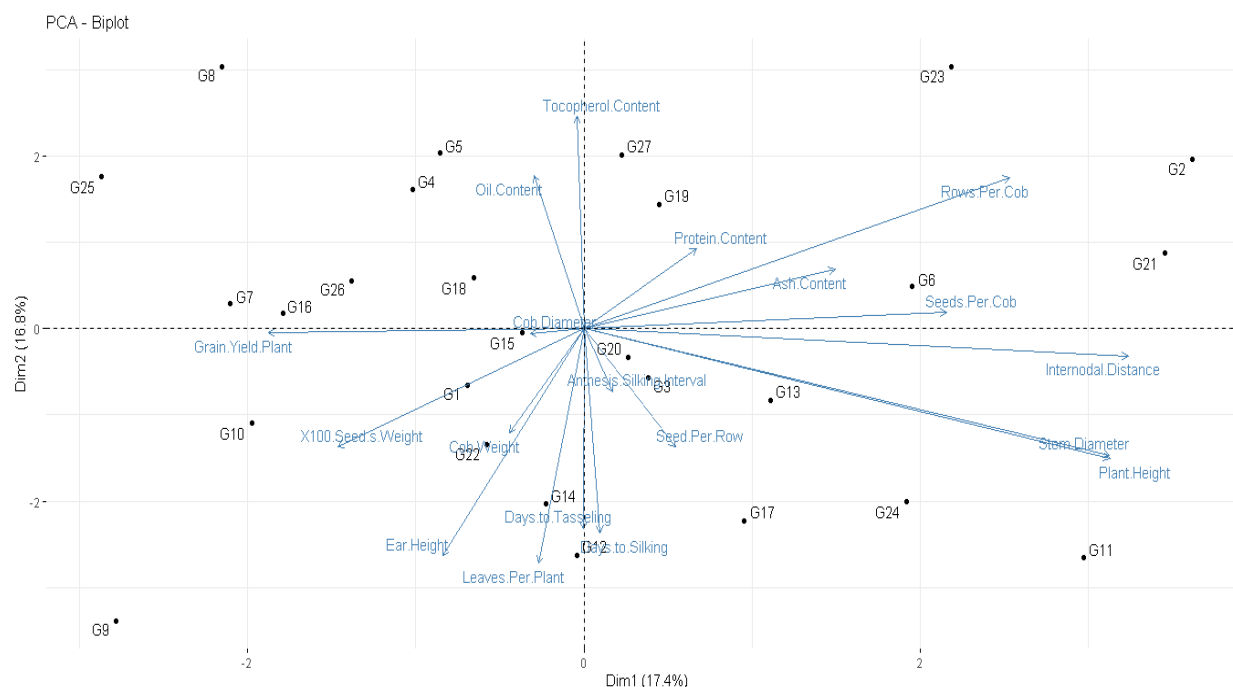
impact. The length of the trait arrows represents the importance of each trait in the analysis. Longer arrows indicate the corresponding trait has a higher effect on the principal components. Considering the grain tocopherol content vector, it resides in the same dimension with the oil and protein content vector, indicating positive interaction among them. Meanwhile, days to tasseling,

silking, and leaves per plant revealed a negative association. For grain yield, 100-seed weight, and oil content, they have a positive interaction as they lie in the same direction, whereas internodal distance and cob weight have a negative interaction. By understanding the nature of the interaction, indirect selection for desired traits can succeed.

**Supplementary Table S1.** Mean values of different agronomic, physiological, and yield-contributing traits of maize genotypes.

