Can we improve heterosis for root growth of maize by selecting parental inbred lines with different temperature behaviour?

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Tolerance to high and low temperature is an important breeding aim for Central and Northern Europe, where temperature fluctuations are predicted to increase. However, the extent to which genotypes differ in their response to the whole range of possible temperatures is not well understood. We tested the hypothesis that the combination of maize (Zea mays L.) inbred lines with differing temperature optima for root growth would lead to superior hybrids. This hypothesis is based on the concept of ‘marginal overdominance’ in which the hybrid expresses higher relative fitness than its parents, summed over all situations. The elongation rates of axile and lateral roots of the reciprocal cross between two flint and two dent inbred lines were assessed at temperatures between 15°C and 40°C. Indeed, the cross between UH005 and UH250 with lateral root growth temperature optima at 34°C and 28°C, respectively, resulted in intermediate hybrids. At temperatures below and above 31°C, the hybrids’ root growth was comparable to the better parent, respectively, thereby increasing temperature tolerance of the hybrid compared with its parents. The implications of and reasons for this heterosis effect are discussed in the context of breeding for abiotic stress tolerance and of putatively underlying molecular mechanisms. This finding paves the way for more detailed investigations of this phenomenon in future studies.

Keywords: flint; dent; corn; marginal overdominance; root; abiotic stress

1. INTRODUCTION

Maize is a chilling-sensitive species of tropical origin, with severely hampered development at atmospheric temperatures below 15°C. In agricultural history, the so-called flint types of maize adapted to higher latitudes, first as the result of selection by native American farmers, later by European farmers [1] and in the past century by modern plant breeders. North of the Alps, maize formerly grew only in the warmer areas such as the alpine foehn valleys [2]. Today, maize is cultivated as far north as southern Scandinavia [3,4]. At least part of this success was achieved by lowering the temperature limit at which maize can still grow [4]. As a side effect, such lines are likely to suffer from heat sensitivity [4]. However, according to climate models for Europe [5], future crops will have to cope with an even greater variability of high and low temperatures throughout the season [6]. It is therefore advisable to select genotypes that are capable of tolerating a wide range of temperature conditions.

Shoot growth and yield strongly depend on the performance of the root system [7]. In maize, it is well known that root zone temperature strongly affects leaf growth and shoot biomass [8,9], mediated by pronounced alterations in shoot metabolism [10] and short-term growth dynamics [11]. Low root zone temperatures induce decreased root growth and this in turn hampers shoot development by altered water relations [12,13] and carbohydrate metabolism [11]. Because root zone temperatures are modulated not only throughout the season, but also throughout day–night cycles in topsoil regions, the question as to how intensely root growth is hampered by sub- or supra-optimal temperatures is highly relevant also in the context of the overall development of the shoot, which is a main trait of plant breeding.

Plant breeders usually develop hybrids to benefit from heterosis. Heterosis is defined as the superior performance of an F1 hybrid compared with the average performance of the distinct homozygous inbred parents. The phenomenon of heterosis has been described not only for shoot traits and yield, but also for root traits of maize [14] and wheat [15,16]. To benefit from heterosis, breeders combine the so-called heterotic groups. Inbred lines are kept and selected separately within these groups. For the cool climate of Central and Northern Europe, the Northern Flints and the Corn Belt Dents are the basis for hybrid crosses [17]. The flint lines are adapted to a cool temperate climate, contributing to chilling tolerance, while the warm-temperate-adapted dent lines contributed to a high-yield potential [4,18].

The success of the flint–dent combination in the cool regions of Europe raises the question of whether...
the combination of different temperature optima of these groups partially explain heterosis. It is well established that plants of different geographical origin [19], as well as cultivars of one and the same species that are adapted to different regions [4,20,21], possess different temperature optima. However, for roots, detailed information about their temperature optima is lacking. A few studies investigating the relation of root growth parameters in different genotypes to temperature have focused either on high or low temperature stress [22,23], not taking into account the dynamic response across a wider range of temperatures and not investigating specific responses of different traits of the root system (e.g. lateral versus axile roots). Although it is likely that genotypes differ with respect to their response of root growth to a range of temperatures, it has not been tested yet (i) whether clear-cut distributions of growth response towards differing temperatures exist and (ii) whether such differences could be used to increase temperature-dependent heterosis. Mc William & Griffith [24] studied temperature-dependent heterosis of shoot growth of maize. In their study, the largest heterosis effect was found at temperature extremes, i.e. below a day temperature of 21°C and above a day temperature of 33°C. However, their study did not involve genotypes with different temperature optima.

