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Cold tolerance of maize inbred lines at the seed germination stage

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Abstract
Maize cultivation in northern regions is limited by the crop's sensitivity to low temperatures. The optimal temperature for its germination and early growth is around 30 °C, making cold stress a key barrier to early sowing. In the context of climate change, improving cold tolerance during germination is essential, particularly at higher latitudes, to support earlier sowing and avoid yield losses caused by summer drought and heat stress. Earlier flowering and reduced grain moisture at harvest are major agronomic advantages of early sowing. This study, conducted at the Agricultural Institute of the HUN-REN Centre for Agricultural Research (Hungary), aimed to evaluate cold tolerance among 56 genetically diverse maize inbred lines, including reference lines W401 (cold-tolerant) and W64A (cold-sensitive). Phenological traits were measured under controlled cold stress conditions. Several lines demonstrated strong cold tolerance, with a percentage of emergence (PE) > 85%, days from sowing to emergence (DSE) < 22 days, and cold tolerance index (CTI) values between 3 and 3.9. These inbred lines can represent promising candidates for future breeding programs targeting cold resilience.

Keywords Cold tolerance · Different genetic backgrounds · Early development · Maize inbred lines · Phenotypical characteristics

Abbreviations

HMv Exp	Hungary, Martonvásár experiment
BSSS	Iowa stiff stalk synthetic
RYD	Reid's yellow dent
EU Dent	European dent line
MYD	Mindszentpuszta yellow dent
CTI	Cold tolerance index
DM	Dry matter content
PE	Percentage of emergence
DSE	Days from sowing to emergence
SPAD	Soil plant analysis development

Introduction

The importance of maize production continues to grow due to the expanding market and the increasing demand for maize for non-food uses (e.g., bioenergy) (Rosegrant et al. 2009). Today, maize is one of the most widely cultivated crops, with an area of 208 million hectares, and it was the second largest cereal crop after wheat in 2023(FAO).

As a plant of tropical origin, maize tolerance to lower temperatures, in northern growing regions, is still a major problem due to low temperatures and water scarcity (Zydelis et al. 2018). Simulation models and reality predict a reduction in maize yield of 1–6% for every 1 °C increase in temperature (Xu et al. 2016; Wu et al. 2021). Climate change, new varieties, and a short vegetation period have made it possible, while growing demand has encouraged the spread of maize cultivation to northern regions with a cooler climate (Soane et al. 2012; Spiertz 2014). Early sowing requires a high germination ability and vigorous early development at lower temperatures (Revilla et al. 2000).

Temperature and water availability are the most important factors influencing the growth and development of maize, such as the basic conditions for germination are sufficient

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moisture, appropriate temperature, and oxygen (Marton and Zsubori 2017; Nagy 2021). The seed can also germinate at a temperature lower than the theoretical minimum temperature (10 °C) required for germination, but in this case the vigor of the seedlings is weaker, germination takes longer (Marton and Zsubori 2017), and the developmental lag may persist until the end of the growing season (Gombos 2021).

Over the past hundred years, several studies have been conducted to determine the cold tolerance in maize during germination in moist, cold soil (Meyers 1924; Hund et al. 2008; Guan et al. 2009; Marton et al. 2013; Meng et al. 2025). Several authors have found that germination in cold soils was largely influenced by the age of the seed and the conditions of seed production (early frost, maturity at harvest, harvesting method, seed purity, grading). Based on these, for cold tolerance tests, it is advisable to use seed grown in the same year, in the same place, under the same conditions (Szundy et al. 2005). Since germination speed is affected by temperature and humidity (Wang et al. 2018; Hund et al. 2008), reliable measurement of laboratory germination rate—typically assessed on day four (Nagy 2021)—requires strictly controlled conditions. According to Sánchez et al (2014), the minimum temperature required for maize to grow and develop is 6.2 °C, while Fischer et al. (2014) defined it as 8 °C. All phenological phases of maize are characterized by slow development at 10 °C (Varga-Haszonits et al. 2006).

There is usually a large difference between laboratory and field germination ability (the percentage of seeds germinated within a given time: 16 days), which is greater the colder the soil in which the maize is sown (Nagy 2021).

