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Research Article

Traits of significance for temperature variability on cold tolerance of maize inbred lines under field conditions

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Abstract

In cool locations like Northern India, for hybrid maize, temperature is the crucial factor in yield and there are relatively few sources of corn that can withstand freezing or cold stress. Under field conditions, the tolerance/resistance of 200 inbred lines to low temperature stress was assessed. The relationship of weather fluctuations was studied on crop growth and cold stress parameters. During the first and second phases of the cold spell (stage I and stage II), inbred plants responded to the cold and frost by starting to yellow and dry their leaves. The findings showed that there was a lot of genetic variation for the tolerance/sensitivity-controlling characteristics. Sixty inbred lines demonstrated minimal leaf yellowing and healthy plant development in the presence of persistent cold stress. Thirty six inbred lines were found moderately tolerant and the rest of them were susceptible to cold/frost at less than 10°C. Among the resistant source inbred lines, the lines with the higher recovery of more than 50 % for yellowing of leaves as well as plant growth of more than 20 % respectively, among the selected resistant/ tolerant inbred lines include HKI 1352-2 and HKI 1348-6-2 of white maize, HKI 766, HKI 463 and HKI 1160 of normal maize and HKI PC 4B of popcorn. The inbred lines HKI 164-7-6 (0, 23.00), HKI 170 (1+2) (0, 41.02), and HKI PC 11 (0, 34.78) were least affected by the cold stress indicated that did not possess the impact of cold stress even in the adverse or suboptimal temperature as well as showed faster plant growth during growth stage II. Hence, the majority of the resistant inbred lines that displayed low leaf yellowing scores and good plant growth may therefore be used for growing in the winter season and also be employed in the development of cold tolerant hybrids.

Keywords cold stress, growth stage, inbred lines, maize, tolerant

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Introduction

In terms of both output and productivity, maize (*Zea mays* L.) is the world's most productive and widespread grain. Known as the queen of cereals, it can be used as a food, fodder, and an industrial crop. Both abiotic factors (drought, cold/frost, low nitrogen input, waterlogging) and biotic stresses (disease and pests) are the most important limiting factors that reduce maize productivity. In North India, winter maize is grown the month of October and November. In the winter season, the maize crop is exposed to cold stress, which more particularly affects young plants after emergence. According to the Compound Annual Growth Rate (CAGR) since 2001, area, production, and productivity have increased during both the *kharif* and *rabi* seasons. However, the growth rate was higher during the *rabi* period than during the *kharif* period. The productivity of maize is higher during *rabi* season because there is negligible insect pest and diseases during this season and secondly the temperature is mild during the grain development stage. Haryana can get very good grain yields during *rabi* season, but there is always the risk of cold/frost damage as the temperature many times reaches to zero degree.

Maize is classified as a cold sensitive species due to the relatively high temperature optimum for germination, development, and dry matter accumulation [1]. The growth of the maize crop is inhibited when exposed for an extended period of time to very low temperatures because it is susceptible to low temperature stress. Low temperature at planting greatly affects germination. During the vegetative phase, prolonged exposure to low temperatures causes leaf tissue to die, which causes yellowing of the leaf, chlorosis, and fire of the leaf tip. When cold stress occurs during the reproductive phase, it has a significant negative impact on flowering and causes reduced tassel size and branches that causes delayed anthesis, pollen grain mortality, smaller silk, and in some cases, no seed germination thereby, considerably affecting the yield and in extreme cases no grain yield at all i.e. cobs without grain.

Cold stress in maize has been shown to reduce leaf size, stem elongation, and root proliferation, disrupt plant-water relationships, and impair water uptake [2]. Researchers [3] also looked into the impact of low growth temperatures below (15°C) on maize's photosynthetic system. They have identified that eight genomic regions in all are extremely pertinent to the manifestation of the target traits. It has been difficult to adjust to spring climates with chilly, humid weather and this is due to late planting and breeding of early-maturing maize hybrids. Some key factors that are likely to be affected by cold stress and suboptimal temperatures include the consistency of time to flowering (this is critical in hybrid production regions where synchronization of the two parental lines is essential), grain filling, and delayed senescence (maintenance of post-flowering green leaf area), physiological maturity of grain and plant moisture at harvest (early), grain or silage yield.