There are several potential explanations for how temperature response of plants might be controlled mechanistically. For example, temperature may act via networks governed by thermodynamic laws (e.g. rise and decline of various enzyme activities from suboptimal via optimal to supraoptimal temperatures). Most likely, subordinate metabolic processes would be controlled by key enzymes. Rubisco activase is a candidate for such a key enzyme controlling the temperature response of photosynthesis [25]. There, the temperature response of rubisco activase determines photosynthesis response to an extent that it may even set boundaries to the geographical distribution of different plant species [25]. However, Parent et al. [26] found that the temperature response of photosynthesis does not follow the same function as growth processes. Alternative to thermodynamic dependences, other molecular control processes exist. For example, heat stress transcription factors and other pathways mediated by abscisic acid, salicylic acid, hydrogen peroxide and ethylene induce heat acclimation (for review, see [27]). It is still unclear which enzymes or pathways would be the major factors governing the response of root growth to temperature in breeding material of maize. The mapping of quantitative trait loci (QTLs) is a straightforward approach to identifying genomic regions controlling root growth [28] and their response to temperature [29]. The protocol developed here may be used for selection or QTL mapping in order to (i) identify genomic regions controlling the response to temperature and (ii) to elucidate whether such regions are involved in temperature-dependent heterosis. Heterosis in a modern context may give rise to hybrids that perform better across the whole temperature range. This concept can be illustrated by two hypothetical combinations: (i) inbred lines that differ with respect to temperature optimum (figure 1a), and (ii) inbred lines that differ with respect to their temperature range and their maximal growth at the temperature optimum (figure 1b). In the first case, the parents contribute both heat and chilling tolerance to the hybrid; in the second case, the parents contribute tolerance to temperature extremes and the ability to perform well at optimal temperatures.

Given the earlier mentioned considerations, we aimed to elucidate (i) whether the temperature behaviour of root growth of maize can be described with a simple, nonlinear function, such as a Gaussian function, (ii) whether inbred lines or heterotic groups differ with respect to the parameter estimates of this function, and (iii) whether the resulting hybrids of these differing inbred lines follow the performance of the better parent, i.e. express marginal overdominance with respect to different traits of root system growth (lateral versus axile roots).

2. MATERIAL AND METHODS
(a) Plant material and environmental conditions
Two flint inbred lines (UH002 and UH005), two dent inbred lines (UH250 and UH301) and their reciprocal hybrids were used. The material was obtained from the University of Hohenheim, Germany. The seed surface was sterilized with 2.5 per cent aqueous sodium hypochlorite (NaOCl (aq)) for 15 min and rinsed three times with water. The seeds were germinated in Petri dishes on moist germination paper at 27°C for 2 days. Germinated seeds were transferred to growth pouches consisting of blue germination blotting paper (24 × 29.5 cm; Anchor Paper, St Paul, MI, USA) covered by black plastic sheets. The pouches
were placed in custom-made cooling boxes (figure 2) allowing root zone temperatures to be set independently from shoot-zone temperatures. The bottom of the pouch was submerged in a nutrient solution (0.23% (v/v) of Wuxal; Aglukon Spezialdünger GmbH, Düsseldorf, Germany). The composition per litre nutrient solution was 230 mg N, 230 mg P₂O₅, 173 mg K₂O, 437 mg Fe, 373 mg Mn, 235 mg B, 186 µg Cu, 140 µg Zn, 23 µg Mo. Each box contained a full set of 12 plants, one per genotype. For the first 2 days after the onset of the experiment, plants were kept at 25°C. After this phase, the temperature in the boxes was set at either 15°C, 16°C, 21°C, 24°C, 28°C, 32°C, 36°C or 40°C. The temperature was measured at the seed level in the pouch. A pump (figure 2a) distributed the nutrient solution along the walls of the container to achieve more uniform temperatures within the box. The root zone temperature was set to the desired temperature by a heat or cold exchanger (Julabo Labortechnik GmbH, Seelbach, Germany). The boxes were placed in a growth chamber (E16; Conviron, Winnipeg, Canada) set at 25°C shoot-zone temperature, photosynthetic active radiation of 300 µmol m⁻² s⁻¹ and relative humidity of 75 per cent.

