

Chilling Tolerance of Central European Maize Lines and their Factorial Crosses

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• **Background and Aims** Chilling-stress tolerance is a prerequisite for maize production under cool climatic conditions. The main goal of this study was to evaluate the Central European dent and flint pools for chilling tolerance during heterotrophic and early autotrophic growth in field trials and growth chamber experiments.

• **Methods** Five European flint and five dent inbreds and their 25 factorial crosses were evaluated in six natural environments, where chilling occurred, for chlorophyll concentration and plant height at the three-leaf stage, and plant height and fresh weight at the seven-leaf stage. In growth chambers, leaf 3 growth was analysed under cold and control conditions.

• **Key Results** Comparing the field and growth chamber data, the strongest association was found between leaf elongation rate during cold nights and plant height at the three-leaf stage, with a weaker association with the seven-leaf stage. In the field, moderate correlations were observed between plant height at the three-leaf stage, and plant height and fresh weight at the seven-leaf stage, respectively. Furthermore, mid-parent and hybrid performance were only moderately correlated.

• **Conclusions** The results suggest that heterotrophic and early autotrophic growth stages are controlled by different genetic factors or that maternal effects play a role. In addition, the findings showed that mid-parent performance is a poor predictor of hybrid performance. Consequently, test cross performance should be the target in quantitative trait locus (QTL) mapping studies with the final goal of establishing marker-assisted breeding programmes for chilling-tolerant hybrids.

Key words: Maize, *Zea mays*, chilling stress, heterotrophic and autotrophic growth.

INTRODUCTION

Maize (*Zea mays*) is considered a chilling-sensitive species with a relatively high temperature optimum for germination, development and dry matter accumulation (Miedema, 1982). Under climates characterized by cool and humid springs, such as in Central Europe, adaptation has been partially successful due to late planting and breeding for early-maturing maize hybrids. These strategies are useful to minimize the risk of field losses due to chilling stress (Stamp, 1986). Improvement of chilling tolerance would support earlier spring planting and, consequently, lead to higher yielding maize hybrids (Lee *et al.*, 2002). Furthermore, earlier soil coverage would help to reduce erosive processes.

At approximately the three-leaf stage of maize, seed reserves are exhausted and the seedling has to rely on its photosynthetic activity for carbon gain (Cooper and MacDonald, 1970). Heterotrophic growth (i.e. germination and growth relying partly on seed reserves) and autotrophic growth (i.e. photosynthesis-based growth after the exhaustion of seed reserves) appear to require different minimum limiting temperatures, which may be attributable to different genetic control (Brandolini *et al.*, 2000). In agreement with this hypothesis, Hodges *et al.* (1997) and Revilla *et al.* (2000) reported low associations between chilling tolerance at heterotrophic and autotrophic growth stages. In contrast, Janowiak and Markowski (1987)

observed a high correlation between the two stages, suggesting a similar genetic control. Hence, further studies comparing heterotrophic and autotrophic plant growth for chilling tolerance in maize are needed.

The correlation between hybrid and mid-parent performance is important for designing optimum breeding strategies and deciding whether selection of superior hybrids could be based on predictions obtained from parental performance. In addition, this correlation is crucial for answering the question of whether quantitative trait locus (QTL) mapping studies for chilling tolerance should concentrate on inbred lines or hybrids. Maryam and Jones (1983) observed a high association between mid-parent and hybrid performance for chilling tolerance in maize, suggesting that prediction of hybrid performance is feasible based on mid-parent performance. In contrast, Hodges *et al.* (1997) reported that reliable prediction of hybrid maize chilling tolerance was not possible from information about the parental inbreds. Hence, further research on the association between hybrid and mid-parent performance for chilling tolerance in maize is required.

Field trials depend largely on the combined effects of multiple environmental parameters changing simultaneously throughout the duration of the experiment. In contrast, plants can be grown in the laboratory under well-controlled conditions in growth chambers. This allows the evaluation of the effects of environmental factors on performance one at a time, which reduces the complexity and enhances the sensitivity of the analysis. Moreover, such experiments do not depend on outside conditions

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and are therefore reproducible over time, and can be performed year-round, which will result in a reduction in the screening time of potentially valuable germplasm. The higher reproducibility and time reduction of the screening process would be of great advantage for future breeding programmes. However, growth chamber trials should reflect the aspects of stress situation occurring in field trials in order to accomplish this advantage.

