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HARNESSING GENETIC VARIABILITY AND TRAIT RELATIONSHIPS FOR COLD TOLERANCE IN MAIZE IN THE WESTERN HIMALAYAN REGION

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ABSTRACT

This study explores the genetic variability, heritability, and correlations among agro-morphological and physiological traits associated with cold tolerance in maize inbreds. Significant variability was observed for all traits, with high phenotypic (PCV) and genotypic coefficients of variation (GCV) for root weight, grain yield per plant, and total dry matter weight, indicating strong genetic control and potential for selection. Heritability estimates (>60%) and genetic advance revealed that traits such as grain yield per plant and root weight are predominantly influenced by additive gene action, making them ideal targets for breeding programs. Correlation analysis highlighted critical relationships, including a strong positive correlation (r = 0.98) between days to anthesis and days to silking, indicating synchronized flowering under cold stress. Moderate correlations between grain yield and total dry matter weight (r = 0.58), and between root volume and root length (r = 0.41), underscore the importance of biomass accumulation and efficient root systems for resilience. Physiological traits such as degree of yellowing and Electrolyte Leakage were also strongly correlated (r = 0.76), reflecting cold-induced stress damage. These findings highlight the potential to improve cold tolerance in maize by selecting for traits with high heritability, genetic advance, and positive contributions to yield and stress resilience. This study provides valuable insights for developing high-yielding, cold-tolerant maize genotypes for cultivation in cold-prone regions.

***Keywords* :** Maize, GCV, PCV, Heritability, Correlation

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops globally, playing a vital role in food security, animal feed, and industrial applications. Despite its economic and nutritional significance, maize production is highly vulnerable to abiotic stresses, such as cold stress, which significantly impede growth, development, and yield. Cold stress delay seed germination (Walne *et al.*, 2020) reduce growth rates, and negatively impact plant vigor (Wijewardana *et al.*, 2015). Under extremely low temperatures, permanent frost damage can occur (Ali *et al.*,2018), particularly in regions where early planting or high-altitude cultivation is essential. As climate variability continues to challenge traditional maize-growing regions, improving the crop's tolerance to cold stress is crucial for ensuring stable productivity and expanding its cultivation to non-traditional areas. Crop's effectiveness is determined by the magnitude of genetic variability present in the population, but also by how heritable it is (Hussain *et al.*, 2011).The exploration of genetic variability within maize inbred lines offers significant potential for breeding programs aimed at enhancing cold tolerance.

Inbred lines, being genetically homozygous and stable, provide a robust platform for identifying traits and mechanisms linked to stress tolerance. By evaluating agro-morphological traits such as grain yield, and root structure, alongside physiological parameters like chlorophyll content, degree of yellowing and electrical conductivity test, researchers can uncover key indicators of cold stress resilience. Understanding trait associations is particularly important in breeding programs because it enables indirect selection for complex traits such as cold tolerance, which are influenced by multiple genes and environmental interactions.

Heritability assumes that individuals who are closely connected are more likely to resemble one another than those who are distantly related (Falconer and Mackay, 1996). Heritability estimate helps breeders to allocate resources effectively to select desired traits and to achieve maximum genetic gain with little time and resources (Smalley *et al.*, 2004).

Genetic variability among inbred lines acts as a repository of diverse traits, enabling the identification of genotypes well-suited for cold environments. Furthermore, understanding the interrelationships among traits plays a crucial role in accelerating the development of cultivars that integrate high yield potential with enhanced stress tolerance (Kumar *et al.*, 2014). Heritability, a key parameter in quantitative genetics, is widely recognized for its predictive value in assessing the reliability of phenotypic traits as indicators of breeding value. Traits with high heritability can be effectively improved through simple selection methods. However, heritability alone is insufficient without considering genetic progress, which represents the extent of heritable genetic improvement achieved under specific selection pressures. Optimal selection outcomes are observed when both heritability and genetic progress are high (Bello *et al.*, 2012).

The coefficient of variation reflects the overall variability across traits, though it does not account for the heritable component. Correlation analysis complements this by offering valuable insights into the nature, magnitude, and direction of trait associations. Specifically, correlation coefficients facilitate the identification of relationships between distinct traits and their mutual variability, providing critical guidance for the selection process (Zeeshan *et al.*, 2013; Bocanski *et al.*, 2009; Nagabhushan *et al.*, 2011).

The objective of this study was to determine the variability as well as their correlation for agro-morphological and physiological traits in maize inbred lines under cold stress conditions.

Materials and Methods

Experimental site

The field experiment were conducted during the year 2022 and 2023 and laid at Mountain Crop Research Station, Larnoo, SKUAST-K, J&K, India (75.331°N, 33.644°E) at an altitude of 2280 msl. The study area location is characterized by cold temperate conditions with moderate summers and severe winters and at Greenhouse, Division of Genetics & Plant Breeding, FoA, Wadura.