(b) Experimental design
The experimental design was a split plot experiment with two independent replications. Temperature was the whole plot and genotype the subplot factor. Each experimental unit consisted of one plant. Thus, each genotype was tested at eight temperatures allocated to four independent growth chamber replications. Each replication harboured four of the eight temperatures. One of the four replications resulted in an overall poor plant performance, likely due to malfunction of the growth chamber, and was discarded from the analysis. The remaining replications summed up to 12 biological replications (plants) per genotype in total. During the treatment phase, the pouches were scanned daily within a period of 5 days to determine root elongation rates. The images were acquired using

Figure 1. Schematic of performance profiles of hypothetical homozygous inbred lines (P₁ (short dashed lines) and P₂ (long dashed lines)) and their heterozygous F₁ (solid lines) hybrid dependent on growth temperature. It is assumed that the trait value Y decreases symmetrically from its maximum value Yₘₐₓ at the temperature optimum µ and that the temperature operating range σ can vary: Y = Yₘₐₓ e⁻[(x-µ)/σ]. Assuming that the alleles of the better parent are always dominant, the F₁ always performs as well as the best parent (solid line). Examples where this leads to marginal overdominance are: both inbred lines show the same σ and Yₘₐₓ but differ in µ by Δx (a), and both inbred lines show the same µ but differ for σ and Yₘₐₓ (b).

Figure 2. Temperature box (a); root growing on germination paper in a pouch (b). The box is shown with two pouches (a), a heat exchanger (b) connection to the Julabo heating and cooling device (not shown) and a water pump (c) connected to a hose with small holes at 2 cm intervals for uniform cooling (d). The box was insulated by 2 cm Styrofoam. When operating, the box was closed with a 2 cm Styrofoam lid, leaving only space for the shoots.
a flatbed scanner (HP scanjet 4600 series, ‘see-through’; Hewlett-Packard Company). Images were processed as described by Hund et al. [34] to obtain binary images containing root objects and background. These were analysed using WinRHIZO 2003b (Regent Instruments, Montreal, Quebec, Canada) to obtain the root-length in diameter class distribution of the scanned roots. Objects below a threshold of 0.5 mm were classified as lateral roots; those above this threshold were considered as axile roots.

### (c) Models and statistical treatment

The elongation rate of the axile roots (ER\(_{Ax}\)) was modelled as linear, and the elongation rate of the lateral roots (ER\(_{Lat}\)) was modelled as exponential as described in more detail elsewhere [34,35]. The corresponding models were

\[
x(t) = x(t_0) \times \text{ER}_{Ax} t
\]

and

\[
\text{ER}_{Ax} = \frac{x(t) - x(t_0)}{t}
\]

for axile root elongation, where \(x(t)\) is the root length at time \(t\) after germination and \(x(t_0)\) is the root length at the first scanning day, and

\[
x(t) = x(t_0) \times e^{k_{\text{lat}} t}
\]

and

\[
k_{\text{lat}} = \frac{\ln(x(t) - x(t_0))}{t}
\]

for lateral root elongation rate.

The temperature response of each genotype was modelled using a Gaussian function

\[
Y = Y_{\text{max}} e^{-\frac{(x-\mu)^2}{\sigma}}
\]

in which the trait value (\(Y\)) is a function of the maximum elongation rate (\(Y_{\text{max}}\)) at the temperature optimum (\(\mu\)) and the temperature operating range (\(\sigma\)). The nonlinear least squares estimated of the Gaussian function and their 95% CI were computed using the function nlsList() in R [36]. The model was fitted as described by Pinheiro & Bates [37, pp. 338ff.]. To estimate the effect of the heterotic group used as a mother and of the combining ability, nonlinear mixed effect models were fitted using the function nlmek() in R [36]. In this case, the genotypes were set as a random factor and the desired contrast was set as a fixed factor following the procedure described by Pinheiro & Bates [37, pp. 354ff]. Since there was no self-starting function available, the starting values were estimated based on the scatterplots of growth rates versus temperature of the whole population.