The initial development of plants is prevented by the appearance of animal and plant pests in the cracks on the pericarp (Roundhawa 1990). Seeds that germinate in cold soil are often attacked by fungal infections (*Fusarium sp.*) (Dinolfo et al. 2022; Czembor et al. 2024). If the seed is placed in moist, cold soil, germination becomes difficult (Meng et al. 2025), and the severity of infection increases (Marton et al. 2015). A study by Marton et al. (2013) confirms that the poorer germination in the field compared to phytotron values was largely due to the occurrence of pathogens, as soil-dwelling fungi usually do not cause damage when germinated in sterile conditions in the laboratory. This is why the so-called cold test type of cold germination method were gradually introduced in the USA from the beginning of the twentieth century (Marton 1990a; Nagy 2021) The idea is that after a shorter cold incubation [5–10 days at 8–10 °C according to Marton and Zsubori (2017), 7–10 days at 6–8 °C according to Nagy (2021)], the seeds are placed at optimal temperature, under conditions that promote rapid germination. A method based on combining the two temperatures is suitable for detecting differences between genotypes. Marton (1990a) also describes in his thesis that the result of the cold test is strongly influenced by four external factors: seed dressing, the duration of the cold test, the temperature applied, and the germination medium. (The positive effect of seed dressing on germination percentage was also demonstrated by Záborszky et al. (2001). On the other hand, the results are influenced mainly by seed quality and to a lesser extent by genotype (Marton 1990a). Even under sterile conditions, the germination rate decreases with increasing length of cold incubation (Kovács 1961).

Climate change is expected to increase the intensity and frequency of both abiotic and biotic stress factors (Salika and Riffat 2021). With the average temperature rising and the growing demand for food by the world's population, climate change is an indication that crops will have to be grown under less favorable conditions (Janda 2023). High temperatures have been identified as detrimental to crop yields (Schlenker and Roberts 2009; Zhao et al. 2017). Therefore, it is important to breed maize hybrids that can adapt to extreme weather conditions. Chen et al. (2024) found that full-season hybrids are more adaptable to critical conditions than the short-season hybrids. Early sowing dates—needs cold tolerance—for maize are advantageous (Mock and McNeill 1979), because the most sensitive phenological phase, flowering and fertilization, can thus be prevented from hot, summer days. Heat around flowering strongly impacts maize yield (Nikolič et al. 2020; Ning et al. 2023).

Hypothesis: Inbred lines from various genetic backgrounds show different cold tolerance. In addition to the existing, known lines, we are seeking new, cold-tolerant inbred lines that can serve as parent components of early-maturing, cold-tolerant hybrids or as valuable source materials for the development of cold-tolerant inbred lines.

The new cold-tolerant maize lines enable the northward expansion of maize cultivation in Europe and earlier planting dates. The earlier sown cold-tolerant maize produces higher yields with lower grain moisture, ultimately resulting in increased profitability.

Materials and methods

Inbred lines tested

More than fifty (56) genetically diverse maize (*Zea mays* L.) inbred lines –from Flint, RYD, Lancaster, Iodent, MYD heterotic groups (Table 1) -were tested at the HUN-REN Agricultural Institute of the Centre for Agricultural Research, in Phytotron. These inbreds are part of a larger, unevaluated sample of nearly 200 samples. Some of the inbred lines

Table 1 Origin of the 56 maize inbred lines

Inbred	Genetic background	Pedigree	Reference
CO158	Miscellaneous	DEKALB 46	
A654	Minnesota 13 complex	A116 x WF9	Dubreuil et al. 1996
A96	Minnesota	A48xH	Schaefer 2013
F107		F115xFv174	Henderson 1984
F186		F108TR	Henderson 1984
F244		F188 x F186	Pollacsek 1992
F271	DENT	50% CO125, 50% W103	Burstin et al. 1994
W401	EU DENT Minnesota 13 complex	(W33 x W25) x W67C	Pollacsek 1992; Messmer et al. 1992, 1993; Bar-Hen et al. 1995; Dubreuil et al. 1996; Smith et al. 1985
HMv Exp 6401	DENT		
HMv Exp 6252	EU DENT		
F259	EU DENT	W401 x CO169	Messmer et al. 1992, 1993
HMv Exp 6079	FLINT	CM7 rel	
HMv Exp 5173	FLINT	CM7 rel	
A34	FLINT		
HMv Exp 62,047	FLINT		
B6	FLINT		Russell and Vega 1973
D503	FLINT	Syn. PF75 (50%), D102 (25%), DK105 (12.5%), F6B.Scag (12.5%)	Messmer et al. 1992
EE-583–127	FLINT		
HMv Exp 6016	FLINT		
F257	EU FLINT	F120 X F192	Messmer et al. 1992, 1993; Dubreuil et al. 1996
F432	FLINT	Blanc Chalosse x Hybrid	Henderson 1984
F286	FLINT (early)	F7 x F564	Barrière et al. 2008
F2	FLINT (Lacaune)		Messmer et al. 1992, 1993; Burstin et al. 1994; Dubreuil et al. 1996
EP1	FLINT (Lizargarate)		Haberer et al. 2020; Revilla et al. 2002; Schaefer 2013; Messmer 1993
CM7	FLINT (Ottawa) Minnesota 13 Complex	W85xCMV3	Dubreuil et al. 1996, Dubreuil and Charcosset 1999
HMv Exp 14-IO-43–103	IODENT		
HMv Exp 17–30-47–125	IODENT		
HMv Exp 25–41-040–80	IODENT		
HMv Exp 37–73-S8	IODENT		
HMv Exp 5112–040-80-ET-S7	IODENT		
HMv Exp ST-6–4	IODENT		
A619	LANCASTER Sure Crop	(A171xOH43)xOH43	Dubreuil and Charcosset 1999; Smith et al. 1985
HMv Exp 6227	LANCASTER	MO17 rel	
HMv Exp 6407	LANCASTER	MO17 rel	
C103	LANCASTER		Lu and Bernardo 2001; Smith et al. 1985
156	MYD		
HMv Exp 0118A-124A_	MYD		
HMv Exp 0118A-124A	MYD		
W64A	RYD Wisconsin	WF9 × 187–2	Pollacsek 1992; Mertz et al. 1964; Schaefer 2013
HMv Exp 73–30-E-UT	RYD		
HMv5395	RYD		
HMv Exp 73–43-103-WX	RYD		
A632	RYD	(Mt42 x B14) x B14^{3}	Pollacsek 1992; Dubreuil and Charcosset 1999; Dubreuil et al. 1996
A632waxy	RYD		
HMv Exp 61028A	RYD		
HMv Exp 6006	RYD		
HMv Exp 6795	RYD		
DH105	RYD		