Plant material and experimental design

A set of diverse 164 maize inbred lines including 4 checks were evaluated for various morpho-physiological and agronomic traits under field condition in an Augmented Block Design (ABD).

Data collection

For all traits, data was obtained from five randomly selected plants from each experimental plot. These selected plants were observed for morpho-physiological and agronomic traits such as root length (cm), root volume (ml), root weight (g), grain yield per plant (g), total dry matter weight (g), chlorophyll content, anthocyanin, days to anthesis, days to silking, anthesis-silking interval (ASI), degree of yellowing was scored based on a 1-5 scoring scale, where “1” indicated highly tolerant and “5” indicated highly susceptible. germination percentage (%) was calculated using the formula:

$$\text{GP}\,(\%) = \frac{(\text{Number of seeds germinated})}{(\text{Total number of seeds sown})} \times 100 \quad (1)$$

Electrical Conductivity was calculated using the formula:

$$\text{EC}\,(\%) = \frac{(\text{Leakage of live tissue})}{(\text{Leakage of heat killed tissue})} \times 100 \quad (2)$$

Data analysis

Excel 2010 was used to process the experimental data, while R-studio (Version 4.3.0) were used to analyze it. Augmented Block Design (ABD) with one-way ANOVA was used to analyze data. The treatment means were compared using the least significant difference (LSD) at 5% level of significance (Gomez and Gomez, 1984). The data on all of the characters were subjected to standard analysis of variance methods (Panse and Sukhatme, 1967). The phenotypic and genotypic coefficients of variation (Singh and Chaudhary, 1985), Heritability (in the broad sense) (Johnson *et al.*, 1955), genetic advance (Burton, 1952), and genetic advance as percentage of the mean (Johnson *et al.*, 1955) were all calculated. The GCV (Eq. 3), PCV (Eq. 4), Heritability (Eq. 5), Genetic advance (Eq. 6), Genetic advance percentage of mean (GAM) (Eq. 7) were calculated using below formulas;

$$\text{GCV}(\%) = \frac{\sigma_g^2}{\overline{X}} \times 100 \qquad (3)$$

$$\text{PCV}(\%) = \frac{\sigma_p^2}{\overline{X}} \times 100 \qquad (4)$$

Where, σ_g^2 = Genotypic variance, σ_p^2 = Phenotypic variance and $\overline{X}$ = Mean of character

Heritability= $h^2 = (\text{b.s.}) = \frac{\sigma_g^2}{\sigma_p^2} \times 100 \qquad (5)$

Where, h^2= Heritability(broad sense), σ_g^2 = Genotypic variance and σ_p^2 = Phenotypic variance

Genetic advance (G.A.)

$$\text{G.A} = k \times \frac{\sigma^2 g}{\sigma^2 p} \times \sqrt{\sigma_p^2} \qquad (6)$$

Where, σ_g^2 = Genotypic variance, σ_p^2 = Phenotypic variance, k= Selection differential (at 5 % selection = 2.06) and $\sqrt{\sigma_p^2}$ = Phenotypic standard deviation

G.A. as percentage of means (GAM)

$$\text{GAM} = \frac{\text{GA}}{\overline{X}} \times 100 \qquad (7)$$

Where, G. A. = Genetic advance, $\overline{X}$ = Character mean

Table 1 : Mean sum of squares for different traits for 164 maize inbreds

Traits	Treatments (df=163)	Error (df=21)
Chlorophyll Content	19.95**	3.23
Root Volume	1.95**	0.63
Root Weight	3.33**	0.29
Root Length	5.88**	0.72
Germination percentage	630.82**	133.93
Days to Anthesis	64.53**	3.87
Days to Silking	63.59**	5.55
Anthesis silking interval	2.46**	0.63
Degree of yellowing	0.80**	0.32
Grain yield per plant	430.75**	12.9
Total dry matter weight	805.63**	64.94
Electrolyte leakage	197.96**	20.18

*, ** significant at 5% and 1% level, respectively

Results and Discussion

Field studies were conducted to determine the genetic variability and correlations in agro-morphological and physiological traits in maize genotypes. The findings were evaluated and discussed, with evidence from earlier studies to back them up.

Genetic variability

All of the maize inbred lines significantly differed for all the characters under study indicating sufficient amount of variability present in the inbreds (Table 1).