Mid-parent values were calculated to compute parent–offspring correlations. As a specific form of mid-parent value (MP), the combination of the range (\(R\)) and temperature shift of the parents (\(O\)) was calculated as

\[
\text{MP} = \frac{R_{P1} + R_{P2}}{2} + \frac{O_{P1} - O_{P2}}{2},
\]

where \(R\) is the temperature range of the respective parent (\(P_1\) or \(P_2\)) and \(O\) is its temperature optimum. This calculation was conducted to take into account that the shift in the temperature optimum of the parents may affect the temperature range of the respective hybrids.

### 3. RESULTS

(a) Differences among genotypes existed for the maximum elongation of ER\(_{Ax}\) and ER\(_{Lat}\) as well as for the temperature optimum of \(k_{\text{lat}}\)

Overall, the Gaussian model was well suited to describe the temperature response of the 12 genotypes (figures 3 and 4). Thus, optimum temperature, temperature range and maximum elongation rate at optimum temperature (‘maximum elongation’) were extracted from this model (table 1). Growth of axile roots of all genotypes practically ceased at temperatures of 15°C and 40°C, respectively, with maximum values being found between 25°C and 30°C (figure 3). For lateral roots, variability of the temperature response for individual genotypes and variability between genotypes were higher (figure 3). Owing to poor germination, inbred line UH250 had five plants only. Accordingly, its parameter estimates could not be estimated reliably, resulting in large 95% CI (table 1). Differences between genotypes with respect to the temperature range and the temperature optimum were least pronounced for axile roots (figure 1). Their CI did not overlap (table 1; see electronic supplementary material, S1 for visualization). However, there was significant variation for the maximum growth. It ranged from 7.9 cm d\(^{-1}\) for UH005 to 33 cm d\(^{-1}\) for UH301, with a population average of 22 cm d\(^{-1}\). For lateral roots, there was a significant variation of the optimum temperature and of the maximum elongation of \(k_{\text{lat}}\) (table 1; electronic supplementary material, S2). The optimum temperature of \(k_{\text{lat}}\) ranged from 28°C for the cross between UH250 and UH002 to 34°C for UH005 with a population average of 29°C. The maximum \(k_{\text{lat}}\) ranged from 0.47 d\(^{-1}\) for UH005 \(\times\) UH250 to 1.08 d\(^{-1}\) for UH250 \(\times\) UH002 with a population average of 0.77 d\(^{-1}\). There was neither a tendency for increased chilling tolerance of the flint group nor for increased heat tolerance of the dent group. Contrary, the flint line UH005 was the most heat tolerant inbred line with highest temperature optimum of \(k_{\text{lat}}\). Thus, the hypothesis that the response of root growth to temperature of the two groups is determined by their geographical origin can be rejected.

(b) The optimum shift among specific inbred lines may be used to select hybrids with altered optimum and increased temperature range

We used parent–offspring correlations to evaluate whether differences among inbred lines could be passed on to their hybrids. For most traits with low variability among the inbred lines, this was not the case (table 2). However, the mid-parent values of the temperature optimum of \(k_{\text{lat}}\) were strongly correlated with \(k_{\text{lat}}\) of the hybrids (\(r = 0.85\)). This was mainly due to the fact that the cross between UH005 and UH250, with an optimum of 34°C and 28°C, respectively, resulted in hybrids with an average of 31°C (cf. figure 4).
As outlined for the temperature behaviour of hypothetical genotypes (figure 1), the temperature range of a hybrid may not only be affected by the temperature range of the parents but also by a shift of their temperature optima. Accordingly, we tested whether the temperature range of the hybrids could be predicted by the combination of the range and temperature shift of their parents (equation 2.4). Indeed, there was a reasonably strong parent–offspring correlation ($r = 0.54$; table 2) for this trait, while the correlation was negative when the temperature shift was not taken into account ($r = -0.45$).

