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Cold Stress Tolerance Evaluation in Maize Double Haploid Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The experimental materials consisting 218 maize double haploids and two commercial checks were screened at CIMMYT-BISA-Ludhiana-Punjab under natural cold stress (winter sowing) during *rabi* 2019-20. Data was recorded on leaf necrosis (seedling stage), ear leaf length, ear leaf width, ear leaf area, number of plants per plant, ears per plot, bareness and grain yield. These traits were observed to be highly correlated with cold tolerance and also exhibited moderate to high heritability except leaf width, bareness. DH_1_24, DH_1_67, DH_1_216, DH_1_17 and DH_1_180 are suitable for sowing in the winter season. Leaf necrosis observed as important criteria for selection of cold tolerant genotypes at vegetative stage and bareness at reproductive stage.

Keywords: Maize; double haploids; leaf necrosis; cold tolerance; selection.

1. INTRODUCTION

Maize (*Zea mays* L.) has wider adaptability and can therefore, be grown during all three principal agricultural growing seasons-*Kharif*, *Zaid* and *rabi* especially, in the plains of Northern India. The acreage under cultivation during the *rabi* season shrinks however, on account of the presence of extreme low temperatures (<15 especially in the northern most plain zone of the country [1,2]. Also, as is being regularly observed on account of global climate change,

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temperatures frequently drops below 15°celsius in the plains of Northern India, during winters. Consequently, *rabi* maize is highly sensitive to cold stress [3].

Cold stress can't be artificially created in field conditions and laboratory observations mainly fail when it comes to field. Hence, these findings may be relevant for finding of best maize hybrids with sustainable yield during cold stress especially for Northern India. A study of effects of cold stress on maize and traits associated with cold injury or cold tolerance will, provide a foundation for breeding maize with improved cold tolerance. Appraisal of cold tolerance in the field however, is highly influenced by environmental conditions. Hence, the study of genetic factors involved in plant responses to cold stress is essential to develop cold tolerant maize genotypes, of shorter duration. Numerous studies (Strigens et al. 2003), Hund et al. [4], Wijewardana et al. [5] and Kandel & Shrestha [4] were conducted to assess cold stress effect on germination percent, leaf area, chlorophyll content, growth traits, pre-flowering and yield attributing traits in maize seedlings under cold stress conditions in maize. For instance Ahmad et al. [7] evaluated 66 inbred lines and pointed out that the early and extra early maturing lines were most tolerant to cold temperatures. They found significant high correlation coefficients between yellowing of leaves and plant growth. Hussain et al. [2] studied the effect of low growth temperature on the photosynthetic apparatus of maize.

Understanding the response of maize genotypes selected for cold stress may thus facilitate the identification of promising hybrids with tolerance to cold stress fairly quickly for further testing, release and deployment. Parents of these hybrids can also be further screened to identify source materials for breeding to further increase their tolerance to much higher levels. Keeping in view this study was, therefore, conducted to (i) assess the extent of variation and response pattern of hybrids to cold stress; (ii) identify traits that contributed to better performance under cold stress; (iii) select the best yielding hybrids coupled with tolerance.

2. MATERIALS AND METHODS

The genetic material that was used in this study consisted of 220 double haploid (DH) maize genotypes including two commercial hybrid checks. These genotypes were evaluated at CIMMYT BISA Ludhiana Punjab. The experimental materials were evaluated in alpha lattice design with two replications. Entries were planted in single-row plots, 4 m long, with 0.75 m spacing between rows and 0.20 m between hills. Two seeds per hill were initially planted and then thinned to one plant per hill at three weeks after emergence for a final plant. Fertilizers were applied at the recommended dose. Average weekly maximum and minimum temperature, rainfall and relative humidity (maximum) recorded from a weather station at CIMMY-BISA-Ludhiana during the growing period in 2019-20 is presented in Fig. 1.