Table 1 (continued)

Inbred	Genetic background	Pedigree	Reference
CM105	RYD/ BSSS	(V3 x B14) B14	Reid et al. 2011; Gethi et al. 2002; Dubreuil et al. 1996
CM105waxy	RYD /BSSS		
CM174	RYD/ BSSS	(V3 x B14) B14	Reid et al. 2011; Dubreuil et al. 1996;
B37	RYD/ BSSS C0		Melchinger et al. 1991; Russell and Vega 1973; Dubreuil et al. 1996; Lu and Bernardo 2001
B73	RYD/ BSSS C5		Melchinger et al. 1991; Gethi et al. 2002; Reid et al. 2011; Lu and Bernardo 2001
B14	BSSS		Melchinger et al. 1991; Russell and Vega 1973; Lu and Bernardo 2001
HMv Exp 6195	unknown		
CH300-M-T-A	unknown		

mentioned are well-known older varieties, making it interesting to compare their results with those of more recent inbred lines.

Experimental design

The experiment involved 56 inbred lines, which were placed in boxes measuring 60 cm × 40 cm × 15 cm, arranged in a randomized design. Each box contained twenty inbred lines, with ten seeds per genotype. For comparison, two inbred lines were included in each box: one cold-tolerant line, W401, and one cold-sensitive line (W64A) as referenced by Mauro et al. (2005). The experiment was performed in two replicates.

From day 10 onwards, growing conditions were 14 h with light at 300 umol m-2 s-1 light intensity, and 10 h without light. Irrigation was applied with tap water immediately after sowing, maintaining a constant water capacity of 70%. Water losses were continuously replenished. The lighting and irrigation conditions were the same in both chambers, while humidity levels were not controlled. The growing medium was brown chernozem soil, sterilized at 100 °C for 24 h. No additional soil parameters were monitored.

Determination of percentage of emergence (PE), days from sowing to emergence (DSE), and cold tolerance index (CTI)

We recorded seedling emergence percentage (PE) daily through visual inspection. Once seedlings reached approximately 1 cm above the soil surface, daily observations were continued until all genotypes reached their final germination.

The days from sowing to emergence (DSE) were computed as a weighted average of days. It was calculated using this formula:

$$X\left(average\right)=\frac{x_1\ {}_1+x_2p_2+\cdots+x_np_n}{\sum_{i=1}^{n}x}$$

$$X\left(average\right)=\frac{\sum_{i=1}^{n}x_ip_i}{\sum_{i=1}^{n}x_i}$$

where x = the number of seedlings, and p = the days from the sowing.

Cold tolerance index (CTI) for each inbred line in the cold experiment was determined following the method of Herczegh (1978) using the formula:

CTI = *PE* /**DSE**.

At the end of the experiment, fresh and dry shoot weight (dried to mass consistency, 100 °C for 48 h) and SPAD values of leaves at the 3–4 leaf stage were measured.

Determination of germination temperature

Herczegh (1970) developed the cold test method to study low-temperature adaptation. The author noted that evaluating genetic and physiological cold tolerance becomes crucial when environmental temperatures exceed 10 °C by a few degrees and germination begins. Under these conditions, seedling emergence may demonstrate considerable variability and an extended duration. Therefore, Herczegh set the experimental temperature at 13–14 °C following cold incubation at 8 °C, as this temperature interval exhibited the greatest variability among the inbred lines in terms of cold tolerance, making it an optimal choice for discriminating differences in cold response.