The main goal of this study was to evaluate the Central European dent and flint pools for chilling tolerance during heterotrophic and early autotrophic growth in field trials and growth chambers experiments. In particular, we (a) examined the associations between mid-parent and hybrid performance for four measured traits; (b) analysed correlations between chilling tolerance-related traits determined at different developmental stages; (c) investigated the effects of low night temperature on seedling growth in growth chambers; and (d) assessed to what extent growth chamber data predict maize performance in the field.

MATERIALS AND METHODS

Plant materials

Five European dent and five European flint inbred lines of *Zea mays* L. were used, as well as their 25 factorial crosses in the F_1 generation. Seeds were produced using the flint lines as pollinators and dent lines as seed parents. The lines were chosen as a representative sample of modern public elite inbreds developed during the 1990s at the University of Hohenheim. The flint lines were F039, F047, F048, F052 and L024. The dent lines were D23, P048, P087, S046 and S070.

Field trials

The ten parental inbred lines and their 25 factorial crosses were evaluated in field trials over 2 years (2005 at 2006) at three locations in South Germany [Hohenheim, 400 m above sea level (asl), silty loam soil texture; Ihingerhof, 500 m asl, loam soil texture; and Oberer Lindenhof, 700 m asl, loam soil texture]. The experiment was laid out as a randomized complete block design with two replicates. Each plot consisted of two rows, and each row was 5 m length with a distance of 0.75 m between rows. Plant density was 88 000 plants ha^{-1} . To analyse the chilling tolerance of maize genotypes, the following four traits were measured: (1) chlorophyll concentration at the fully expanded three-leaf stage for 20 plants per plot (measurements were taken at leaf 3 at three positions and values were averaged); (2) plant height at the three-leaf stage; (3) plant height at the seven-leaf stage immediately before harvest; and (4) fresh weight per plant at the seven-leaf stage. Chlorophyll concentration was estimated with a handheld portable SPAD-502 chlorophyll meter (Minolta Corporation, Ramsey, NJ, USA). This instrument non-destructively measures the relative amount of chlorophyll in plant leaves. High values observed for the four traits indicate chilling tolerance of the respective lines or hybrids. Soil temperatures were recorded throughout the growing

period from planting till harvest using automatic data loggers installed at the three locations. Temperature data for Hohenheim in the year 2006 are incomplete, because of technical problems with the temperature sensor.

Growth chamber experiments

The maize lines were germinated in peat pellets (Jiffy International As, Norway) at 25 °C and 70 % humidity, with a 16 h photoperiod and light intensity of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Thereafter, the seedlings were transferred to pots with a height of 20 cm and a diameter of 4.6 cm filled with soil (N⁰, Structural, Kaprijke, Belgium) and placed in a growth chamber (type vb1014, Vötsch industrietechnik, Balingen, Germany) at 70 % relative humidity, 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR at plant level provided by a combination of fluorescent tubes (Osram-77 and Osram-31-830, Osram, Munich, Germany). The diurnal cycle was 16/8 h (day/night) with a gradual decrease and increase of radiation intensity over 0.5 h. Temperature was kept at 25 °C during the photoperiod and decreased to 18 °C (control) or to 4 °C (treatment) during the last 6 h of the night.

To calculate leaf elongation rates (LERs) during the photoperiod and the night, the length of leaf 3 (ten plants per treatment) was measured at the beginning and end of the photoperiod from leaf emergence to maturity, using the soil level as a reference point.

Statistical analyses

Analyses of variance were computed for the hybrids and inbreds across the environments using PROC MIXED in SAS (Version 9.1, SAS Institute, 2004). Environments were treated as fixed effects, and genotypes and genotype \times environment interactions as random effects. A Wald test was used to test whether variances were significantly greater than zero. Heritability (h^2) on an entry mean basis was calculated for all traits, according to Hallauer and Miranda (1981). Best linear unbiased predictions (BLUPs) were estimated for all four traits. Differences between the mean performance of the dent and flint lines were tested with a t -test. Pearson's correlation coefficients (r) between the BLUP values of the traits measured in the field trials and the growth chambers were calculated for inbreds and hybrids. Significance tests of r were performed using tabulated values based on Fisher's (1921) z transformation.

