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Araştırma Makalesi / Research Article

Evaluation of Phenotypic Variation among Turkish Maize (*Zea mays* L.) Hybrids for Tolerance to Chilling Stress

Fatma AYDINOĞLU[1*], Ömer İLTAŞ[1]

[1] *Gebze Technical University, Faculty of Science, Molecular Biology and Genetics Department, Kocaeli.*

** Corresponding author: e-posta: faydinoglu@gtu.edu.tr ORCID ID: https://orcid.org/0000-0002-9974-045X*

iltas.omer@gmail.com ORCID ID: https://orcid.org/0000-0003-4614-6673

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Keywords

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Hybrid seed;
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Abstract

Maize (*Zea mays* L.) is a tropical crop and chilling temperatures (below 15 ºC) cause growth retardation and yield losses. The development of chilling-tolerant maize varieties is one of the goals of plant breeders growing maize in cool climates. Hybrids are more vigorous than their parents, including being more tolerant to diverse stresses. However, stress screening is a problematic. This study aims to evaluate chilling stress tolerance of Turkish maize hybrids and to determine suitable indicators for selecting the most tolerant hybrid. Nine hybrids were subjected to low night-time temperatures following germination until the third leaf was fully enlarged. Hybrids were evaluated at the morphological, cellular and physiological levels by comparison with control seedlings. The data were subjected to kinematic analysis and statistical tools. The findings showed that all indicators differed significantly among the hybrids. Indicators such as leaf elongation rate, mature cell length and cell production increase our understanding of stress tolerance by establishing connections between phenotype and cellular functions. Shoot fresh and dry weight emerged useful indicators for revealing association between growth and the physiological stress response of seedlings. In conclusion, this study identified beneficial indicators for breeding studies at early seedling screening of maize hybrids exhibiting genetic variation in terms of chilling stress tolerance.

Türk Mısır (*Zea mays* L.) Hibridlerinin Üşüme Stresi Toleranslarında Fenotipik Varyasyonların Belirlenmesi

Anahtar kelimeler

Zea mays;
Hibrit tohum;
Üşüme stresi;
Stres belirteçleri;
Fenotipik varyasyon

Öz

Mısır (*Zea mays* L.) tropikal orjinli bir bitkidir ve düşük sıcaklıklar (15 °C'nin altında) büyüme inhibisyonuna yol açarak verim kayıplarına neden olur. Bu nedenle, üşüme stresine dayanıklı mısır çeşitlerinin geliştirilmesi, serin iklimlerde mısır yetiştirebilmek için mısır ıslahçılarının temel amaçları arasındadır. Hibridler, çeşitli streslere daha toleranslı olduklarından ebeveynlerine göre üstündür. Ancak, stres taramasının yapılması zordur. Bu bağlamda, çalışma, Türk mısır hibritlerinin üşüme stres toleranslarını değerlendirmeyi ve en toleranslı hibrit seçiminde uygun belirteçleri belirlemeyi amaçlamaktadır. Bu doğrultuda dokuz farklı genotipe sahip mısır hibridi, çimlenmelerinin ardından üçüncü yaprakları tamamen olgunlaşıncaya kadar düşük gece sıcaklığına maruz bırakılmıştır. Üşümeye maruz bırakılan hibridler, kontrol şartlarında yetiştirilen fideler ile karşılaştırılarak stres toleransları morfolojik, hücresel ve fizyolojik seviyelerde değerlendirilmiştir. Veriler kinematik analiz ve istatistiksel araçlar ile analiz edilmiştir. Bulgulara göre, tüm stres belirteçleri hibridler arasında önemli derecede farklılık göstermiştir. Yaprak uzama oranı (LER), olgun hücre uzunluğu (MCL) ve hücre üretimi (CP) gibi belirteçler, fenotip ve hücresel fonksiyonlar arasında bağlantı kurmaya olanak sağladığından stres tolerans mekanizmasını anlamamızda faydalı olduğu görülmüştür. Bununla birlikte, taze ve kuru fide ağırlığının (SFW ve SDW) fidelerin büyüme ile fizyolojik stres tepkisi arasındaki ilişkiyi ortaya çıkarmak için yararlı göstergeler olduğu saptanmıştır. Sonuç olarak, bu çalışma, genetik varyasyon sergilediği gözlenen üşüme stresi toleransı geliştirmeyi amaçlayan ıslah çalışmalarında mısırın erken aşamada taranabilmesine olanak sağlayan bir yaklaşım sunmaktadır.