Geno\Traits	PH	EH	ID	SD	NLP	DS	DT	ASI	NRC	NSR	NSC	CW	CD	Ash	Oil C	Prot	GTC	HSW	GYP
G25	135.67	59.33	12.95	4.07	10.67	51.33	50.33	1	14.67	23.67	346	212.4	46.99	5.27	2.73	14.23	29.83	33.47	218.05
G27	192	89	14.87	6.27	12.67	49.33	48.33	1	18	27.67	498	131.2	35.99	5.17	3.13	13.73	33.7	24.75	206.03
G8	146.33	67	16.83	4.63	9	50	47.67	3.33	13.33	23	304.67	113.37	44.45	3.73	3.07	11.8	35.47	20.81	202.26
G17	263.67	81.67	20.67	8	12.67	52.33	50.33	2	12.67	30.33	384	181	40.99	4.83	2.57	12.23	22.3	29.28	199.02
G16	194.33	95.33	15.4	6.23	13	48	48	0	13.33	31	414.67	177	40.22	3.73	3.03	10.97	35.67	32.37	192.64
G9	209.67	120.67	15.07	6.33	14.33	53.33	52.33	1	10.67	25.33	268.67	173.33	44.47	3.47	2.6	13.3	25.23	35.79	178.44
G26	198	93.67	14.6	5.67	14	52.33	48.33	4	14.67	21.33	312.67	187.33	15.5	5.33	3.03	14.13	37.57	33.68	177.17
G14	204	105.67	14.67	6.33	14.33	51.33	46.33	5	14.67	39.67	581.33	246.37	47.77	4.4	2.8	13.53	26.73	32.1	155.15
G13	219.9	92.23	17.53	7.13	12.67	54.33	49.33	5	14.67	29.33	429.33	118.17	34.44	5.37	2.67	12.27	26.33	21.44	154.56
G7	173.33	64	14.33	5.1	12	48.33	47	1.33	12	28.67	344	178.1	39.56	4.37	2.63	10.6	24.37	29.02	153.47
G12	195	97	14.6	5.63	13	62	56.67	5.33	14.67	35.33	517.33	173.8	43.32	4.83	2.77	12.6	28.3	22.17	151.89
G6	256.33	75.67	18.17	7.93	12.67	51.33	46.33	5	15.33	31.67	483.33	145.07	39.03	4.63	2.93	12.3	34.47	24.27	140.06
G11	270.67	82	22.5	8.87	12.33	55	54.33	0.67	14.67	31.67	463.33	206.53	36.23	5.2	2.67	12.07	21	24.95	139.36
G10	143.33	73	12.53	4.1	11.67	57.33	53.33	4	13.33	31.33	416	252.17	37.7	4.67	2.57	13.5	24.77	25.17	135.88
G22	197.67	96.67	15.07	6.13	13	54	49.33	4.67	14	29.33	410.67	190.63	31.28	4.87	2.63	13.57	28.8	32.11	135.66
G18	187	85.33	15.53	5.4	12.33	52	47.33	4.67	12.67	32	403.33	164.23	34.45	4.87	2.97	12.83	32.2	22.79	134.41
G15	187	89.67	14.57	5.5	13	50.33	49.67	0.67	16.67	30.67	508	233	35.99	5.07	2.8	11.53	33.03	32.56	127.47
G20	218.33	73.33	18	6.3	12.67	50	48.67	1.33	12.67	28.33	362	108.33	43.84	4.73	2.37	13.23	25.77	26	126.61
G5	159.33	63.67	16.2	5.13	10	50.33	47.33	3	13.33	26.67	354	143.87	43.59	4.8	2.83	12.7	26.77	22.73	125.29
G24	266.67	83.33	21.83	8.5	12.33	57.33	52.67	4.67	14	20.67	289.33	159.27	38.21	5.67	2.67	12.53	26.1	30.89	118.88
G2	243.33	62.33	21.37	7	10.67	50.33	47.33	3	19.33	28.33	547.33	169.8	36.5	5	2.67	14.43	30.17	21.47	117.41
G19	176.33	84	14.07	5.8	13	49	48.33	0.67	15.33	30.33	467.33	140.77	43.49	7.13	2.8	12.23	33.63	24.28	113.13
G23	229.67	53.67	19.4	6.73	10.33	49	47.67	1.33	17.33	24.67	426	201.7	44.58	5.53	2.87	15.87	35.77	26.49	112.87
G4	139.67	72.33	15.03	4	10.67	48	46.67	1.33	14	31.67	443.33	162.17	36.66	4.73	2.53	12.8	25.5	23.43	103.91
G21	260.67	75.67	21	8.37	11.67	49.33	47.33	2	18	30.67	555.33	183.1	38.12	4.43	2.83	13.27	33.23	26.64	100.55
G1	208.33	103	15.73	6.3	13.33	49.33	44.33	5	11.33	31.33	354.67	202.1	45.24	4.7	2.87	13.8	27.9	27.3	96.72
G3	181.67	72	15.47	5.97	12	54.33	48.33	6	14.67	30.33	444	166.3	46.28	5.13	2.47	11.1	23.6	21.47	95.2