Following this method, seeds were incubated at 8 °C for 10 days and then at 13.5 °C for 30 days. During this period, germination and the initial development of the plants were observed until they reached the 3–4 leaf stage.

Control measurements were taken in a PGV-36 chamber, which was kept at a constant 23 °C. Additionally, a cold tolerance experiment was conducted within the same PGV-36 chamber.

Table 2 Analysis of variance for the main characteristics of the cold tolerance: percentage of emergence (PE), days from sowing to emergence (DSE), and cold tolerance index (CTI) of inbreds by treatment

Factor	df	F-value		
		PE	DSE	CTI
Inbred lines	55	4.08***	1.45 $^{+}$	2.66***
Treatments	1	415.20***	4879.98***	4067.49***
Inbred lines x Treatments	55	2.75***	0.87 ^{NS}	0.73 ^{NS}
C.V. [%]		15.56	11.62	15.77

***Indicates significant difference at $P<0.001$ level. $^{+}$ indicates significant difference at $P<0.05$. NS = no significant difference

Statistical analysis

For the statistical analysis of the obtained data, we utilized Agrobase 99® for Microsoft Windows® (Agronomix Inc.), the Randomized Complete Block Design (RCBD) ANOVA analysis technique, and Microsoft Excel for data management. The results of the ANOVA are presented in Table 3. A two-factor ANOVA was employed to estimate the significance of differences in the interactions between inbred line and treatment (climate program). Additionally, we performed a two-variable linear regression to determine the correlation coefficient between traits across different treatments (Sváb 1981).

Results

Germination was delayed and reduced in cold conditions. Considerable variation was observed among the inbred lines regarding this trait. For the evaluation of cold tolerance, the most relevant physiological parameters are percentage emergence (PE) and days from sowing to emergence (DSE) (Table 2).

Significant differences were found between treatments for all tested traits, except dry matter content (DM).

Only 58.7% of the plants germinated at low temperature, which is 34% less than the number of plants that germinated in the control trial. Emergence rates showed notable differences among lines in the cold-test study (Fig. 1). The PE reflects the response of specific maize inbred lines to low temperature stress. In our cold stress experiment, inbred line C103 exhibited a markedly reduced germination rate (5% under cold versus 70% under optimal temperature), while inbreds HMV EXP 6401, W401, A619, and A632 maintained germination rates exceeding 90% in both cold and warm conditions (Fig. 1).

In the low-temperature study, the emergence time was significantly extended compared to the control conditions. At a constant temperature of 23 °C, the average germination time was 7.5 days. In contrast, under cold stress, germination occurred on average after 25 days (Fig. 2). The shorter the emergence period, the greater the inbred line's tolerance to early cold stress. Specifically, the cold-tolerant reference line W401 germinated in approximately 25 days, whereas the cold-sensitive W64A line required nearly 27 days to germinate, representing a delay of about 2 days (Fig. 2).

The inbred lines W401, HMv Exp 6401, HMv Exp 5173, CM7, F271, HMv Exp 0118A-124A, A619, and A632 exhibited higher CTI values in both treatments. These lines exhibited a higher number of germinating individuals within a shorter time frame compared to the other tested lines, indicating a promising cold tolerance phenotype (Fig. 3).

Based on the parameters evaluated for cold tolerance, among the top 10 maize inbred lines exhibiting the highest