1. Introduction

Due to its tropical origin, maize (*Zea mays* L.) is very sensitive to low temperatures which dramatically inhibit its growth and yield by causing chilling stress. Chilling stress for maize is particularly crucial in the cool climate of northern countries, in which damage to the plant accounts for between 65% and 87% of the total crop loss (Tokuhisa and Browse 1999). Crop losses are likely to increase in the future due to climate change. It has been estimated that the earth's temperature will rise, and that climate patterns will shift towards the polar regions, with crops also accompanying this shift. Although the effect of climate change at the local level remains unpredictable, some regions may become consistently colder. These risks, together with the increased future food demand of the growing world population demonstrate the importance of the development of chilling tolerant maize varieties.

Chilling in maize causes sharp reductions in growth rate and development and disrupted metabolism after exposure to nonfreezing low temperatures below 15 °C. These are accompanied by reduced or retarded germination and seedling emergence, wilting and chlorosis of leaf tissue, electrolyte leakage and tissue necrosis. Chilling injury is a complex interaction between stress and various other factors and alters with the duration of stress and with developmental stages. For example, chilling damage is often exacerbated by high light intensity, whereas water stress has been shown to reduce injury (Takahashi et al. 1994). Further investigations have established that one major factor may be the disruption of the circadian clock due to low temperature, resulting loss of coordination in the expression of critical enzymes controlling photosynthetic metabolism (Jones et al. 1998).

Hybrid seed development is a good strategy for increasing the chilling tolerance of maize. Hybrid seeds are produced by cross-pollination of different inbred lines generated by consecutive self-pollination (Duvick 2001). Hybrids provide many advantages such as higher yield and better growth performance under unfavorable conditions than their parents, thanks to the phenomenon known hybrid vigor. However, the unstable genetic structure of maize causes loss of hybrid vigor within repetitive planting. Hybrids must therefore be reproduced consistently, which requires efficient screening methods with reasonable costs requirements.

Chilling tolerance is mostly evaluated via visual assessment such as observing leaf discoloration or withering. This phenotypic screening is practical, but it is difficult to evaluate the results consistently due to the subjectivity of the ratings. In addition, some physiological parameters such as electrolyte leakage, antioxidant levels, lipid peroxidation, hormones, polyamines and sugars are also used as chilling stress indicators (Kim and Tai. 2011). However, it may be difficult to reproduce the results for these biochemical tests. The purpose of this study was therefore to evaluate potential chilling stress indicators by comparing contrasting maize hybrids developed by the Turkish Maize Research Institute at the seedling stage. The study thus offers a holistic understanding of chilling stress tolerance mechanisms as a result of quantitative phenotypic screening followed by cellular imaging and kinematic analysis.

2. Materials and Methods

2.1 Plant materials and chilling treatment

Nine maize hybrids developed by the Maize Research Institute (Turkey) were used in this study (Table 1). Nine seeds for each hybrid were germinated in vials under control conditions. At germination the seedlings were transferred to 1.5 l pots filled with peat and were placed in a growth chamber (Panasonic MLR-352H) to be exposed to chilling treatment or control conditions (Figure 1A). Chilling stress and control conditions were as follows; a photoperiod with a 16-/8-h day/night cycle, at 25/4 °C for stress and 25/18 °C for control and at a 250 µmol m^{-2} s^{-1} light intensity and a 70% humidity for both conditions.

2.2 Morphological, physiological and cellular screening and kinematic analysis

For phenotypic screening, the growth of the third leaf was observed from leaf appearance to full maturity (Figure 1B). The lengths of the third leaves of each seedling were measured daily using the soil level as a reference point. The leaf elongation rate (LER) (mm h^{-1}) of the third leaf was calculated as a derivative of the leaf length over time (Fiorani et al. 2000). The third leaf of each seedling was harvested when it was fully expanded, and the leaf area (LA) (mm^2) was determined by Image J software (Schneider et al. 2012).