PH: Plant Height (cm), EH: Ear Height (cm), ID: Internodal Distance (cm), SD: Stem Diameter (cm), NLP: Number of Leaves per Plant, DS: Days to Silking, DT: Days to Tasseling, ASI: Anthesis Silking Interval, NRC: Number of Rows per Cob, NSR: Number of Seeds per Row, NSC: Number of Seeds per Cob, CW: Cob Weight (g), CD: Cob Diameter (cm), Ash: Ash Content (%), Oil c: Oil Content (%), Prot: Protein Content (%), GTC: Grain Tocopherol Content ( $\mu\text{gg}^{-1}$ ), HSW: 100-Seed Weight, and GTP: Grain Yield per Plant (g).



**Figure 1.** Principal component biplot for different agronomic, physiological, and yield-contributing traits in maize. The biplot indicates the distribution of genotypes along the parameters to determine the most suitable combination of genotypes for their respective parameters.

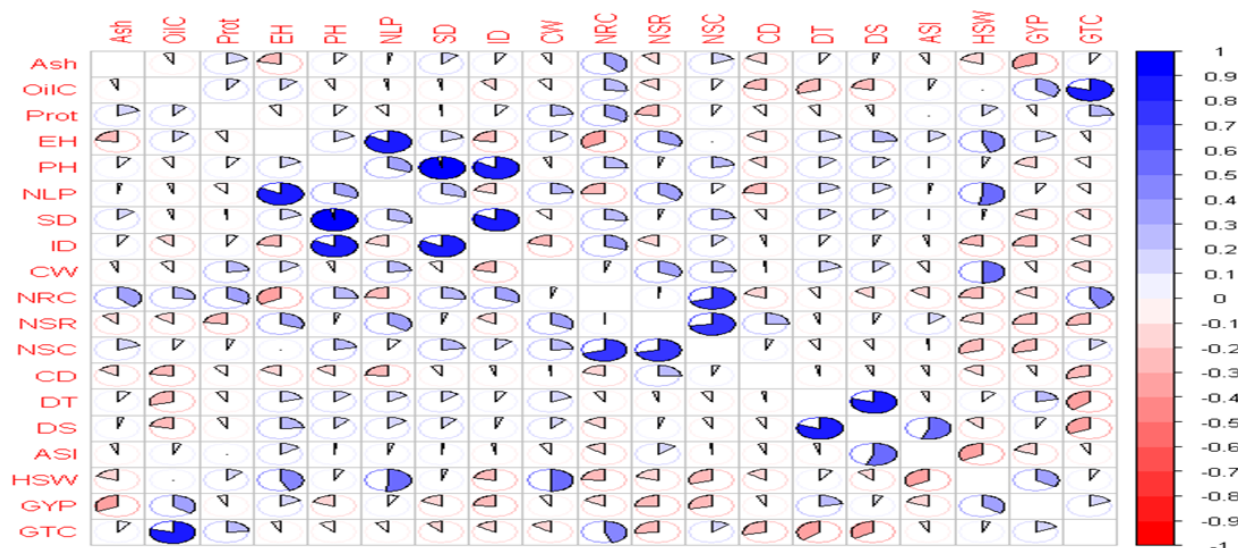
PH: Plant Height (cm), EH: Ear Height (cm), ID: Internodal Distance (cm), SD: Stem Diameter (cm), NLP: Number of Leaves per Plant, DS: Days to Silking, DT: Days to Tasseling, ASI: Anthesis Silking Interval, NRC: Number of Rows per Cob, NSR: Number of Seeds per Row, NSC: Number of Seeds per Cob, CW: Cob Weight (g), CD: Cob Diameter (cm), Ash: Ash Content (%), Oil c: Oil Content (%), Prot: Protein Content (%), GTC: Grain Tocopherol Content ( $\mu\text{g g}^{-1}$ ), HSW: 100-Seed Weight, and GTP: Grain Yield per Plant (g).