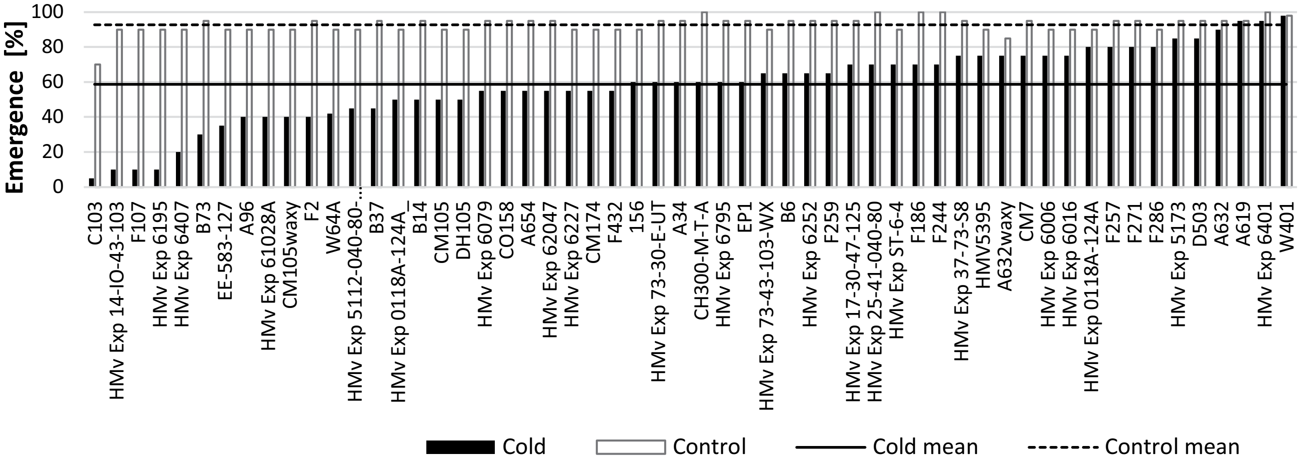

Fig. 1 Development of emergence percentage (PE) in maize inbred lines under cold stress compared to control conditions. PE values under cold conditions are depicted by black bars, while control values are shown by white bars. The mean PE values for the cold and control treatments are indicated by solid and dashed lines, respectively. Highly significant differences were observed between lines ($LSD_{5\%}$ = 16.6935***) and between treatments ($LSD_{5\%}$ = 3.1548***). Data from the cold treatment are sorted in ascending order. (*** indicates significance at $P<0.001$)

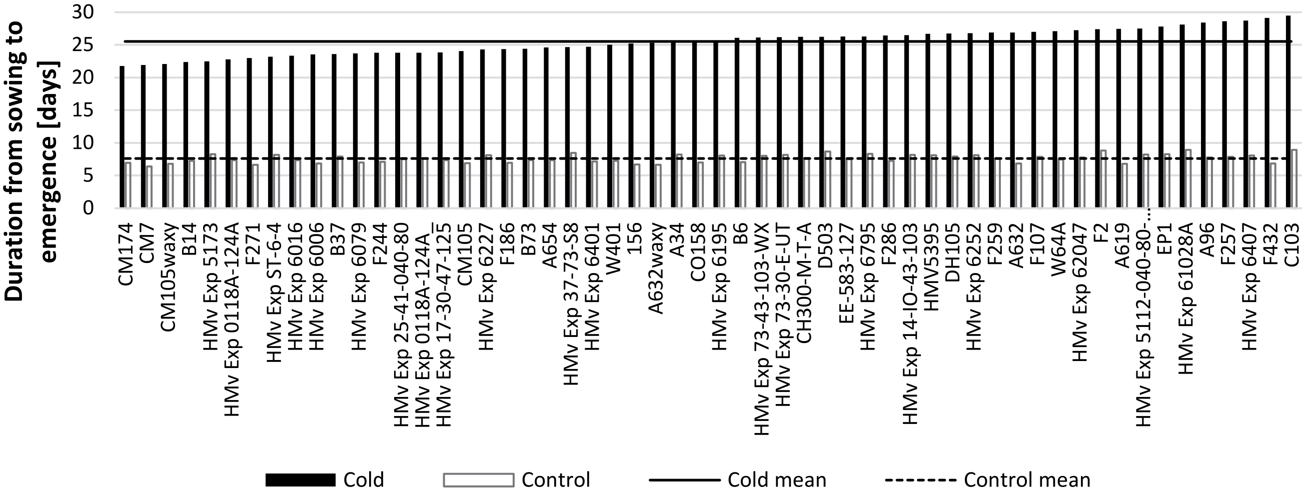

Fig. 2 Days from sowing to emergence (DSE) in maize inbred lines under cold and control conditions. DSE values under cold conditions are depicted by black bars, while control values are shown by white bars. The mean DSE values for the cold and control treatments are indicated by solid and dashed lines, respectively. Germination time ranged from 6 to 9 days in the control (23 °C) and 21–29 days in the cold treatment. The difference between treatments was statistically significant ($LSD_{5\%}=0.5082^{***}$), while a significant difference was observed between lines ($LSD_{5\%}=2.6891^{+}$). (*** indicates significance at $P<0.001$; $^{+}$ indicates significance at $P<0.05$)