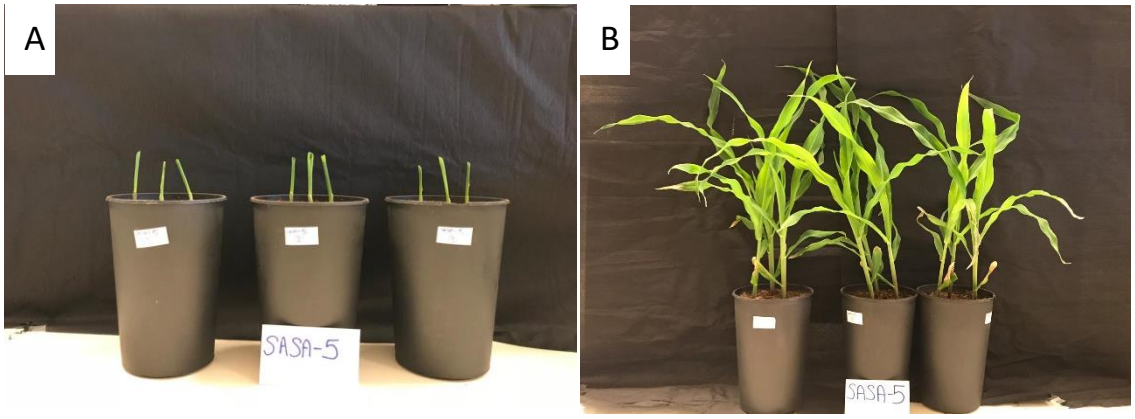

Figure 1. A representative picture of maize seedlings at the beginning (A) and at the end (B) of chilling treatment when third leaf fully got matured.

When the third leaf was fully expanded, the shoot length (SL) (mm) was measured from soil level to the leaf base. The above-ground part of the seedlings was harvested and weighted precision scales to determine the shoot fresh weight (SFW) (g). Subsequently, the shoots were dried in an oven set to heat (65 °C) for two days before determining the shoot dry weight (SDW) (g). scales to determine the shoot fresh weight (SFW) (g).

In order to determine the mature cell length (MCL) (µm), 1 cm of leaf segment was harvested from half of the fully expanded third leaf and fixed in lactic acid as described elsewhere (Rymen et al. 2007). The length of cells localized in an abaxial epidermal cell file adjacent to the stomatal rows was measured by imaging with a confocal microscope (Carl-Zeiss LSM 710) (Figure 2).

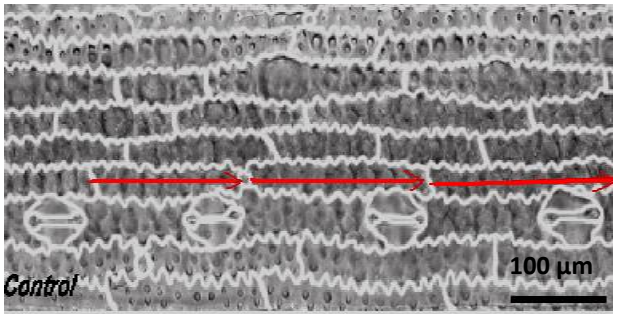

Figure 2. A representative picture of mature cells of a third leaf of maize seedlings at 10x microscope magnification.

Cell production (CP) (cells h^{-1}) in the fully expanded third leaf was calculated by dividing LER by MCL as described in kinematic analysis in a previously published study (Fiorani et al. 2000).

The total chlorophyll content (CC) of the fully expanded third leaf was estimated using a portable SPAD (Minolta SPAD-502 meter) chlorophyll meter.

The results were represented as mean ± standard deviation values of nine individual seedlings for each hybrid. Percent changes were also calculated for stress versus control. The information from different possible chilling tolerance inhibitors was weighted equally in order to select the tolerant genotypes responses to chilling stress.