These strategies aid in lowering the likelihood of field losses resulting from chilling stress [4]. An increase in chilling tolerance would enable earlier spring planting, which would result in higher yielding maize hybrids [5]. In addition, maize is chilling-sensitive in cool environmental conditions, making plant establishment in early spring difficult [6]. There are differences in maize genotypes for vulnerability to frost damage [7-9]. The growth of maize has been reported to be affected by temperatures between 0°C and -1.5°C, while the plants were severely harmed by temperatures between - 2°C and - 3°C [7]. Production of maize in cool climates requires the ability to withstand cold stress. Inbred lines used as parents to produce hybrid maize seeds are more susceptible to cold stress.

Considering the facts for getting good yield in *rabi* maize we should have cold tolerant hybrids. To create cold-tolerant hybrids, we require inbred lines that can withstand the cold. Therefore, the main purpose of this study was to assess the ability of inbred lines to withstand cold temperatures in field conditions during the winter.

Methodology

A total of two hundred inbred lines were evaluated in this study. The experiment was conducted during *rabi* season, 2017-18 in three replications in a randomized block design (RBD) at the research farm of CCS HAU Regional Research Station, Karnal. The experimental field's soil type is clay loam. Climate data collected throughout the trial period revealed that the minimum temperature in January was less than 10°C for more than a month and intermittently fell as low as 3°C. With 0.15 cm between rows, 0.60 m between them, and a 4m long single plot was used to plant each inbred line. Field observations were recorded for leaf yellowing and leaf drying (1 to 9 scales) at two stages. The first date of observations was immediately after the severe cold i.e. 10th January 2018 while observing the recovery of the maize plants on a second date was done when the temperature had somewhat risen i.e. on 30th January 2018. Leaf yellowing and dryness data were recorded on a scale of 1 (no yellowing/drying) to 9 (severe yellowing/drying) according to [10]. Plant growth was also recorded on a scale of 1 (poor plant growth) to 9 (excellent plant growth). Recordings are taken as a percentage index for the yellowing of leaves and plant growth to calculate percentage recovery for both traits. The highest rating of yellowing of leaves was taken towards more yellowing and the lowest rating of plant growth was taken towards poor growth because both the scales are opposite. The transformation was done by Percentage index for yellowing of the leaf and plant growth was calculated for both stages.

$$\%\ \text{index for yellowing of leaf} = \frac{\text{average of all ratings}}{\text{total number of plants X highest rating of yellowing}}\ \text{X}\ 100$$

$$\%\ \text{index for Plant Growth} = \frac{\text{average of all ratings}}{\text{total number of plants X lowest rating of plant growth}}\ \text{X}\ 100$$

Comparison of means of % index for yellowing of leaf and plant growth was done by simple ANOVA and paired t-test by OPSTAT software.

Results and Discussion

The optimum temperature required for maize roots, shoots, and leaf elongation is 30 -35o C. The growth of maize is completely inhibited between 6 and 8 °C temperature [1]. The average minimum temperature in the first two weeks, combined with short daylight hours (5.9 hours) and high wind speeds (2.5 km/h), can have a cumulative effect on plant cold sensitivity [10]. Changes in temperature, sunshine hours, and average wind speed were recorded throughout the cold January period (data provided by the Department of Meteorology at his CCSHAU Regional Research Station in Karnal). The average low temperature for the first two weeks was 5.0 °C, while the average temperature for the second week was 6.5 °C, slightly higher than the first two weeks Figure 1. The first two-week minimum temperature was below 5oC for eight days, while the second-week minimum temperature was recorded for only four days. In comparison, there was a difference between the average high temperature for the first two weeks (17.0°C) and the average high temperature for the second week (19.7°C). During the first fortnight average sunshine hours were observed lower in comparison with the second fortnight and average wind speed was observed higher in comparison with the second fortnight. During the second week, there was an increase in mean temperature (6.5°C), a decrease in sunshine hours (5.9), and an increase in wind speed (3.5) as depicted in Figure 1. In contrast to earlier studies of [10], there was not much variation in overall cold spells during the whole month in weather parameters except for the increase in minimum temperature, hence concluded that after two weeks, environmental conditions warmed somewhat and the plants showed recovery from cold symptoms and showed better growth even within the prevalent cold stress.