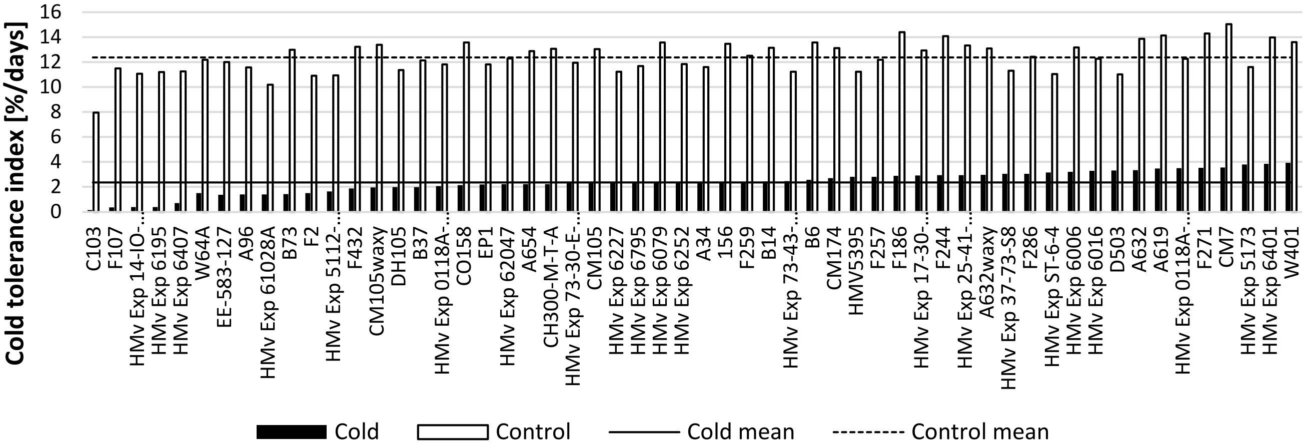

Fig. 3 Cold Tolerance Index (CTI) of maize inbred lines under cold stress and control conditions. CTI values under cold conditions are depicted by black bars, while control values are shown by white bars. The mean CTI values for the cold and control treatments are indicated by solid and dashed lines, respectively. Higher CTI values reflect increased tolerance to low-temperature stress. Statistically significant differences were identified both among inbred lines ($LSD_{5\%}=1.6420^{***}$) and between treatments ($LSD_{5\%}=0.3103^{***}$), highlighting substantial genetic variation in cold tolerance among the evaluated maize lines

cold tolerance, five belong to the Dent type, four to the Flint type, and one to the Lancaster heterotic group (Table 3).

For each inbred line, the SPAD measurement was calculated as the average of readings taken at three distinct points on the leaf surface of each individual plant, adjusted for leaf surface area. Significant differences between inbred lines were observed ($LSD_{5\%}=9.6646^{***}$), although these results should be interpreted with caution due to the limited measurement area. The relative chlorophyll content among the lines ranged from 11 to 31.5 SPAD units.

A correlation analysis was conducted to evaluate the relationship between SPAD values and dry matter yield under cold stress conditions (Fig. 4).

Our results support that the chlorophyll content moderately reflects the physiological status of the plants.

Discussion

The findings above are interpreted in the following discussion.

Maize is a strategically important and widely cultivated crop worldwide. Known for its unique adaptability and usability. Due to its starch content, it is an essential food and feed source, just as it is a raw material for ethanol production. Maize is a tropical-origin, C4 plant. It requires heat

Table 3 Summary table presenting the key traits of the ten best-performing inbred lines

Inbreds	HETEROTIC GROUPS	PE		DSE		CTI	
		Cold conditions	Control conditions	Cold conditions	Control conditions	Cold conditions	Control conditions
HMv Exp 6016	Flint	75	90	23.32	7.34	3.29	12.26
D503	Flint	85	95	26.26	8.65	3.32	11.02
A632	RYD	90	95	26.90	6.85	3.34	13.87
A619	Lancaster	95	95	27.46	6.79	3.47	14.14
HMv Exp 0118A-124A	MYD	80	90	22.81	7.34	3.50	12.27
F271	DENT	80	95	22.97	6.66	3.54	14.29
CM7	FLINT	75	95	21.94	6.40	3.56	15.05
HMv Exp 5173	FLINT	85	95	22.46	8.25	3.80	11.61
HMv Exp 6401	DENT	95	100	24.71	7.15	3.85	13.99
W401	DENT	98	98	25.00	7.27	3.94	13.59
$\mathrm{LSD}_{5\%\mathrm{INBREDS}}$		16.6935***		2.6891 $^{+}$		1.6420***	

The most relevant traits for cold tolerance, namely PE (percentage of emergence), DSE (days from sowing to emergence), and CTI (cold tolerance index), were compared. The statistical evaluation was conducted on the entire experimental dataset, and no significant differences were observed among the highlighted maize lines listed in the table regarding PE. *** indicates significant difference at $P<0.001$ level. $^{+}$ indicates significant difference at $P<0.05$

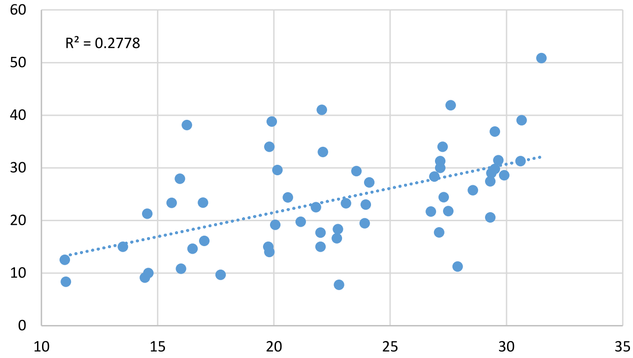

Fig. 4 Relationship between SPAD values (relative chlorophyll content) and dry matter yield (mg) in maize inbred lines. A moderate correlation was observed. * indicates significance at $P<0.05$. Correlation levels were interpreted according to Guilford (1950). Absolute value of R: <0.19—Slight; almost no relationship; 0.20–0.39—Low correlation; definite but small relationship; 0.40–0.69—Moderate correlation; substantial relationship; 0.70–0.89—High correlation; strong relationship; 0.90–1.00—Very high correlation; very dependable relationship