Genotypes closer to particular trait arrow exhibit higher values for that trait, while genotypes farther away have lower values. The projection for G23, G8, G25, and G27 resides on the tocopherol content factor, indicating a higher concentration of tocopherol. Conversely, the genotypes G25, G8, G7, and G10 are closer to the grain yield vector, implying them as high-yielding genotypes. Similarly, previous research suggested tocopherols have great genetic variability and had interaction with other traits (Kassem *et al.*, 2024). Genotypes G25 and G8 are almost equally good in grain yield and tocopherol, while G7 is only for yield, and G27 is only for tocopherol content. Several other studies on the maize gene pool expressed that it has a broad genetic base and great variability in yield and yield-contributing traits, as this was similar to the presented study (Ferdoush *et al.*, 2017).

### Correlation using correlogram

The correlation coefficient among the traits attained quantification by Pearson correlation and is graphically represented by a correlogram in Figure 2. The direction and degree of the correlation coefficient helped to understand the association among the traits. A high positive correlation with statistical significance can be a good marker for indirect selection.

The number of leaves per plant has a strong positive association with ear height. Plant height has a strong positive correlation with stem diameter and internodal distance. Grain rows per cob gave a positive association with ash content but showed a negative association with ear height. The grain total tocopherol content displayed a strong positive association with oil content and the number of



**Figure 2.** Correlogram indicates the relationship among the studied parameters of the studied genotypes. The right side bar colored from dark blue to dark red is the indicator of correlation strength that ranged from 1 to -1.

PH: Plant Height (cm), EH: Ear Height (cm), ID: Internodal Distance (cm), SD: Stem Diameter (cm), NLP: Number of Leaves per Plant, DS: Days to Silking, DT: Days to Tasseling, ASI: Anthesis Silking Interval, NRC: Number of Rows per Cob, NSR: Number of Seeds per Row, NSC: Number of Seeds per Cob, CW: Cob Weight (g), CD: Cob Diameter (cm), Ash: Ash Content (%), Oil c: Oil Content (%), Prot: Protein Content (%), GTC: Grain Tocopherol Content ( $\mu\text{g g}^{-1}$ ), HSW: 100-Seed Weight, and GTP: Grain Yield per Plant (g).

rows per cob and provided a negative linkage with days to tasseling. Another study conducted in soybean crops indicated an association of tocopherol with oil content, but the mechanism is still in question (McCord *et al.*, 2004). This signified that grain oil content could be the indirect marker for high tocopherols in genotypes. Scientific investigation revealed the foliar application of tocopherol increases the biomass, dry matter, and then, the yield (Ali *et al.*, 2020). However, the need to investigate the genetics behind the tocopherol content and the shortening in the vegetative phase in maize crops is essential. Indirect selection for grain yield can succeed by considering the degree and direction of the association among the tasseling traits. Grain yield per plant has a strong positive correlation with a hundred-seed weight and grain oil content, although it has a negative association with ash content.

## CONCLUSIONS

Genetic diversity is vital for identifying and categorizing to develop high-tocopherol maize varieties. Diversity in the grain's tocopherol content has strong effects on other plants' agrochemical traits. Tocopherol has a strong positive association with oil content and grain rows per cob and a negative correlation with days to tasseling. In future studies, these morphological and biochemical parameters could be beneficial for the indirect selection of high-tocopherol lines. The G25 and G8 genotypes are good for both traits, yield and tocopherol content, while G27 is excellent for tocopherol only, and G7 is a good performer for yield. By using these promising inbred lines in future breeding programs, tocopherol-biofortified hybrid development can be successful.