For all the traits, the GCV values were lower than PCV value, showing that the characters were more influenced by their surrounding environments (Table 2). Similar results were found by Magar *et al.* (2021) and Pranay *et al.* (2022). According to Sivasubramanian and Menon (1973), the traits evaluated in this study had low (less than 10% phenotypic and genotypic coefficients of variation), moderate (10–20% phenotypic and genotypic coefficients of variation, and high (more than 20% phenotypic and genotypic coefficients of variation. The analysis of genetic variability showed high phenotypic coefficient of variation (PCV) for root weight (65.28%), germination percentage (45.83%), grain yield per plant (62.72%), and total dry matter weight (43.16%), indicating significant variability influenced by genetic and environmental factors. Moderate PCV (20–30%) was observed for chlorophyll content (37.65%), root volume (36.23%), root length (31.28%), anthesis silking interval (29.74%), and electrolyte leakage (22.08%), supporting effective selection. Traits like days to anthesis (9.56%), days to silking (9.03%), and degree of yellowing (25.01%) had low PCV, indicating minimal variability. High genotypic coefficient of variation (GCV) was found for root weight (62.88%), grain yield per plant (61.18%), germination percentage (40.71%), and total dry matter weight (41.16%), showing strong genetic control and potential for direct selection. Moderate GCV (20–30%) was recorded for chlorophyll content, root volume, root length, anthesis silking interval, and electrolyte leakage, while days to anthesis, days to silking, and degree of yellowing showed low GCV (<20%), reflecting limited genetic variability (Table 2). These findings are in good agreement with the observations of Vishnu *et al.* (2023) and Grace *et al.* (2018) The presence of a significant degree of genetic diversity is indicated by GCV, but the amount of heritable variation can only be assessed using heritability estimates and genetic gain (Rao and Rao, 2015).

Table 2 : Estimation of PCV, GCV, Heritability, Genetic Gain and GAM for various morpho-physiological and agronomic traits under cold stress.

Trait	PV	GV	EV	PCV	GCV	ECV	hBS	GA	GAM
CC(SPAD)	22.61	19.38	3.23	37.65	34.86	14.22	85.73	8.41	66.59
RV(ml)	2.33	1.7	0.63	36.23	30.95	18.85	72.95	2.3	54.53
RW(g)	4.04	3.75	0.29	65.28	62.88	17.54	92.78	3.85	124.95
RL(cm)	6.37	5.65	0.72	31.28	29.46	10.52	88.69	4.62	57.23
GR(%)	635.08	501.15	133.93	45.83	40.71	21.05	78.91	41.03	74.61
DTA	61.4	57.53	3.87	9.56	9.25	2.4	93.7	15.15	18.48
DTS	62.2	56.65	5.55	9.03	8.61	2.7	91.08	14.82	16.96
ASI	2.59	1.96	0.63	29.74	25.87	14.67	75.66	2.51	46.41
DOY	0.62	0.3	0.32	25.01	17.34	18.02	48.08	0.78	24.81
GYPP(g)	265.44	252.54	12.9	62.72	61.18	13.83	95.14	31.98	123.11
TDMW(g)	718.96	654.03	64.94	43.16	41.16	12.97	90.97	50.32	80.99
EL(%)	151.87	131.68	20.18	22.08	20.56	8.05	86.71	22.04	39.49

*CC-Chlorophyll Content, RV- Root Volume, RW-Root Weight, RL-Root Length, GR- Germination percentage, DTA-Days to Anthesis, DTS-Days to Silking, ASI- Anthesis silking interval, DOY- Degree of yellowing, GYPP- Grain yield per plant, TDMW-Total dry matter weight, EL-Electrolyte leakage

Heritability and genetic advance

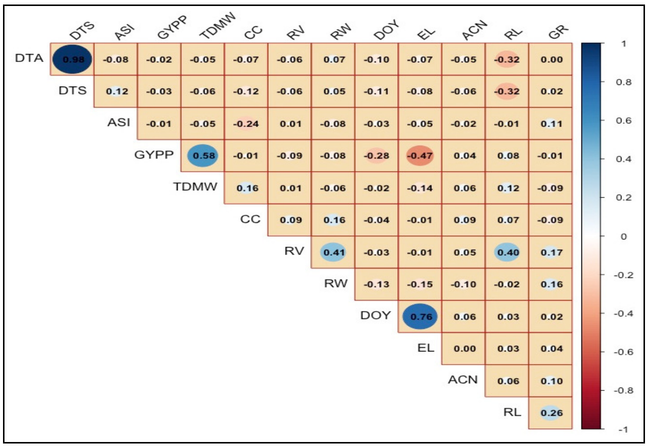
When additive gene effects controlled a characteristic, it usually resulted in both higher heritability and genetic advance, whereas when non-additive gene actions controlled a trait, it might result in high heritability but poor genetic advance (Mohana Krishna *et al.*, 2009). High estimates of heritability for most of the variables suggested that variations were passed down to progeny, implying that a high-yielding variety may be developed by selecting desirable genotypes. High heritability provides more options for selecting plant material with the desired features. The broad-sense heritability analysis revealed high heritability (>60%) for several traits, including chlorophyll content (85.73%), root weight (92.78%), root length (88.69%), germination percentage (78.91%), grain yield per plant (95.14%), and total dry matter weight (90.97%). These traits are predominantly controlled by genetic factors, offering strong potential for genetic improvement through selection. Moderate heritability (30–60%) was observed for degree of yellowing (48.08%) and anthesis silking interval (75.66%), suggesting moderate genetic control. None of the traits showed low heritability (<30%) (Table 2). These findings are in good agreement with the observations of Rezaei *et al.* (2020)