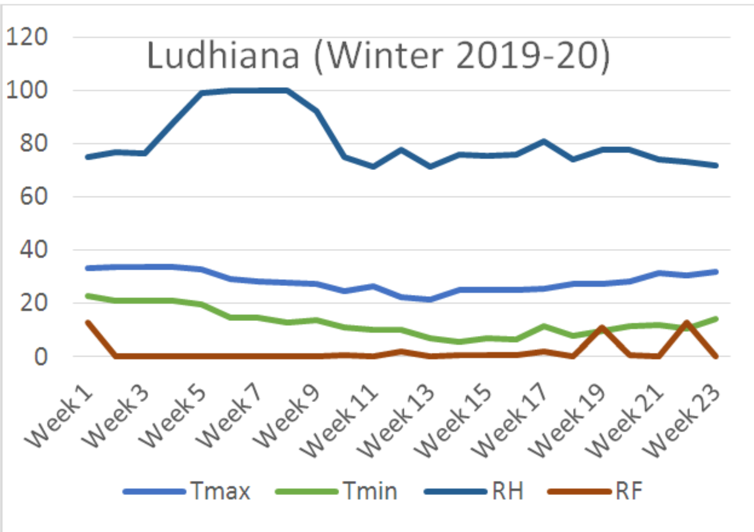

Fig. 1. Weekly weather data recorded from a weather station at BISA in Ludhiana during the growing period 2019-20

All ears harvested from each plot were shelled for determining the percentage grain moisture and grain weight, using moisture meter and weighing balance; respectively. Grain yield (GY) of each plot was estimated on an average as shelling percentage of 80% and standardized to 15% moisture content and then, converted to t/ha. Number of ears per plot (EPP) was recorded by counting the total number of ears, harvested in each plot. Through this, bareness induced by stress can be estimated, using the formula, Barrenness = 1 – EPP.

Length and width of the ear leaf was measured in centimetre. Observations were recorded on five representative plants within each plot and noted as average.

LA of ear leaf was estimated by multiplying the length of ear leaf by its maximum width and then by the factor 0.75 [8]. Observations were recorded on five representative plants within each plot and noted as average.

$$LA = LL \times LW \times 0.75$$

Leaf necrosis per plot was recorded on a 0-10 scale [9] based on, percentage of leaf area showing necrosis symptoms.

0 = No necrosis
1 = 10% of leaves necrotic in a plot
2 = 20% of leaves necrotic in a plot
3 = 30% of leaves necrotic in a plot
4 = 40% of leaves necrotic in a plot
5 = 50% of leaves necrotic in a plot
6 = 60% of leaves necrotic in a plot
7 = 70% of leaves necrotic in a plot
8 = 80% of leaves necrotic in a plot
9 = 90% of leaves necrotic in a plot
10 = complete necrosis

The analysis of variance (ANOVA) was performed using ADEL-R (Analysis and design of experiments with R for Windows), Version 2.0. The phenotypic mean data for each genotype adjusted in the form of Best Linear Unbiased Predictors (BLUPs) using META-R software. Phenotypic coefficients of correlations for grain yield with additional traits were computed under cold stress conditions to further determine the traits contributing to grain yield using META-R.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance (ANOVA)

ANOVA discerned significant differences among all genotypes indicating the presence of sufficient variability among the DH AM panel for all traits except leaf width (LW) and leaf area (LA) taken for the study (Table 1). This result indicated presence of genotypic variability for most of the traits. Such variability was contributed due to presence of DH genotypes derived from parents of different origin or pedigree. Revilla et al. [10] evaluated days to emergence, percent emergence, root dry weight and shoot dry weight of sweet corn hybrids under cold conditions. They found that variation exists for days to emergence, root dry weight and shoot dry weight under cold stress. Similar results were obtained by Hund et al. [4] and Wijewardana et al. [5] for seedling stage parameters studied under cold stress. Further, Kandel & Shrestha [6] also observed significant differences among maize hybrids for phenological traits, growth traits and, yield and yield components under winter planting.