Stress index was calculated to show the stress effect on the plants as in the following formula (Petrozza et. al. 2014). This gives a ranking from -1 (less-affected) to +1 (highly-affected).

$$Stress\ Index = \frac{(Control - Chilling\ Stress)}{(Control + Chilling\ Stress)} \quad (1)$$

2.3 Statistical analyses

Statistical analyses of variance were computed for the hybrids using SPSS (IBM SPSS Statistics Version 22). Statistical relations between

hybrids and different conditions were tested using two-way multivariate analysis of variance (two-way MANOVA). Conditions were treated as fixed effects and growth parameters as dependent variables. For mean comparisons Duncan's multiple range test (DMRT) was used at a probability level of 5% for classifying the hybrids according to the stress index. Bivariate Pearson correlation analysis was applied to determine a sample correlation coefficient, *r*, which measures the strength and direction of linear relationships (R^2) between potential chilling tolerance indicators.

3. Results and Discussion

The purpose of this study was to test the effectiveness of potential chilling tolerance indicators for selecting of the most chilling-tolerant maize genotype across hybrid populations. Accordingly, LER, final leaf length (FLL), LA, SL, SFW, SDW, MCL, CP and CC of the third leaf were measured and compared in chilling-treated versus control seedlings for all nine hybrids. Data were collected from phenotypic and microscopic observations as well as colorimetric measurements and were subjected to kinematic analysis by and statistical tools. Potential chilling stress indicators were also categorized into two groups-growth-related and physiological. The analysis showed that all stress indicators differed significantly between the hybrids ($P < 0.05$) (Table 1-3).

Table 1. Morphological related chilling tolerance indicators of the third leaf of hybrid maize lines grown under optimum condition and chilling stress treatment.

Hybrids	LER (mm h^{-1})		FLL (mm)		LA (mm^2)		SL (mm)	
	C	S	C	S	C	S	C	S
Sasa99	2.6±0.2	1.8±0.1	455±15	370±19	45±6	35±6	116±6	104±5
% dif.		(-30)*		(-19)**		(-22)**		(-10)**
Sasa5	2.3±0.1	1.8±0.1	408±22	327±14	38±5	30±4	102±4	94±5
% dif.		(-23)**		(-20)**		(-22)**		(-8)**
Sasa166	2.5±0.3	1.9±0.2	514±46	453±25	52±8	48±5	114±14	134±10
% dif		(-24)**		(-12)**		NS		17**
Sasa139	2.2±0.1	1.5±0.1	408±14	312±17	42±2	29±3	97±8	92±5
% dif.		(-31)**		(-24)**		(-30)**		NS
Sasa152	2.2±0.2	1.8±0.1	424±22	345±23	50±5	34±5	103±6	95±4
% dif.		(-21)**		(-19)**		(-32)**		(-8)**
Sasa137	2.5±0.2	1.9±0.1	504±23	435±17	54±5	48±4	115±11	124±7
% dif.		(-24)**		(-14)**		(-11)**		NS
Sasa186	2.5±0.1	1.7±0.1	472±26	347±36	48±7	37±7	122±10	105±11
% dif.		(-31)**		(-27)**		(-23)**		(-14)**
Ada1650	2.6±0.1	2.1±0.2	444±29	371±22	47±5	40±5	122±10	110±7
% dif.		(-20)**		(-16)**		(-14)**		(-10)**
Adasa16	2.1±0.2	1.7±0.1	374±32	314±18	46±5	39±6	86±11	83±6
% dif.		(-17)**		(-16)**		(-15)*		NS

LER: Leaf elongation rate, FLL: Final leaf length, LA: Leaf area, % dif., Percent differences between control (C) and chilling stress (S) conditions. -% represents percent reduction, +% represents percent increased. n=9, mean ± SD. NS, Not significant, * Significant at $P < 0.05$, ** Significant at $P < 0.01$ according to Student t-test.