RESULTS

Field trials

Mean daily soil temperatures from sowing to harvest ranged from 8.8 to 29.3 °C with an average of 17.3 °C in 2005, and from 7.2 to 25.9 °C with an average of 17.2 °C in 2006 (Fig. 1). Lowest minimum soil temperatures were measured in both years at Ihingerhof, with 5.1 °C in 2005 and 4.2 °C in 2006 (Supplementary Information 1, available online). Temperatures at Oberer Lindenhof, which is the location with the highest elevation, were consistently lower than at

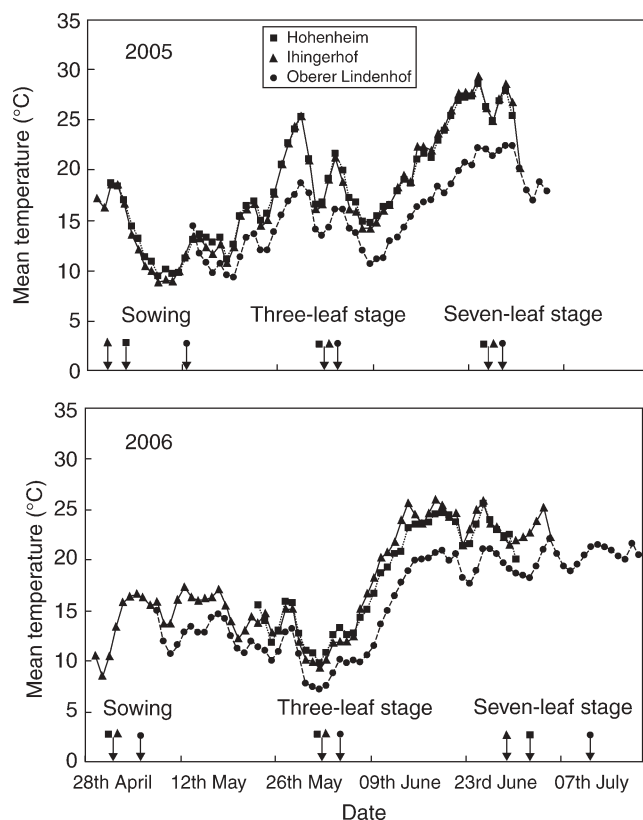


FIG. 1. Daily mean soil temperatures during 2005 and 2006 for the three locations in South Germany: Hohenheim, Ihingerhof and Oberer Lindenhof (circles).

Ihingerhof and Hohenheim (on average 3.7 °C). Nevertheless, the temperature trends were similar across the three locations.

For all traits except chlorophyll concentration, mean performance of the hybrids was higher than for inbred lines (Table 1). Genotypic variances (σ_G^2) of the hybrids and inbreds were significant ($P < 0.05$) for the measured traits except for plant height at the three-leaf stage for the inbred lines. The magnitude of variances associated with genotype \times environment interactions ($\sigma_{G \times E}^2$) was similar to estimates of σ_G^2 for all traits. A more detailed analysis of the performance of the ten inbred lines and 25 hybrids within location \times year combinations revealed a complex clustering of environments (Supplementary Information 2, available online). This complex pattern cannot be explained by single factors such as temperature, rainfall or soil texture alone, but rather points to an interaction of various external factors. Heritability (h^2) estimates ranged from 0.65 to 0.91 for the inbred lines and from 0.72 to 0.83 for the hybrids.

For inbreds and hybrids, no significant phenotypic correlations were observed between chlorophyll concentration and the other three traits (Table 2). Plant height at the seven-leaf stage and fresh weight at the seven-leaf stage were tightly correlated ($r = 0.89$) for both inbreds and hybrids. The phenotypic correlations between (a) plant height at the three-leaf stage and (b) plant height and fresh weight at the seven-leaf stage were higher for hybrids than for inbreds. Mid-parent performance was

TABLE 1. Variance component estimates for five dent and five flint maize inbred lines and their 25 factorial crosses evaluated for four traits in six environments in South Germany

Statistics	Chlorophyll concentration at three-leaf stage (SPAD units)	Plant height at three-leaf stage (cm)	Plant height at seven-leaf stage (cm)	Fresh weight at seven-leaf stage (g plant ⁻¹)
Hybrids				
Mean	23.37	12.90	61.52	152.75
σ_G^2	1.88**	0.33**	8.35**	224.34**
$\sigma_{G \times E}^2$	1.39***	0.44***	5.95*	130.48*
σ_{Error}^2	2.28	0.70	22.29	629.29
Heritability	0.83	0.72	0.76	0.77
Inbred lines				
Mean	23.64	10.51	39.17	47.11
σ_G^2	6.92*	0.30	22.79*	75.29*
$\sigma_{G \times E}^2$	2.55**	0.81***	16.56**	62.80**
σ_{Error}^2	3.52	0.34	17.80	105.82
Heritability	0.91	0.65	0.84	0.80

*Significant at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

positively correlated with hybrid performance for all traits, with the highest correlation observed for fresh weight ($r = 0.71$) and the lowest for plant height at the three-leaf stage ($r = 0.34$).