## REFERENCES

- Akram NA, Bashir R, Ashraf G, Bashir S, Ashraf M, Alyemeni MN, Bajguz A, Ahmad P (2023). Exogenous  $\alpha$ -tocopherol regulates the growth and metabolism of eggplant (*Solanum melongena* L.) under drought stress. *Plants*. 12(2): 237. <https://doi.org/10.3390/plants12020237>.
- Ali Q, Javed MT, Haider MZ, Habib N, Rizwan M, Perveen R, Ali S, Nasser M, Alyemeni HA, Serehy EI, Al-Misned FA (2020).  $\alpha$ -Tocopherol foliar spray and translocation mediates growth, photosynthetic pigments, nutrient uptake, and oxidative defense in maize (*Zea mays* L.) under drought stress. *Agronomy*. 10: 1235-1273. <https://doi.org/10.3390/agronomy10091235>.
- Ammar A, Aslam M, Khan M, Ahmad R (2024). Exploring the genetic potential of maize (*Zea mays* L.) for high-temperature stress tolerance. *SABRAO J. Breed. Genet.* 56(5), 2004-2014. <https://doi.org/10.54910/sabao2024.56.5.23>.
- Berardo N, Mazzinelli G, Valoti P, Lagana P, Redaelli R (2009). Characterization of maize germplasm for the chemical composition of the grain. *J. Agric. Food Chem.* 57: 2378-2384. <https://doi.org/10.1021/jf803688t>.
- Chishti MS, Shahbaz M, Kaleem M, Shafi S, Mehmood A, Zhang Q, Mansha M, Shehzadi N, Rana S, Shahid H, Hashem A, Alfagham AT, Abd Allah EF (2025). Fertigation with alpha-tocopherol enhances morphological, physiological, and antioxidant responses in radish (*Raphanus sativus* L.) under drought stress. *BMC Plant Biol.* 25(1):30. <https://doi.org/10.1186/s12870-025-06052-5>.
- Feng F, Wang Q, Liang C, Yang R, Li X (2015). Enhancement of tocopherols in sweet corn by marker-assisted backcrossing of ZmVTE4. *Euphytica*. 206: 513-521. <https://doi.org/10.1007/s10681-015-1519-8>.
- Ferdoush A, Haque M, Rashid M, Bari M (2017). Variability and traits association in maize (*Zea mays* L.) for yield and yield associated characters. *J. Bangladesh Agric. Univ.* 34: 265-289. <https://doi.org/10.3329/jbau.v15i2.35062>.
- GOP (2025). Pakistan Economic Survey 2024-25. In: Finance and Economic Affairs Division (Ed.), Ministry of Finance, Govt. of Pakistan, Islamabad, Pakistan. [https://www.finance.gov.pk/survey/chapter\\_25/2\\_Agriculture.pdf](https://www.finance.gov.pk/survey/chapter_25/2_Agriculture.pdf).
- Gunjević V, Zurak D, Grbeša D, Kiš G, Međimurec T, Pirgozliev V, Kljak K (2023). Bioaccessibility of tocopherols in commercial maize hybrids determined by an in vitro digestion model for poultry. *Molecules* 28(13), 5015. <https://doi.org/10.3390/molecules28135015>.
- Haberer G, Young S, Bharti AK, Gundlach H, Raymond C, Fuks G, Butler E, Wing RA, Rounsley S, Birren B (2005). Structure and architecture of the maize genome. *Plant Physiol.* 139: 1612-1624. <https://doi.org/10.1104/pp.105.068718>.
- Hectors K, Van Oevelen S, Geuns J, Guisez Y, Jansen MA, Prinsen E (2014). Dynamic changes in plant secondary metabolites during uv acclimation in *Arabidopsis thaliana*. *Physiol. Plant.* 152: 219-230. <https://doi.org/10.1111/ppl.12168>.
- Horwitt MK (2001). Critique of the requirement for vitamin E. *Am J Clin Nutr.* 73(6), 1003-1005. <https://doi.org/10.1093/ajcn/73.6.1003>.
- Jilani T, Iqbal MP (2018). Vitamin E deficiency in South Asian population and the therapeutic use of alpha-tocopherol (Vitamin E) for correction of anemia. *Pakistan J Med. Sci.* 34(6): 1571. <https://doi.org/10.12669/pjms.346.15880>.
- Kassem MA, Knizia D, Meksem K (2024). A summary of two decades of quantitative trait loci (QTL) and candidate genes that control seed tocopherol contents in maize (*Zea mays* L.). *Genes* 15:472-489. <https://doi.org/10.3390/genes15040472>.
- Kumar S, Singh R, Nayyar H (2013).  $\alpha$ -Tocopherol application modulates the response of wheat (*Triticum aestivum* L.) seedlings to elevated temperatures by mitigation of stress injury and enhancement of antioxidants. *J. Plant Growth Regul.* 32: 307-314. <https://doi.org/10.1007/s00344-012-9299-z>.
- Li Q, Yang X, Xu S, Cai Y, Zhang D, Han Y, Li L, Zhang Z, Gao S, Li J (2012). Genome-wide association studies identified three independent polymorphisms associated with  $\alpha$ -tocopherol content in maize kernels. *PLoS One*. 7: 36807. <https://doi.org/10.1371/journal.pone.0036807>.
- Maeda H, Song W, Sage TL, DellaPenna D (2006). Tocopherols play a crucial role in low-temperature adaptation and phloem loading in *Arabidopsis*. *Plant Cell*. 18: 2710-2732. <https://doi.org/10.1105/tpc.105.039404>.
- Majeed S, Iqbal M, Asi MR, Iqbal SZ, Selamat J (2017). Analysis of nutritional traits and aflatoxin contamination in selected maize varieties from Pakistan. *Journal of Food*