3.2 Genetic Component of Variation

Under cold stress maize genotypes exhibited moderate GCV (15.09%), ECV (17.43%) with lower GA (1.11) and moderate GAM (27.05%) and h^2_{bs} for GY and EPP (Table 1). The DH genotypes manifested moderate GCV (13.83%), ECV (16.11%) with GA (8.07) and higher GAM (66.81%) accompanied by, h^2_{bs} for EPP (60%) under cold conditions. Several authors delineated have been conducted to determine the relative amounts of genetic variability for cold tolerance in corn in controlled growth chambers, predominantly targeting on germination and early growth stages [11,12,5,13].

3.3 Hybrids Performance

GY of maize genotypes ranged from 2.03 (Z542-185) to 5.53 t/ha (Z542-23) with mean GY of the DH AM panel as 4.12 t/ha (Table 1). The top 10 DH lines produced a mean grain yield of 5.0 t ha^{-1}, compared to the mean of commercial checks (4.28 t/ha). Among DH lines, the top 10 hybrids produced 12% higher mean GY than the best commercial check (CAH153) under cold stress.

Table 1. Analysis of variance and genetic components of yield and component attributes in maize genotypes under cold condition

Statistic	Genotype Variance	Residual Variance	GCV	ECV	GA	GAM	h^2 (%)	Grand Mean	Range	LSD	CV	Genotype significance	
GY	0.39	0.52	15.09	17.43	1.11	27.05	60	4.12	2.03-5.53	0.80	17.43	**	5E-10
NPP	1.26	2.43	10.08	13.98	3.88	34.79	51	11.15	5.97-14.26	1.57	13.98	**	5E-07
EPP	2.79	3.79	13.83	16.11	8.07	66.81	60	12.08	5.31-16.68	2.12	16.11	**	1E-10
LL	2.55	2.22	5.24	4.88	6.85	22.46	70	30.48	26.42-33.79	1.79	4.88	**	1E-15
LW	0.01	0.40	2.76	18.16	0.04	1.07	4	3.48	3.45-3.73	0.19	18.16	NS	7E-01
LA	19.27	244.87	5.52	19.67	73.98	93.01	14	79.54	76.67-96.79	8.06	19.67	NS	3E-01
BAR	0.01	0.12	87.48	330.54	0.03	30.95	12	0.10	0.07-0.27	0.17	330.54	NS	4E-01
Necrosis	0.41	0.34	16.27	14.91	1.10	27.81	70	3.94	2..26-7.25	0.70	14.91	**	2E-14

''Significant at 5% level of significance, '**'Significant at 1% level of significance, 'NS' non-significant*

Here, GY=Grain yield; NPP= Number of plants per plot; EPP=Ears per plot; LL= Ear Leaf Length (cm); LW= Ear Leaf width (cm); LA= Ear Leaf area (cm^2); BAR=Bareness; LSD= Least Square of Difference; CV= Coefficient of Variation; GCV= Genotypic coefficient of Variation; PCV= Phenotypic coefficient of Variation; GA= Genetic advance; GAM= Genetic advance as percentage of mean; h^2= broad-sense heritability

Table 2. BLUPS values of the top ten DH lines relative to commercial checks

Name	GY	NPP	EPP	LL	LW	LA	BAR	Necrosis
DH_1_24	5.53	11.66	12.43	31.56	3.47	79.71	0.09	3.69
DH_1_67	5.17	11.71	12.49	31.09	3.47	79.64	0.09	3.95
DH_1_216	5.16	11.79	15.83	31.96	3.48	80.56	0.1	3.59
DH_1_17	5.13	13.1	16.68	33.79	3.48	81.99	0.09	3.94
DH_1_180	4.94	11.76	14.78	32.06	3.49	81.01	0.09	3.62
DH_1_35	4.92	11.2	14.89	31.58	3.49	81.26	0.1	2.92
DH_1_148	4.83	11.34	12.99	32.17	3.48	80.74	0.21	3.26
DH_1_110	4.82	11.76	12.47	30.48	3.47	79.23	0.09	3.93
DH_1_53	4.79	11.99	12.88	32.9	3.48	81.21	0.09	3.65
DH_2_43	4.78	11.97	13.2	32	3.48	80.5	0.09	4.01
Mean of top 10 DH lines	**5.0**	**11.8**	**13.9**	**32.0**	**3.5**	**80.6**	**0.1**	**3.7**
Commercial Checks								
CAH153	4.46	11.27	11.86	31.44	3.47	79.76	0.09	3.99
NK6240	4.1	11.54	12.02	30.39	3.47	79.01	0.1	4.27
Mean	**4.28**	**11.41**	**11.94**	**30.92**	**3.47**	**79.39**	**0.095**	**4.13**