The first response of plants to stress factors is growth inhibition (Avramova et al. 2016). Since organ growth is a result of cell division and cell expansion processes, an organ's size depends on its cell number and cell size. Therefore, LER, LA, FLL, SL, MCL and CP were therefore assigned as potential growth-related chilling indicators. Analysis of LER between the hybrids identified, Adasa16 as the least significantly affected with a 17% reduction ($P<0.01$), while Sasa186 was significantly most affected with a 31% reduction ($P < 0.01$) (Table 1). LER has been described in detail under salinity and water deficiency as a stress indicator in various studies, and the results underlined that LER is essential for maintaining the productivity of grasses (Cramer and Bowman 1991, Durand et al. 1997, Neves-Piestun and Bernstein 2001). Other studies of Gramineae roots and leaves have emphasized the effect of

temperature on tissue dynamics in the elongation zones (Pahlavanian and Silk 1988, Gastal et al. 1992, Ben-HajSallah and Tardieu 1995, Tonkinson et al. 1997). Considering all these findings, this study suggested that LER is also a useful indicator for chilling screening.

LER is sufficient for predicting leaf area expansion, but not FLL, because FLL also depends on leaf growth duration (Durand et al. 1999). When hybrids were evaluated in terms of FLL, chilled leaves of Sasa166 were shortened by 12 %, and this hybrid was therefore regarded as the best expanded under stress, while Sasa186 leaves were significantly the most shortened, by 27% ($P < 0.01$) (Table 1). LA in Sasa166 was not significantly affected by chilling ($P > 0.05$), but LA in Sasa152 was significantly reduced by 32% ($P < 0.01$) (Table 1). SL in Sasa137, Sasa139 and Adasa16 were not affected by chilling ($P > 0.05$), while the other hybrids displayed significant similar reduction profiles ranging from 8% to 10% according to Duncan's Multiple Range test ($P < 0.01$) (Table 1).

Table 2. Cellular and kinematic-related chilling tolerance indicators of third leaf of maize hybrids grown under optimum condition and chilling stress treatment.

	MCL (µm)		CP (cells h^{-1})	
	C	**S**	**C**	**S**
Sasa99	149±14	125±18	0.017±0.003	0.015±0.002
% dif	(-16)**		(-15)*	
Sasa5	145±6	119±12	0.016±0.001	0.015±0.001
% dif	(-18)**		NS	
Sasa166	140±6	125±5	0.018±0.003	0.015±0.001
% dif	(-11)**		(-15)**	
Sasa139	137±6	110±9	0.016±0.001	0.014±0.001
% dif	(20)**		(-14)**	
Sasa152	134±11	101±9	0.017±0.002	0.018±0.002
% dif	(-35)**		NS	
Sasa137	139±12	134±14	0.018±0.002	0.014±0.001
% dif	NS		(-22)**	
Sasa186	153±14	136±12	0.016±0.001	0.013±0.002
% dif	(-11)**		(-22)**	
Ada1650	160±7	125±18	0.017±0.001	0.017±0.003
% dif	(-22)**		NS	
Adasa16	138±15	128±10	0.015±0.001	0.014±0.002
% dif.	NS		(-11)*	

MCL: Mature cell length, CP: Cell production; % dif.: Percent differences between control (C) and chilling stress (S) conditions. -% represents percent reduction. +% represents percent increased. n=9, mean ± SD. NS: Not significant; * Significant at $P < 0.05$; ** Significant at $P < 0.01$ according to Student t-test.

MCL in Sasa137 and Adasa16 was not significantly affected by chilling treatment. However, Sasa152 was the most affected hybrid at the cellular level, as its MCL was shortened by 35% ($P < 0.01$) (Table 2). CP remained unchanged in Sasa5, Sasa152 and Ada1650, while Sasa137 and Sasa186 were the most affected, with 22% reductions ($P < 0.01$) (Table 2). Considering all the results from growth-related indicators together, Sasa152 exhibited the greatest shrinkage in LA (32%) due to chilling. However, its CP remained constant. These findings indicate that the reduction in leaf size was caused by shortened MCL, rather than CP. Maintaining leaf size is a principal stress adaptation process for plants, since leaves constitute the site of photosynthesis (Nelissen et al. 2018). Sasa166 was relatively the best adapted to chilling, as it reached the same final leaf size by slowing down the growth processes.