As depicted in Figure 2, It was observed that there was continuous and wide variation for the minimum temperature over the whole month and even in the day and night temperature also, consequently, variations in the link between temperature and damage are unclear, but they could be due to variations in the cold stress and that were in conformity with earlier findings of [7, 11] under controlled environment conditions. Long term cold stress affects the plant in numerous ways but leaf colour and plant growth were significantly affected. In maize, the inbred response to cold was represented by the onset of leaf yellowing and initiation of drying of leaves during initial data

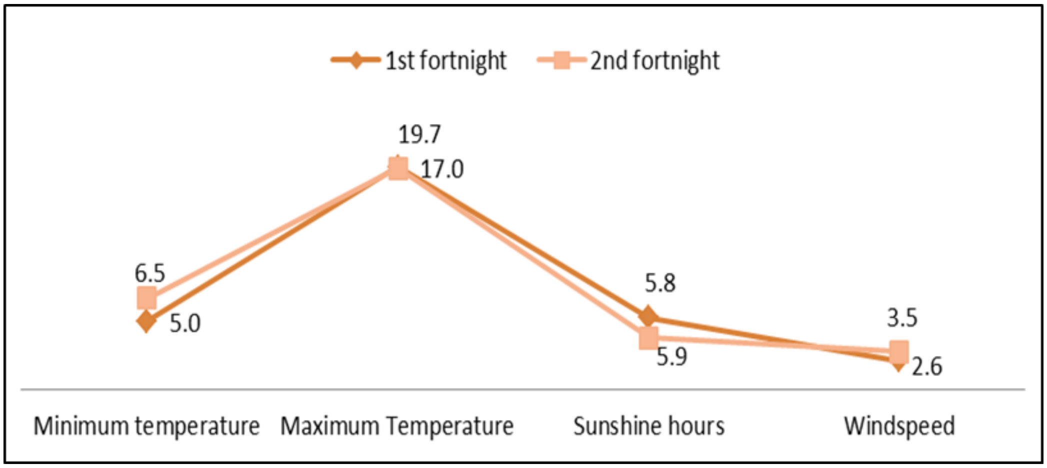

Figure 1. Average weather parameters during January 2018

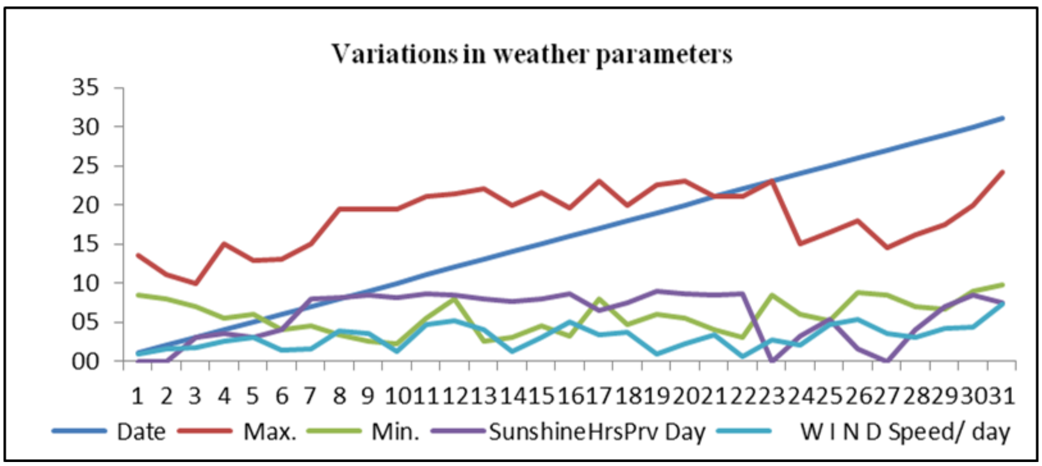

Figure 2. Description of variations in weather parameters during the whole month of January 2018

collection. The first recording showed the expression of the cold response of the inbred lines, whereas the second recording showed restoration of plant growth and reduced leaf yellowing. Comparing the best and worst types of inbreds, leaf yellowing showed significant differences between inbreds under cold stress. The greatest reduction in leaf yellowing due to new leaf development and accelerated plant growth was observed in early and mid-bred lines of different types of maize mainly in the second half (second fortnight) of the month and predicted as recovery rates (in percentage) which were found highest for cold resistant/tolerant inbred lines.