For plant height and fresh weight, the mean performance of the flint lines was significantly ($P < 0.01$) higher than that of the dent lines (Table 3). For chlorophyll concentration, no significant difference was found between the mean of the flint and dent lines. D23 was the best performing dent inbred line for all traits. F047, F048 and F052 were the best performing flint lines. Hybrids produced from flint parents F039 and F052 and dent parents D23 and S046 showed higher plant height and fresh weight compared with other hybrids.

Phenotypic evaluation in growth chambers

In the growth chamber experiments, cold was only applied during the night, while daytime temperatures were the same for control and cold treatments (Rymen *et al.*, 2007). For all inbred lines and hybrids, this treatment resulted in a reduced overall leaf growth (Fig. 2). Similar to the field data, the hybrids performed better than the inbred lines under both conditions. Moreover, both the mean reduction and the range of variation in mature length of leaf 3 were smaller for the hybrids compared with those of the inbred lines.

Correlation analyses of field and growth chamber data revealed that mature leaf length determined in the growth chamber trials was significantly associated with plant height of the inbred lines evaluated in the field at the three-leaf stage (Table 4). Interestingly, for performance of the hybrids, higher correlations were observed between field data and the difference of cold vs. control treatment than between field data and cold treatment. Analyses of correlations between growth chamber and single environmental

TABLE 2. Phenotypic correlations among four traits measured for five dent and five flint maize inbred lines (above the diagonal) and their 25 factorial crosses (below the diagonal)

	Chlorophyll concentration at three-leaf stage	Plant height at three-leaf stage	Plant height at seven-leaf stage	Fresh weight at seven-leaf stage
Chlorophyll concentration at three-leaf stage	0.50**	−0.03	0.32	0.39
Plant height at three-leaf stage	−0.19	0.34	0.65*	0.59
Plant height at seven-leaf stage	−0.20	0.78***	0.51**	0.89***
Fresh weight	−0.14	0.70***	0.91***	0.71**

Elements on the diagonal (in bold) are correlations between mid-parent and hybrid performance for each trait.

*Significant at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 3. Best linear unbiased predictions of five dent and five flint maize inbred lines and their 25 factorial crosses evaluated for four traits in six environments in South Germany and for leaf growth in the laboratory