Following stress-induced growth retardation, plants seek to maintain homeostasis via physiological rearrangements resulting in accumulation of various metabolites, thus causing weight differences (Tokuhisa and Browse 1999). In addition, sustaining efficient photosynthesis in response to stress is also crucial for biomass accumulation (Gama et al. 2009, Greer et al. 2011). SFW, SDW and CC were therefore assigned as potential physiological-related chilling stress indicators. The results showed that SFW in Sasa99, Sasa5, Sasa139, Sasa186 and Adasa16 was not affected by stress, but that Sasa166 and Sasa137 exhibited 41% and 36% increases, respectively ($P < 0.01$) (Table 3). However, SFW in Sasa152 and Ada1650 decreased by 35% and 13%, respectively. SDW in Sasa5, Sasa152, Sasa186 and Adasa16 was not affected, while SDW in Sasa166 and Sasa137 increased by 70% and 40%, respectively ($P < 0.01$) (Table 3). CC data evaluation showed that photosynthesis activity was not affected by chilling treatment in most hybrids. However, CC increased by 25% in Sasa137 and by 9% in Adasa16 ($P < 0.01$) (Table 3).

The increase in weight in Sasa166 and Sasa137 under stress suggested the possibility that stress protectants are accumulated more efficiently when growth processes are inhibited. These findings

indicate that the unshrunk LA in Sasa166 was maintained by slowing down growth processes with decreases in LER and CP, and increases in SFW and SDW, and with continuous photosynthesis activity. In conclusion, the hybrid that grew slowly under chilling was relatively better adapted to the stress condition through high accumulation of stress-protective metabolites. The observation that SDW exhibited no correlation with any other growth parameters except for SL also supported this conclusion (Table 4).

The highest positive correlation was observed between FLL and LA (r = 0.7; P < 0.01), while the lowest insignificant correlation was calculated between CC and LA (r = 0.2, P < 0.05) (Table 4). While SDW was only correlated with SL, MCL exhibited no correlation with any of the other indicators measured. CP and MCL exhibited significantly high negative correlation (r = -0.7, P < 0.01). CP was also correlated with LER, but not with the other indicators. Quantifying parameters for use in screening is always problematic, because each hybrid exhibits a different performance based on different parameters (Table 5). The leaf of a particular hybrid may be shortened, but at the same time may be enlarged in area due to stress. This occurs due to the complexity of stress tolerance mechanisms regulated by numerous genes (Roy et al. 2011). Breeders can determine which parameters are most suitable for their selection goals. On the other hand, selection can be performed based on the overall performance of the hybrid under stress treatment versus control conditions.

Table 3. Physiological-related chilling tolerance indicators of third leaf of maize hybrids grown under optimum condition and chilling stress treatment.

Hybrids	SFW (g)		SDW (g)		CC (SPAD)	
	C	S	C	S	C	S
Sasa99	5.8 ± 1	5.7 ± 1	1.9 ± 0.1	1.6 ± 0.1	41 ± 3	40 ± 2
% dif.	NS		(-16)**		NS	
Sasa5	5.2 ± 0.7	5.1 ± 0.9	1.4 ± 0.1	1.4 ± 0.2	40 ± 2	39 ± 3
% dif.	NS		NS		NS	
Sasa166	5.4±1.5	7.6 ± 1.5	1.4 ± 0.2	2.3 ± 0.4	33 ± 3	37 ± 4
% dif.	41**		70**		NS	
Sasa139	4.9 ± 0.6	4.6 ± 0.7	1.5 ± 0.1	1.3 ± 0.1	33 ± 3	34 ± 3
% dif.	NS		(-15)*		NS	
Sasa152	6.5 ± 1	4.2 ± 0.4	1.5 ± 0.4	1.1 ±0.1	37 ± 4	36 ± 4
% dif.	(-35)**		NS		NS	
Sasa137	5.5 ± 1	7.5 ± 0.8	1.4 ± 0.2	2 ± 0.2	30 ± 2	37 ± 3
% dif.	36**		40**		25**	
Sasa186	6.4 ± 1.3	6.7 ± 0.8	1.6 ± 0.2	1.7 ± 0.1	37 ± 3	36 ± 5
% dif.	NS		NS		NS	
Ada1650	7 ± 0.5	6.1 ± 1	1.9 ± 0.2	1.6 ± 0.1	35 ± 3	37 ± 3
% dif.	(-13)*		(-15)*		NS	
Adasa16	7.1 ± 1.8	6.5 ± 1.5	1.9 ± 0.2	1.6 ± 0.1	36 ± 3	39 ± 3
% dif.	NS		NS		9*	