Table 3. Phenotypic correlation coefficients among yield and component traits in cold condition at BISA Ludhiana (2019)

Traits	NPP	EPP	Bareness	LL	LW	LA	Necrosis
EPP	0.79**						
Bareness	-0.11	-0.09					
LL	0.31**	0.44**	-0.07				
LW	0.13	0.10	-0.03	0.09			
LA	0.22**	0.26**	-0.05	0.50**	0.90**		
Necrosis	-0.53**	-0.56**	-0.01	-0.40**	-0.04	-0.20	
GY	0.62**	0.69**	-0.02	0.57**	0.10	0.32**	-0.48**

The top 10 hybrids on average showed two ears increase in EPP compared to the mean of the commercial checks (12). Likewise, the top 10 hybrids on average showed 1.2 cm^2 increase in EPP and decrease in necrosis (-0.5) compared to the mean of the commercial checks. The mean of the DH genotypes for leaf necrosis based on biomass was 3.94. However, there was no difference between the top 10 hybrids and the commercial checks in NPP, LW and bareness (Table 2). Several authors delineated cold tolerance mechanism in controlled growth chambers, predominantly targeting on germination and early growth stages. Cholakova-Bimbalova & Vassilev [14] also observed reduced fresh mass accumulation, diminished net photosynthetic rate and chlorophyll content in maize under cold stress. Mahajan et al. [1] stated that cold stress causes yellowing of leaves which may be used as a selection criteria for cold tolerance at vegetative stage. Recovery of the plants and silk emergence in maize get affected due to yellowing of leaves [7].

3.4 Phenotypic Correlation Coefficients

Analyses of correlation coefficients provide a better understanding of the nature, extent and direction of selection to access secondary traits contributing to cold tolerance and grain yield of a maize genotype. Thus knowledge of trait association is of immense importance to reveal the cause of relationship among yield and its components for enhancing the usefulness of, selection. Correlation coefficients between pairs of yield and component traits are displayed in Table 3. All the characters that had significant phenotypic association with each other also showed significant genotypic relationship. Henceforth, only phenotypic correlations are discussed below. GY was positively and significantly associated with EPP (0.69), NPP

(0.62), LL (0.57) and LA (0.32) at both 5% and 1% levels of significances. EPP showed highly significant positive association with NPP (0.79) at 1% level of significance. Similarly, Leaf necrosis showed highly significant and negative association with EPP (-0.56), NPP (-0.53) and LL (-0.40). Though, our findings are in conformity with Revilla et al. [10] and Mahajan et al. [1] who reported similar significant relationships for agronomic traits under cold stress conditions. Mahajan et al. [1] found significant correlation between electrical conductivity and yellowing of leaves and thus, considered selection of genotypes with less yellowing. Kandel & Shrestha [6] also found positive and significant correlation between grain yield and growth traits like leaf length and leaf area.

4. CONCLUSION

It was observed that yield and component traits of maize genotypes were adversely affected due to early cold stress. The, morpho-physiological traits namely: leaf area, necrosis along with, grain yield play a predominant role in index selection of suitable germplasm for cold stress tolerance. Selection and development of early maturing, cold-tolerant corn hybrids have been considered, and they should be more economic, efficient, and, in cold conditions. The outstanding genotypes could be recommended for further evaluation for commercial use against the colder environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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