- Protection* 80: 1993-1998. <https://doi.org/10.4315/0362-028X.JFP-17-117>.
- McCord KL, Fehr WR, Wang T, Welke GA, Cianzio SR, Schnebly SR (2004). Tocopherol content of soybean lines with reduced linoleate in the seed oil. *Crop Sci.* 44: 772-776. <https://doi.org/10.2135/cropsci2004.7720>
- Muzhingi T, Palacios-Rojas N, Miranda A, Cabrera ML, Yeum KJ, Tang G (2017). Genetic variation of carotenoids, vitamin E and phenolic compounds in provitamin A biofortified maize. *J. Sci. Food Agric.* 97: 793-801. <https://doi.org/10.1002/jsfa.7798>.
- Niu Y, Zhang Q, Wang J, Li Y, Wang X, Bao Y (2022). Vitamin E synthesis and response in plants. *Front. Plant Sci.* 13: 994058. <https://doi.org/10.3389/fpls.2022.994058>.
- Pearson K (1901). On lines and planes of closest fit to a system of points in space. *Philos. Mag.* 2, 6th series. <https://doi.org/10.1080/14786440109462720>.
- Popa C, Călugăr RE, Russu F, Varga A, Muntean LS (2023). Maize and its use in human food. *Hop Med. Plants.* 31(1-2): 138-150. <https://doi.org/10.15835/hpm.v31i1-2.14744>.
- Rocheford TR, Wong JC, Egesel CO, Lambert RJ (2002). Enhancement of vitamin E levels in corn. *J. Am. Coll. Nutr.* 21: 191-198. <https://doi.org/10.1080/07315724.2002.10719265>.
- Serna-Saldivar SO (2023). Maize, ICC Handbook of 21st Century Cereal Science and Technology. Elsevier, pp. 131-143. <https://doi.org/10.1016/B978-0-323-95295-8.00030-7>.
- Shamim AA, Schulze K, Merrill RD, Kabir K, Christian P, Shaikh S, Wu L, Ali H, Labrique AB, Mehra S (2015). First-trimester plasma tocopherols are associated with risk of miscarriage in rural Bangladesh. *Am. J. Clin. Nutr.* 101:294-301. <https://doi.org/10.3945/ajcn.114.094920>.
- Steel R, Torrie J, Dickey D (1997). Principals and Procedures of Statistics. A Biometrical Approach. 3rd Ed. McGraw Hill Book Co. Inc., New York, USA. 13:1409-1414. <https://doi.org/10.2307/2530180>.
- Yusuf MA, Kumar D, Rajwanshi R, Strasser G, Tsimilli-Michael M, Sarin B (2010). Overexpression of  $\gamma$ -tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: Physiological and chlorophyll a fluorescence measurements. *Biochim. Biophys. Acta - Bioenerg.* 1797:1428-1438. <https://doi.org/10.1016/j.bbabi.2010.02.002>.
- Zhang Z, Xu P, Zhou H, Peng X, Qi J, Zhang Y (2025). Research on corn quality detection based on near-infrared spectroscopy. *Spectrosc Lett*, 58(4), 394-403. <https://doi.org/10.1080/00387010.2024.2445691>