SFW: Shoot fresh weight, SDW: Shoot dry weigh, CC: Chlorophyll content; % dif.: Percent differences between control (C) and chilling stress (S) conditions. -% represents percent reduction, +% represents percent increased. n=9, mean ± SD. NS: Not significant; * Significant at P < 0.05. ** Significant at P < 0.01 according to Student t-test.

The severity of stress is of crucial importance for screening studies. Most stress experiments have exposed plants to either short or severe cold shock (Riva-Roveda et al. 2016, Meng and Sui 2019). However, stress tolerance is a process, and it is not possible to observe gradual adaptation through the powerful inhibition of plant growth with severe stress (Tokuhisa and Browse 1999). In this context, maize seedlings in the present study were subjected to a low night-time temperature (4 °C), while maintaining an optimal daytime temperature (25 °C) following germination, until the third leaf was fully enlarged.

Bhosale et al. (2007) evaluated five European flint and five dent maize inbred lines and their 25 factorial crosses in six natural environments exposed to chilling. They therefore concluded that mid-parent performance is a poor predictor of hybrid performance. They therefore proposed that test-cross performance should be the target in quantitative trait locus mapping studies proceeding

to marker-assisted breeding of chilling-tolerant maize. They also compared field and growth chamber data and found strong associations between leaf elongation rates during cold nights and plant height at the three-leaf stage. The parameters tested in this study were offered as possible chilling-induced growth and physiological indicators of stress tolerance, as a result of comparative studies. These indicators may be beneficial for the early prediction of hybrid performance under chilling stress and thus in terms of savings of time, money and labor required to achieve chilling-tolerant variet.

Tablo 4. Simple correlation coefficients matrices of possible chilling tolerance indicators.

	FLL	LA	CC	SL	SFW	SDW	MCL	CP
LER	.523**	.400**	.316**	.393**	.267*	.033	-.096	.526**
FLL	1	.729**	.319**	.633**	.422**	.224	.098	.142
LA		1	.283*	.388**	.582**	.183	.092	.161
CC			1	.504**	.363**	.254	.091	.086
SL				1	.605**	.428*	.212	.021
SFW					1	.285	.218	.050
SDW						1	.287	.175
MCL							1	.678**

LER: Leaf elongation rate; FLL: Final length of leaf; LA: Leaf area; CC: Chlorophyll content; SL: Shoot length; SFW: Shoot fresh weight; SDW: Shoot dry weight; MCL: Mature cell length; CP: Cell production. n = 9, mean ± SD. **: Correlation is significant at the 0.01 level (2-tailed). *: Correlation is significant at the 0.05 level (2-tailed).

Table 5. Sorting of the maize hybrids according to chilling stress index (SI).