		Dent inbred				
Flint inbred	<i>Per se</i>	D23	P048	P087	S046	S070
Chlorophyll concentration at three-leaf stage (SPAD units)						
<i>Per se</i>		26.06	20.46	27.59	21.96	23.86
F039	22.34	22.43	22.49	24.95	22.90	24.67
F047	23.43	23.48	22.51	24.89	23.22	24.95
F048	23.61	22.06	21.05	24.15	20.98	22.53
F052	26.72	23.10	22.19	25.28	23.49	24.64
L024	20.38	23.51	22.49	25.13	23.12	24.03
s.e. (lines) = 0.87; s.e. (hybrids) = 0.30						
Plant height at three-leaf stage (cm)						
<i>Per se</i>		10.99	9.87	9.85	10.28	10.39
F039	10.35	12.96	12.42	12.33	13.77	13.23
F047	10.97	13.10	13.06	12.89	13.42	13.54
F048	10.59	13.17	13.35	12.05	12.85	12.52
F052	10.68	13.08	12.60	12.81	13.15	13.21
L024	11.09	13.09	12.45	11.58	12.99	12.93
s.e. (lines) = 0.22; s.e. (hybrids) = 0.14						
Plant height at seven-leaf stage (cm)						
<i>Per se</i>		41.67	36.08	33.67	34.78	37.35
F039	36.71	63.47	59.90	59.72	65.14	62.89
F047	43.26	63.09	63.39	61.49	64.37	61.95
F048	43.05	62.64	62.77	58.81	59.63	59.86
F052	47.20	64.39	63.01	63.14	62.62	61.82
L024	37.96	63.60	58.16	54.80	59.63	57.61
s.e. (lines) = 1.65; s.e. (hybrids) = 0.67						
Fresh weight (g per plant)						
<i>Per se</i>		55.37	38.53	36.28	40.78	46.69
F039	49.67	175.05	143.83	145.83	172.26	158.78
F047	50.66	160.50	154.96	155.57	161.44	158.18
F048	53.14	161.19	157.70	148.74	145.53	147.99
F052	59.05	174.10	147.62	158.85	155.36	151.12
L024	40.90	159.23	129.10	121.28	141.56	132.97
s.e. (lines) = 3.08; s.e. (hybrids) = 3.46						
Mature leaf 3 length in the cold treatment (cm)						
<i>Per se</i>		39.22	29.51	31.81	32.41	33.45
F039	30.13	49.82	44.35	44.42	41.47	45.41
F047	33.12	50.09	46.37	46.19	43.24	41.83
F048	39.84	48.57	48.80	44.61	42.53	43.85
F052	35.36	49.49	42.32	46.38	46.38	40.33
L024	38.96	55.11	49.01	50.13	52.77	48.67
s.e. (lines) = 0.86; s.e. (hybrids) = 1.23						
LER at night (cold treatment vs. control treatment) (%)						
<i>Per se</i>		−67	−71	−72	−70	−68
F039	−64	−46	−48	−49	−53	−59
F047	−54	−52	−56	−47	−38	−41
F048	−66	−51	−48	−53	−55	−62
F052	−65	−41	−59	−62	−62	−51
L024	−65	−41	−64	−61	−38	−60

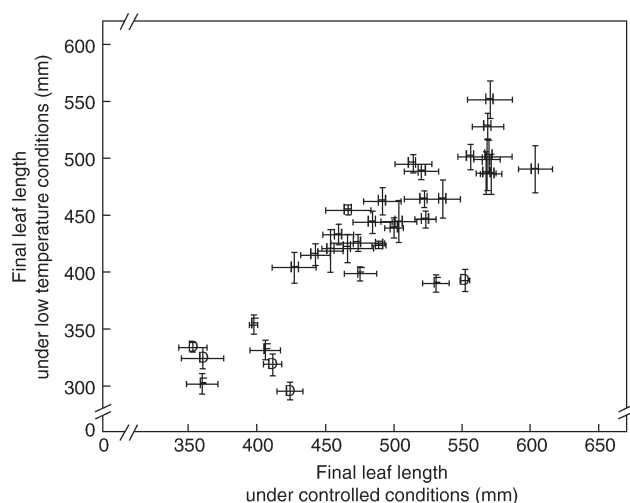


FIG. 2. Final length of leaf 3 measured for five flint inbred lines (F), five dent inbred lines (D) and 25 hybrid lines (H) grown in growth chambers under control conditions [25/18 °C (day/night)] and low night temperature conditions [25/4 °C (day/night)]. Symbols are means \pm SE ($n=10$).

data (Supplementary Information 3, available online) demonstrated that growth chamber experiments allow modelling of temperature stress naturally occurring in a wide range of locations.

The measurements of day and night LER in combination with the cold treatment during the night allowed the growth dynamics during daytime and at night to be investigated separately, and for the general temperature effect and the induced cold stress effect to be estimated (cf. Rymer *et al.*, 2007). This analysis revealed for all genotypes a typical growth curve with an initial steady-state LER, followed by a decline in LER (Rymer *et al.*, 2007). The

steady-state growth contributed most to the overall growth of the leaf and was used for the subsequent calculations. LER was affected by the cold during daytime and at night. The largest reduction occurred at night when the cold was applied, with an average LER reduction of -56% (range -38% to -72%). During the daytime, LER was also reduced for most genotypes, although some compensated their reduced growth at night by a faster growth during the day (e.g. the hybrids S070 \times F039 and D23 \times F047 had an increased LER during the day of 24 and 22 %, respectively).

Significant associations were found between the LER and the reduction in LER at night when the cold was applied and plant height measured in the field experiments (Table 4). In contrast, no significant associations were observed between the field and LER data during the daytime, indicating the general temperature response rather than the induced stress effect as the main contributor to the performance of the plants under field conditions.