Similarly, the lines that have good growth even in the suboptimal temperature and later in the second fortnight, showed faster growth as compared to the first fortnight are collectively considered as cold resistant inbred lines. Also, on comparing the means of both the stages for both the traits i.e. Leaf yellowing I and Leaf yellowing II and Growth stage I and Growth stage II, t–test values were found significant which implies a significant difference between the means of the two stages (Table 1). Depending on the yellowing of leaves and crop growth stages sixty inbred lines of different types of maize were found resistant against cold/frost and showed less yellowing in the leaves with the highest growth rate and is shown in Figure 3. These lines showed recovery of yellowing of plants up to -5.79 to -79.64 % and plant growth up to 3.67 to 41.02 %, i. e. the leaves become green and plants showed faster growth in the second half of January when climate conditions became warmed as depicted from (Table 1). The negative values of recovery in cold stress indicated resistance, while positive values of recovery in plant growth indicated significant plant growth.

a. Initially non yellowing of leaves with good growth at Growth Stage I (Resistant/ Tolerant)

b. Finally non yellowing of leaves with good growth at Growth Stage II (Resistant/ Tolerant)

c. Initially average yellowing in leaves with average growth at Growth Stage I (Moderately Resistant)

d. Finally recovered from yellowing of leaves with good growth at Growth Stage II (Moderately Resistant)

e. Initially yellowing of leaves with poor growth at Growth Stage I (Susceptible)

f. Finally not recovered for yellowing of leaves and growth as well at Growth Stage II (Susceptible)

Figure 3. Different reactions of inbred lines to cold at Stage I and Stage II in the field. Stage I shows the status of plants immediately after cold spell and Stage II shows the status of plants after temperature started increasing

The lines with the higher recovery of more than 50 % for yellowing of leaves as well as plant growth of more than 20 % respectively, among the selected resistant/ tolerant inbred lines are as follows; viz., inbred line HKI 1352-2 (-50.38, 20.00) and HKI 1348-6-2 (-50.38, 21.36) of white maize, HKI 766 (-79.64 and 23.00), HKI 463 (-50.38, 34.78) and HKI 1160 (-50.38, 23.35) of normal maize and HKI PC 4B (-50.38, 28.38) of popcorn as depicted from (Table 1). The inbred lines that were least affected by the cold stress or do not even develops symptoms and remained dark green and hence showed faster plant growth during growth stage II include HKI 164-7-6 (0, 23.00), HKI 170 (1+2) (0, 41.02) and HKI PC 11 (0, 34.78) as depicted from (Table 1).

Table 1. Performance of superior maize inbred line for tolerance to cold expressed as percentage