LER (mm h^{-1})	**SI**	**Sasa139**	**Sasa186**	**Sasa99**	**Sasa137**	**Sasa166**	**Sasa5**	**Ada1650**	**Sasa152**	**Adasa16**
		0.19^{b}	0.19^{b}	0.17^{b}	0.14^{ab}	0.13^{ab}	0.12^{ab}	0.11^{ab}	0.11^{ab}	0.09^{a}
FLL (mm)	**SI**	**Sasa139**	**Sasa186**	**Sasa5**	**Sasa99**	**Sasa152**	**Ada1650**	**Adasa16**	**Sasa137**	**Sasa166**
		0.13^{b}	0.15^{b}	0.11^{ab}	0.10^{ab}	0.10^{ab}	0.09^{ab}	0.09^{ab}	0.07^{ab}	0.06^{a}
LA (mm^2)	**SI**	**Sasa152**	**Sasa139**	**Sasa186**	**Sasa99**	**Sasa5**	**Adasa16**	**Ada1650**	**Sasa137**	**Sasa166**
		0.19^{ab}	0.18^{b}	0.13^{ab}	0.12^{ab}	0.12^{ab}	0.09^{ab}	0.08^{ab}	0.06^{ab}	0.04^{a}
SL (mm)	**SI**	**Sasa186**	**Sasa99**	**Ada1650**	**Sasa5**	**Sasa139**	**Sasa152**	**Adasa16**	**Sasa137**	**Sasa166**
		0.08^{a}	0.05^{a}	0.05^{a}	0.04^{a}	0.03^{a}	0.03^{a}	0.02^{a}	-0.04^{a}	-0.08^{a}
SFW (g)	**SI**	**Sasa152**	**Ada1650**	**Adasa16**	**Sasa139**	**Sasa99**	**Sasa5**	**Sasa186**	**Sasa137**	**Sasa166**
		0.19^{b}	0.07^{ab}	0.05^{ab}	0.03^{ab}	0.02^{ab}	0.01^{ab}	-0.03^{ab}	-0.16^{a}	-0.18^{ab}
SDW (g)	**SI**	**Sasa139**	**Sasa99**	**Adasa16**	**Sasa5**	**Sasa186**	**Ada1650**	**Sasa152**	**Sasa137**	**Sasa166**
		0.09^{c}	0.08^{c}	0.08^{c}	-0.01^{c}	-0.06b^{c}	0.07^{c}	-0.17^{ab}	-0.17^{ab}	-0.25^{a}
MCL (µm)	**SI**	**Sasa152**	**Ada1650**	**Sasa139**	**Sasa5**	**Sasa99**	**Sasa166**	**Adasa16**	**Sasa137**	**Sasa186**
		0.14^{ab}	0.13^{b}	0.11^{ab}	0.10^{ab}	0.09^{ab}	0.06^{ab}	0.03^{ab}	0.02^{a}	-0.06^{ab}
CP (cells h^{-1})	**SI**	**Sasa137**	**Sasa166**	**Sasa139**	**Sasa99**	**Sasa186**	**Adasa16**	**Ada1650**	**Sasa5**	**Sasa152**
		0.12^{b}	0.08^{ab}	0.08^{ab}	0.08^{b}	0.06^{ab}	0.05^{ab}	0.04^{a}	0.03^{ab}	0.00^{ab}
CC (SPAD)	**SI**	**Sasa186**	**Sasa99**	**Sasa5**	**Sasa152**	**Sasa139**	**Ada1650**	**Adasa16**	**Sasa166**	**Sasa137**
		0.12^{a}	0.01^{a}	0.01^{a}	0.01^{a}	-0.01^{a}	-0.02^{a}	-0.04^{a}	-0.06^{a}	-0.11^{a}

LER: Leaf elongation rate; FLL: Final leaf length; LA: Leaf area; SL: Shoot length; SFW: Shoot fresh weight; SDW: Shoot dry weight; MCL: Mature cell length; CP: Cell production; CC: Chlorophyll content; SI: Stress index calculated by the formula represented materials and methods, ranging from -1 (least affected) to +1 (most affected); Within rows means followed by the same letter (a-e) are not significantly different at the 0.05 probability level using Duncan's multiple range test (DMRT). n = 9, mean ± SD.

4. Conclusion

In conclusion, comparison of the genotype-experimental conditions interaction revealed genetic variation between the maize hybrid seedlings in terms of for chilling stress tolerance. Plant breeders require accurate, fast and inexpensive screening methods. This study demonstrates that chilling stress tolerance screening is applicable at the third leaf stage of hybrids. The results showed that monitoring of LER is a good predictor of the tolerance levels for early detection. In addition to LER, FLL and LA were also identified as principal predictors and they can be chosen according to the main goal of many breeding programs. MCL was also found to be informative because of its association with cellular stress adaptation mechanisms.

5. References

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