DISCUSSION

Correlation between mid-parent and hybrid performance

The range of correlations between mid-parent and hybrid performance observed in the present study was similar to estimates obtained for various complex agronomic traits (for a review, see Hallauer and Miranda, 1981). The observed differences between mid-parent and hybrid performance can be fully explained by a simple model with only additive and dominance genetic effects (Smith, 1986). In addition, linkage and/or epistasis can cause deviations of mid-parent from hybrid performance. In summary, the observed magnitude of the correlation for the four investigated traits suggests that mid-parent performance is a poor predictor of hybrid performance. In addition, test cross performance should be the target in QTL mapping studies with the final goal of establishing marker-assisted breeding programmes for chilling-tolerant hybrids.

The involvement of the photosynthetic apparatus in chilling tolerance

Marocco *et al.* (2005) proposed to group physiological response to chilling stress into effects due to: (a) mild chilling stress ($12-17\text{ }^{\circ}\text{C}$) in the light, with reduced photosynthesis and growth as well as the induction of photoprotective mechanisms in response to excess light energy captured by chlorophyll; (b) strong chilling stress ($2-10\text{ }^{\circ}\text{C}$) in the light with cold-induced water stress, because the rate of transpiration exceeds the rate of water uptake by roots due to inhibition of root hydraulic conductivity; and (c) chilling stress in the dark, not associated with oxidative stress in the chloroplast or with water stress, but rather with changes in gene expression (cf. Rymer *et al.*, 2007). In the growth chamber experiments, the focus was on the third class, with only application of low temperature during the night period. The significant association between the LER measured in the growth chambers and the field data indicated that the effects of chilling stress occurring

TABLE 4. Pairwise correlation coefficients between field trial and growth chamber data for five dent and five flint maize inbred lines and their 25 factorial crosses

	Leaf height at three-leaf stage		Leaf height at seven-leaf stage	
	Lines	Factorial crosses	Lines	Factorial crosses
Cold treatment				
Mature leaf length	0.73*	-0.13	0.54	0.11
LER during daytime	0.52	0.20	0.33	0.28
LER at night	0.73*	0.39	0.33	0.24
Control treatment				
Mature leaf length	0.61	-0.37	0.29	-0.34
LER during daytime	0.49	0.14	0.41	0.27
LER at night	0.21	-0.16	-0.13	-0.27
Difference				
Mature leaf length	-0.15	0.52**	0.11	0.47*
LER during daytime	-0.09	-0.01	-0.21	-0.09
LER at night	0.70*	0.40*	0.61	0.37

*Significant at $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

under field conditions are similar to the third class of chilling stress suggested by Marocco *et al.* (2005). Moreover, it suggests that the observed growth reductions can only be partially explained by photoinhibition due to a combination of high light and cold during the day. The findings are in accordance with previous studies of Fracheboud *et al.* (2004) and Jompuk *et al.* (2005) reporting that the photosynthetic apparatus did not noticeably affect biomass accumulation, but are in contrast to the results of Stirling *et al.* (1993) and Andrews *et al.* (1995), who observed a significant effect of photoinhibition on CO₂ assimilation. The conclusion that growth reduction in the field can only partially be explained by photoinhibition is consistent with the low correlation observed between chlorophyll concentration and growth in the field experiment (Table 2). Nevertheless, a decrease in chlorophyll concentration is only one possible factor for reduced photosynthesis. Further analyses based on the ratio of the level of variable to maximal fluorescence of dark-adapted leaves (cf. Andrews *et al.*, 1995) are warranted to exclude photoinhibition as a main factor for growth reduction.

Chilling tolerance during heterotrophic and autotrophic growth

During the heterotrophic growth phase, from sowing until the end of May, chilling stress was more severe than during the autotrophic growth phase (Fig. 1). Nevertheless, severe chilling stress also occurred during the early autotrophic growth phase. Thus, the environments were suitable to identify genotypes with increased chilling tolerance at both growth phases, which was confirmed by significant σ_G^2 observed for inbreds and hybrids (Table 1).

The focus of the present experiments was on plant height at the three-leaf stage in the field and a detailed trajectory analysis of the growth of leaf 3 in the laboratory as an indicator of chilling tolerance during heterotrophic growth, and on plant height and fresh weight at the seven-leaf stage in the field as an indirect indication of chilling tolerance during autotrophic growth. The response to chilling stress of inbred lines and hybrids investigated in this study changed from heterotrophic to autotrophic plant growth (Tables 2 and 3). Consistently, we found a lower association between the growth of leaf 2 in the laboratory and plant height at the seven-leaf stage than at the three-leaf stage in the field experiments (Table 4). The latter can be interpreted as an indicator for differences in chilling tolerance during heterotrophic and autotrophic growth, but may also be explained by the different temperature conditions during the two growth phases (Fig. 1).