Table 4 The mean values of the days from sowing to emergence (DSE) of the cold-treated heterotic groups compared with the results of an earlier cold tolerance study (Herczegh 1983)

Heterotic groups	RYD	Iodent	Flint	MYD	Lancaster
DSE	23.4	23.8	23.8	24.1	24.5
Herczegh (1983)	22.1	–	–	–	24.7

in all phenological phases for optimal development, but can adapt to various climates too. The seedling stage is one of the most sensitive phenophases, when the maize is yet not prepared to tolerate the negative effects of low temperatures, below 10 °C, can presumably only be ensured by a narrow genetic background. Our research aims to select individuals with optimal cold tolerance parameters, based on phenotypic and physiological traits during early development, to enhance the efficiency of genetic mapping. European varieties, particularly those in the ‘Flint’ category, tend to be more tolerant of cooler temperatures compared to tropical varieties (Burnett and Kromdijk 2022). As a result, ‘Flint’ lines are frequently utilized in maize breeding programs in northern Europe to enhance chilling tolerance (Riva-Roveda et al. 2016). Our results confirm the authors’ finding that inbred lines belonging to the “Flint” group generally have good cold tolerance. Interestingly, however, we found several lines in the “Dent” heterosis group had higher cold tolerance than some Flint lines. While most “Flint” cold-tolerant maize lines were selected under climatic conditions characterized by a long, cool spring period, the “Dent” cold-tolerant lines, adapted to climates with a short cold period, exhibit the advantage of rapid growth following this brief cold spell.

According to the literature (Marton 1990b), CM174 is the most cold-tolerant maize inbred line based on earliness.

Herczegh (1983) determined the DSE for heterotic groups at low temperature: at Lancaster elite lines in 24.7 days, at Reid Yellow Dent elite lines in 22.1 days, and at the European-originated lines in 22.4 days. In our experiment, the DSE for the different heterotic groups is presented in Table 4.

The most important indicator of cold tolerance is the CTI, which provides crucial information about a plant’s response to low temperatures. The CTI is designed to better predict potentially problematic seed lots (Burris and Navratil 1979).

The higher the numerator (PE)—and the lower the denominator (DSE)—the higher the CTI value, i.e. the better the cold tolerance of the line, even though the information on days from sowing to emergence and percentage of emergence are separately greater than the CTI (Marton 1990b).

In Fig. 3, the position of the lines used as standards (W401 and W64A) confirms the characteristic described in the literature, their adaptation to low temperatures, that W401 is a cold-tolerant line (Csepregi-Heilmann et al. 2023), and W64A is a cold-sensitive line (Challou et al. 1998; Mauro et al. 2005).

The dry matter accumulation of young seedlings was additionally evaluated to assess early biomass development. Normally, young seedlings contain about 10% dry matter (Marton et al. 1997). A higher dry matter of seedling shoot content indicates a disorder. The dry matter content of the plant provides information on its health status (Marton et al. 1997). Among the inbred lines evaluated, several exhibited robust responses to cold stress with dry matter content below 10% (F107, W401, HMv Exp ST-6–4, HMv 6401), whereas one inbred line demonstrated a notably higher dry matter content approaching 25%. Based on this assessment, the genotype F186 appears to have been adversely affected by low temperature stress.

Chlorophyll content can also indicate the plant's response to temperature extremes. Leaf chlorophyll content at the 3- to 4-leaf stage was quantified using a Minolta SPAD-502 chlorophyll meter, which measures relative chlorophyll concentrations and assesses nitrogen status (Széles 2007; Zotarelli et al. 2003) over a 6 mm^2 leaf area (Scharf and Lory 2002).

Our investigations revealed that the inbred lines exhibited differential responses under distinct environmental conditions (cold stress vs. control). Furthermore, the ranking of lines varied across traits and treatments, indicating genotype-by-treatment interactions (Table 5).

The control and cold treatments for DM showed low correlation (Tab.4). For the main traits (PE, DSE, CTI), a moderate correlation with a significant association was found between the treatments. Therefore, it is necessary to study further cold tolerance under cold conditions.

Today, genome-wide association studies (GWAS) in maize have made significant progress by identifying numerous genetic loci and potential genes associated with complex traits, including responses to both abiotic and biotic stresses (Sahito et al 2024). Recent research on maize cold tolerance has primarily concentrated on genetic mapping and selection breeding. However, limited studies specifically examine the phenotypic and physiological traits associated with chilling tolerance, particularly during the seedling stage.