SN.	Inbred Lines	% index for Leaf yellowing I	% index for Leaf Yellowing II	% Recovery	% index for Growth stage I	% index for Growth stage II	% Recovery	GYPP (gm)
White maize								
1	MBR 139	15.53	11.13	-39.52	19.18	21.68	11.53	36.2
2	HKI 1348-6-2	13.30	11.13	-19.46	19.18	21.68	11.53	31.5
3	HKI 1352	20.00	11.13	-79.64	18.33	20.00	8.38	37.6
4	HKI 1344	15.53	11.13	-39.52	18.51	20.83	11.11	34.7
5	HKI 1354-2	15.53	11.13	-39.52	19.27	20.00	3.67	37.2
6	HKI 1351-1-1	20.00	13.30	-50.38	18.09	20.94	13.64	31.8
7	HKI 1352-2	20.00	13.30	-50.38	17.34	21.68	20.00	31.5
8	HKI 1348T (W)	15.53	11.13	-39.52	18.33	20.83	12.00	38.7
9	HKI 1348-6-2	20.00	13.30	-50.38	17.04	21.68	21.36	38.0
10	HKI 1348-T	20.00	11.13	-79.64	18.09	20.00	9.57	39.4
11	HKI 766-2-WG	16.65	13.35	-24.72	16.20	20.00	19.00	35.5
Normal Yellow								
12	HKI 164-D-3-3	13.30	11.13	-19.46	20.00	20.83	3.96	34.2
13	HKI 164-7-2	13.35	11.13	-19.91	18.33	24.77	26.02	35.6
14	HKI 3-4-7	20.00	13.30	-50.38	20.00	24.77	19.26	31.8
15	HKI 332	15.53	11.13	-39.52	19.27	20.00	3.67	32.8
16	HKI 536YN	15.53	11.13	-39.52	18.51	20.00	7.44	39.5
17	HKI 164-3-2	15.53	13.30	-16.79	20.00	23.80	15.97	32.4
18	HKI 20 (3+4}	15.53	13.30	-16.79	20.00	23.80	15.97	33.6
19	HKI 295	20.00	11.13	-79.64	20.00	21.91	8.74	37.1
20	HKI 288-2	20.00	13.35	-49.81	20.00	20.00	0.00	40.2
21	HKI 536CBT	15.53	11.13	-39.52	17.04	28.90	41.02	32.5
22	HKI 1155-1-2	15.53	11.13	-39.52	19.06	20.00	4.71	36.2
23	HKI 1344	20.00	15.53	-28.76	20.00	23.80	15.97	35.5
24	HKI 1654	15.53	11.13	-39.52	20.00	20.00	0.00	34.4
25	HKI 1354-7	16.65	15.53	-7.19	18.33	23.80	23.00	33.0
26	HKI 1155	15.53	14.68	-5.79	18.09	20.00	9.57	38.5
27	HKI 1128	20.00	15.53	-28.76	18.09	20.00	9.57	40.1
28	HKI 766	20.00	11.13	-79.64	18.33	23.80	23.00	32.6
29	HKI 463	20.00	13.30	-50.38	16.68	25.57	34.78	31.8
30	HKI 1160	20.00	16.65	-20.12	20.00	20.00	0.00	34.5
31	HKI 139	15.53	11.13	-39.52	18.09	24.43	25.98	34.6
32	HKI 577	15.53	13.30	-16.79	20.00	20.00	0.00	36.2
33	HKI 1347	20.00	13.35	-49.81	19.06	23.80	19.93	34.7
34	HKI 1025	20.00	13.35	-49.81	19.18	23.80	19.43	35.7
35	HKI 1042	16.65	15.53	-7.19	18.33	23.80	23.00	34.2
36	HKI 1664	20.00	14.68	-36.24	18.51	20.00	7.44	34.0
37	HKI 1660	20.00	13.30	-50.38	18.20	23.74	23.35	37.1
38	HKI 1670	16.70	13.30	-25.56	18.51	28.90	35.95	34.5
39	HKI L287	15.53	11.13	-39.52	20.00	20.00	0.00	35.2
40	HKI 2067 (Y)	15.53	11.13	-39.52	20.00	20.00	0.00	36.9
41	HKI 1041-1	13.30	13.30	0.00	20.00	20.00	0.00	32.6
42	HKI C-141	20.00	16.70	-19.76	18.33	28.90	36.59	34.0
QPM								
43	HKI QPM 2015/20	20.00	13.30	-50.38	16.68	20.00	16.63	37.2

Continued Table 1.

44	HKI QPM 2015/25	20.00	13.30	-50.38	18.09	20.94	13.64	36.5
45	HKI 5072-2BT	20.00	16.65	-20.12	20.00	23.80	15.97	32.7
46	HKI 164-7-6	20.00	20.00	0.00	18.33	23.80	23.00	38.2
47	HKI 170 (1+2)	20.00	20.00	0.00	17.04	28.90	41.02	36.5
48	HKI 164-7-4	13.30	11.13	-19.46	18.09	24.43	25.98	31.8
19	HKI 163	20.00	15.53	-28.76	20.00	20.00	0.00	39.4
50	HKI 165	15.53	11.13	-39.52	20.00	20.00	0.00	38.8
51	HKI 161	20.00	13.35	-49.81	18.09	20.00	9.57	39.2
52	HKI 193-1	20.00	15.53	-28.76	16.20	20.00	19.00	40.0
53	HKI 193-2	15.53	11.13	-39.52	20.00	25.57	21.77	39.5
54	HKI 164-4 (1-3)	20.00	15.53	-28.76	20.00	20.00	0.00	34.2
55	HKI 194-6	13.30	11.13	-19.46	17.04	24.77	31.19	32.8
Popcorn								
56	HKI PC 8	15.53	13.30	-16.79	18.33	20.00	8.38	34.6
57	HKI PC 3	20.00	13.35	-49.81	19.27	20.00	3.67	32.5
58	HKI PC 4	20.00	11.13	-79.64	18.09	20.00	9.57	33.8
59	HKI PC 4B	20.00	13.30	-50.38	17.04	23.80	28.38	32.8
60	HKI PC 11	20.00	20.00	0.00	16.68	25.57	34.78	34.6
	Mean	**17.69**	**13.25**	**-35.49**	**18.64**	**22.23**	**14.98**	**35.38**
	SD± SE	**2.52±0.32**	**2.33±0.30**	**13.53±2.62**	**1.15±0.15**	**2.64±0.34**	**11.41±1.47**	**-**
	t value	**54.47***	**44.05***	**13.53***	**126.00***	**65.36***	**10.16***	**-**