The differences between the effects on the autotrophic and heterotrophic growth are in accordance with previous studies in maize analysing hybrids from Canada (Hodges *et al.*, 1997) and Europe (Revilla *et al.*, 2000). The different response to chilling stress during heterotrophic and autotrophic plant growth was more pronounced for inbreds (Tables 2 and 3), which is due to the higher capacity of individual buffering for hybrids compared with inbreds (Hallauer *et al.*, 1988). The differences in response to chilling stress at heterotrophic and autotrophic

developmental stages can be explained by: (a) a control by different genetic factors (Hodges *et al.*, 1997); (b) an overriding importance of maternal effects during heterotrophic but not during autotrophic growth; and/or (c) reduced chilling stress during autotrophic growth. Significant maternal effects associated with heterotrophic growth potential were reported by several authors (Eagles and Hardacre, 1979; Maryam and Jones, 1983), but they are in contrast to the findings of Aidun *et al.* (1991) reporting the absence of maternal effects among six reciprocal maize hybrids analysed at early growth stages. A significant ($P < 0.05$) correlation was observed between seed weight and plant height at the three-leaf stage of the hybrids ($r = 0.48$; data not shown), which points to the importance of maternal effects during heterotrophic growth. Nevertheless, further analyses including reciprocal crosses are required to verify whether reciprocal effects are present.

Flint inbred lines were on average more chilling tolerant than dent inbred lines (Table 3). This can be explained by the history of the Central European heterotic groups (Reif *et al.*, 2005). Hybrid breeding was started in the 1950s and as a promising heterotic pattern, non-adapted high-yielding US lines were crossed with flint lines. The flint inbreds were developed by selfing from European open-pollinated varieties, which were adapted to the cool climatic conditions of Central Europe. However, the results for line *per se* performance for fresh weight also indicate that the dent line D23 possesses a high level of chilling tolerance. Therefore, D23 is an excellent source to improve the chilling tolerance of the European dent pool further.

For inbreds and hybrids, plant height at the seven-leaf stage was highly correlated with fresh weight (Table 2). This indicates that plant height is suitable to predict fresh weight of the genotypes under chilling stress to a considerably high level. Consequently, plant height may serve as a non-destructive measurement for biomass at early developmental stages. This non-destructive determination of chilling tolerance would facilitate investigations on associations of chilling tolerance at early developmental stages and grain yield at harvest. Alternatively, canopy reflectance may be used as a non-invasive method to determine biomass at early developmental stages of maize (cf. Montes *et al.*, 2007).

Associations between field trials and growth chamber experiments

The use of laboratory conditions for evaluating plant performance in breeding could be advantageous. Laboratory conditions allow plants to be grown under well-defined conditions, independent of the climate. This could reduce the cost of the breeding of chilling-tolerant hybrids, due to reduction in the time needed and higher reproducibility of the screening process. Unfortunately, it is known that plants under laboratory conditions often perform differently from under field conditions, especially under stress conditions (Mittler, 2006). One possible reason is that plants in the field are constantly exposed to several stresses at the same time. Therefore, it is important

to tune the laboratory conditions to the field conditions of interest. In this study it was shown that the effect of low temperature on specific aspects of seedling growth in growth chambers is significantly associated with the growth observed in the field obtained with the same genotypes at the same stage of development, but that it is more difficult to extend this to later stages (Table 4). These findings are in accordance with a study of Hodges *et al.* (1997) evaluating 12 Canadian hybrids in laboratory and field experiments. This indicates that growth chambers can be used for screenings for tolerant lines, followed by field trials for the final selection.

SUPPLEMENTARY INFORMATION

Supplementary information is available online at <http://aob.oxfordjournals.org/> and provides the following data. (1) Pairwise correlation coefficients between field trial data generated in six environments and growth chamber data. (2) Principal co-ordinate analyses of field data for five flint and five dent lines for chlorophyll concentration, plant height, plant fresh weight and site location. (3) Daily minimum soil temperatures at the three locations during the course of the study.

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