According to Shikha et al. (2023), using growth chambers for small-scale studies at low temperatures is a reliable evaluation method. Although these conditions may not be suitable for large-scale germplasm screening in breeding programs due to high costs and labor requirements. Yi et al. (2021) evaluated a large association panel of 836 maize inbreds. They identified 187 significant single-nucleotide polymorphisms (SNPs), which were incorporated into 159 quantitative trait loci (QTLs) related to emergence and early growth traits. Zhang et al. (2020), integrating GWAS and RNA-seq data, identified two candidate genes, Zm00001d04319 and Zm00001d039219, which have been associated with cold-stress responses during maize germination.

Table 5 Correlation analysis of traits under cold and control treatment conditions

	PE	DSE	CTI	DM
Correlation (r)	0.499	0.477	0.5697	0.2541

Absolute value of r: < 0.19, Slight; almost no relationship; 0.20–0.39, Low correlation; definite but small relationship; 0.40–0.69, Moderate correlation; substantial relationship; 0.70–0.89, High correlation; strong relationship; 0.90–1.00, Very high correlation; very dependable relationship (Guilford's interpretation of the magnitude of significant correlations). PE, percentage of emergence; DSE, days from sowing to emergence; CTI, cold tolerance index; DM, dry matter content

In future work, we intend to extend our research program by examining the phenotypic and genetic characteristics of inbred lines from nearly 200 different genetic backgrounds and geographical regions.

We expect to identify inbred lines that presumably contain cold tolerance genes. This comprehensive comparison is expected to improve our understanding of cold tolerance mechanisms. Inbred cold-tolerant lines should be selected under the same conditions in which the hybrids are to be grown.

Conclusions

Our study aimed to identify cold-tolerant lines that could be used as parental components to produce good cold-tolerant hybrids and/or as sources of starting materials for new cold-tolerant inbred lines.

For this reason, 56 inbred lines with different genetic backgrounds were tested for cold tolerance in two replicates in the Phytotron, in the PGV-36 chamber, in the Agricultural Institute of the Centre for Agricultural Research HUN-REN in Martonvásár. During the tests, notable differences were observed among the inbred lines in some phenological traits.

The characteristics that determine cold tolerance include the PE, DSE, and CTI. Most of the inbreds showed an emergence rate of more than 75%. In addition to the inbred line used as a standard, W401, the outstanding lines in terms of the traits mentioned were the lines HMv Exp 5173 (HMv5173 in the previous study, Csepregi-Heilmann et al. 2023), CM7, HMv Exp 6401, and A632.

The PE above 85% in cold conditions is a remarkable achievement for a plant from the tropics. These lines are particularly notable in terms of emergence percentage:

W401, HMv Exp 6401, A619, A632, D503, and HMv Exp 5173.

Based on earliness, the following lines germinated within 22 days in cold conditions: CM174, CM7, CM105waxy, B14, HMv Exp 5173, F271.

The lines W401, CM7, A632, and A619 demonstrated superior performance across all evaluated traits:

- Higher percentage of emergence and
- Shorter emergence period after sowing.

In addition to exhibiting strong cold tolerance, these lines also possess favorable combining ability. They are the parental components of several commercially available hybrids. The cold tolerance of the old line CM7 (Flint) has been documented in the literature (Szalai et al. 2005), and the line A632 is referenced in Dőry (1990). However, the cold tolerance of the inbred lines W401 (EU Dent), A632 (B14, Iowa Stiff Stalk Synthetic heterotic group), and A619 (Lancaster) has not yet been reported. In our experiments, we identified W401 old line as the most cold-tolerant; additionally, we observed several newly tested inbred lines exhibiting comparable performance, namely HMv Exp 6401 (Dent), HMv Exp 5173 (Dent), and HMv Exp 0118A-124A (MYD). Conversely, the most cold-sensitive lines included C103 (Lancaster), HMv Exp 14-IO-43–103 (Iodent), HMv Exp 6407 (Lancaster), and F107. We confirmed that lines with distinct genetic backgrounds display varying levels of cold tolerance. Although no specific heterotic group is explicitly associated with cold tolerance, certain individual lines demonstrate notable cold tolerance.

Conclusions for future biology

The 56 maize inbreds mentioned earlier are part of a larger group of almost 200 inbred lines that will be tested. After evaluating these lines based on the phenological traits discussed, the next phase of the research will involve genotyping all the inbred lines at the germination stage using genome-wide association studies (GWAS) and DArTseq technology. Our research aims to identify trait-marker correlations that are crucial for establishing cold-tolerant genotypes, thereby accelerating their selection process. The selected cold-tolerant lines may serve as either parental components for producing cold-tolerant hybrids or starting materials for developing new cold-tolerant inbred lines.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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