These lines do not even develop symptoms of cold stress, for both or either of the traits was found highly tolerant/resistant to cold stress, indicating that these lines are tolerant to cold stress upto that extent that did not possess an impact on crop growth and yellowing of leaves even in the adverse or suboptimal temperature. The results coincide with the earlier findings of [10, 12]. Further, these lines also possess good grain yield per plant ranging between 32.5 to 40.2 gm.

Among the total lines, thirty six inbred lines were found moderately tolerant against cold/frost, and these lies in the range of 5.0-6.50 for yellowing of leaves and moderate growth rate (5-6) in Ist fortnight and IInd fortnight as depicted from (Table 1). However, moderately tolerant inbred lines developed some recovery in the yellowing of leaves but the growth stage was not to that extent when compared with resistant lines (Figure 3). These lines recorded average grain yield per plant ranged from 25 to 29.6 gm.

Moderately susceptible inbred lines showed no signs of normalization in either trait as depicted in Figure 3. However, in some cases, cold initially showed yellowing of the leaves (the leaves become pale), followed by drying of the leaf from the tip where it develops to the main shoot, which caused a complete drying of the whole plant and was considered to be the sensitive inbred line. The drying effect was so strong that the completely dried plant could not recover even if the weather conditions improved in the second two weeks of the month or later Figure 3. In addition to the visual score of leaf yellowing, cold stress strongly affected the variability of developmental traits such as plant growth.

Also, the grain yield per plant recorded was very poor ranging from less than 20gm. This conforms with the interpretations made by Lee et al., [5] where they revealed that in inbred lines of maize, seedlings cultivated under cold stress (15/3°C; 16-hour photoperiod) developed leaves at a rate that was around three times slower than that of seedlings grown in normal conditions (25/15°C; 16-hour photoperiod). Performance of an excellent maize inbred line for cold resistance expressed as percent leaf yellowing index (1-9 scale) and percent growth stage index.

Correlation studies showed that there is a significantly high correlation between leaf yellowness and plant growth at both stages (Table 2). It was observed that leaf yellowing stage I had a strong and negative relationship with plant growth (–0.523** and –0.368*) in both stages. For two populations of maize that are acclimated to the Central US Corn Belt, similar outcomes have been found by Mock and Eberhart [13] also for 144 plant introductions of maize studied [14]. Also in the II stage of leaf yellowing, there was a significant negative relationship with plant growth in both phases (-0.439** and -0.299*), but the strength is slightly lower than in stage I of leaf yellowing. The results are in agreement with the earlier findings of [12]. Therefore, it was concluded that leaf yellowness is a mandatory criterion and had a high correlation with plant recovery, also expressed as yield. It was imperative that genetic variation is essential and exists in some of the key physiological functions and developmental stages affected by adverse temperature

Table 2. Association between leaf yellowing and plant growth at two stages

	Leaf yellowing I	Leaf yellowing II	Growth stage I	Growth stage II
Leaf yellowing I	1.000	0.900**	-0.523**	-0.368*
Leaf yellowing II		1.000	-0.439**	-0.299*
Growth stage I			1.000	0.818**
Growth stage II				1.000

and cold stress. It was also supported by earlier findings of [15] who reported that below 10 °C drop in temperature, genetic variations exist for cellular and tissue injury.

Conclusion

From this, we can conclude that the assessment of resistance should be prioritized as the primary selection indicator for yield potential for more efficient selection of cold stress resistance. The essential conception behind improving performance under cold stress is to accumulate advantageous alleles in elite genetic backgrounds. The idea also relies on the ability to detect and analyze genetic variation for resistance traits of various components. Thus, by choosing resistant inbred lines that tolerate cold stress, particular physiological processes or developmental stages have been found that change during growth at suboptimal or inadequate temperatures. Hence, most of the resistant inbred lines with less leaf yellowing and good plant growth are promising for winter cultivation and can also be used to develop cold-tolerant hybrids.

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