Genetic advance and genetic advance as a percentage of the mean highlighted traits with high genetic potential. High genetic advance as a percentage of the mean (>20%) was observed for root weight (124.95%), grain yield per plant (123.11%), total dry matter weight (80.99%), germination percentage (74.61%), and chlorophyll content (66.59%). The high values indicate a significant contribution of additive gene action, making these traits highly effective for selection and improvement. Medium genetic advance as a percentage of the mean (10–20%) was recorded for days to anthesis (18.48%) and days to silking (16.96%), suggesting moderate effectiveness for selection. Low genetic advance as a percentage of the mean (<10%) was noted for degree of yellowing (24.81%) and electrolyte leakage (39.49%), indicating lower genetic gain through selection. Similar results were observed by Magar *et al.* (2021) and Korsa *et al.* (2024).

Overall, these findings provide valuable insights into the heritability and genetic advance of various traits, emphasizing the traits with strong genetic control and high genetic gain potential for targeted breeding programs.

Phenotypic correlation coefficient

The correlation analysis reveals key traits contributing to cold tolerance in maize inbreds (Fig 1). A strong positive correlation between days to anthesis and days to silking (r = 0.98) suggests synchronized flowering, critical for reproductive success under cold stress. A moderate positive correlation between grain yield per plant and total dry matter weight (r = 0.58) highlights that higher biomass supports better yield in cold conditions, while root volume and root length (r = 0.41) emphasize the importance of robust root systems for nutrient uptake. The positive correlation between degree of yellowing and electrolyte leakage (r = 0.76) points to the physiological damage caused by cold stress, which negatively affects yield, reflected in the weak negative correlation between grain yield and electrolyte leakage (r = -0.47). Weak negative correlations between flowering time and root length (r = -0.32) suggest that early flowering inbreds may have shorter roots, but still, efficient root systems are critical for nutrient uptake under prolonged cold. Focusing on biomass, root growth, and minimizing stress-induced damage could enhance cold tolerance and yield in maize inbreds.



	DTS	ASI	GYPP	TDMW	CC	RV	RW	DOY	EL	ACN	RL	GR
DTA	0.98	-0.08	-0.02	-0.05	-0.07	-0.06	0.07	-0.10	-0.07	-0.05	-0.32	0.00
DTS		0.12	-0.03	-0.06	-0.12	-0.06	0.05	-0.11	-0.08	-0.06	-0.32	0.02
ASI			-0.01	-0.05	-0.24	0.01	-0.08	-0.03	-0.05	-0.02	-0.01	0.11
GYPP				0.58	-0.01	-0.09	-0.08	-0.28	-0.47	0.04	0.08	-0.01
TDMW					0.16	0.01	-0.06	-0.02	-0.14	0.06	0.12	-0.09
CC						0.09	0.16	-0.04	-0.01	0.09	0.07	-0.09
RV							0.41	-0.03	-0.01	0.05	0.40	0.17
RW								-0.13	-0.15	-0.10	-0.02	0.16
DOY									0.76	0.06	0.03	0.02
EL										0.00	0.03	0.04
ACN											0.06	0.10
RL												0.26

Fig. 1 : Correlation heat map showing association between various morpho-physiological traits under cold stress.

Conclusion

This study reveals significant genetic variability and heritability among traits related to cold tolerance in maize inbreds. Traits like root weight, grain yield per plant, and total dry matter weight, with high heritability and genetic advance, are predominantly governed by genetic factors, making them ideal for selection. Strong correlations, such as between days to anthesis and silking (r = 0.98) and grain yield and total dry matter weight (r = 0.58), highlight the importance of synchronized flowering, biomass accumulation, and robust root systems for stress resilience. Physiological markers like degree of yellowing and electrolyte leakage (r = 0.76) reliably indicate cold-induced damage. These findings provide a basis for breeding high-yielding, cold-tolerant maize varieties by targeting traits with high genetic control and strong contributions to stress tolerance.

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