(c) Dent inbred lines as a mother increased the maximum elongation rate

For both root orders, i.e. the axile and lateral roots, the hybrids with the dent as a mother (figures 3 and 4; D*F) had a consistently higher elongation rate compared with those with the flint as a mother. These parent-or-origin effects suggest maternal genetic effects, or genomic imprinting. We tested the significance of these effects using the hybrids as random variable and the heterotic group of the mother (flint or dent) as a fixed factor. Using the dents as mother increased the maximum $k_{lat}$ by 0.20 cm d$^{-1}$ ($p < 0.001$) and the maximum $ER_{Ax}$ by 9.6 cm d$^{-1}$ ($p < 0.001$ data not shown), when compared with the flints as mother.

For both root orders, the pattern of the maximum elongation rate (cf. table 1 and electronic supplementary material, S2) suggested that the flint line UH002 as a parent led to an increased elongation rate compared with the flint line UH005. Adding this contrast into the fixed term of the model revealed that UH002 increased $k_{lat}$ of the hybrids compared with UH005 by 0.15 d$^{-1}$ ($p < 0.05$; data not shown). However, this effect could statistically not be verified for $ER_{Ax}$. In summary, the highest $k_{lat}$ was achieved by a combination of the dent group as mother and UH002 as father. An altered temperature range or optimum temperature among the hybrids depending on the heterotic group or genotype could not be verified statistically.

4. DISCUSSION

We aimed to evaluate whether the response of root growth to temperature can be described using a non-linear model. The model of choice was a Gaussian function, because it allows retrieving biologically meaningful parameters in a parsimonious model and because it is consolidated also by other experimental root growth data [19]. Moreover, this simple model is sufficient to test the clear-cut hypothesis investigated here, while in future studies working with a broader
base of genotypes, more differentiated models might be adequate. Also, this model may not be adequate for other traits or studies, where the response to temperature results in a more asymmetric distribution of values above and below the temperature optimum. In these cases, the beta function proposed by Yin...
Table 2. Parent–offspring correlations for parameter estimates, describing the response of the elongation rates of axle (ERAx) and lateral (klat) root to temperature. The parameter ‘range and temperature shift’ adds the difference in the temperature optima between parents to their temperature range (equation (2.4)) before correlation with the temperature range of the hybrids.

<table>
<thead>
<tr>
<th>parameter</th>
<th>ERAx</th>
<th>klat</th>
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<tbody>
<tr>
<td>temperature range</td>
<td>−0.07</td>
<td>−0.45</td>
</tr>
<tr>
<td>range and temperature shift</td>
<td>0.07</td>
<td>0.54</td>
</tr>
<tr>
<td>temperature optimum</td>
<td>−0.43</td>
<td>0.85</td>
</tr>
<tr>
<td>maximum elongation</td>
<td>0.33</td>
<td>−0.028</td>
</tr>
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</table>

et al. [38] or the thermodynamic function proposed by Johnson & Lewin [39] may be better suited. Parent et al. [26], for example, used Johnson and Lewin’s function to model the temperature response of in vitro enzyme activities, developmental processes or net photosynthesis. These more complex models, however, have the disadvantage that they require estimating more than three model parameters and are therefore less parsimonious.

The temperature response of the flint and dent parents did not differ in all aspects investigated. For example, the temperature optimum of axle root growth did not differ between flint and dent lines, indicating that a systematic utilization of marginal over-dominance has not been the background of historical development of these heterotic groups. This is in line with the findings of McWillam & Griffing [24] who did not find a strong difference with respect to the temperature optima of dent and flint. However, our results suggest that some genotypes possess the potential to improve the hybrid’s reaction of root growth to temperatures. Particularly, the cross between UH005 and UH250 demonstrates this effect for klat. It is unclear why only the temperature optimum of lateral roots was affected and not that of axle roots. However, a differential response of axle and lateral roots to temperature was also reported earlier [22].

It is well conceivable that similar marginal over-dominance effects of root elongation response to temperature are of relevance in laboratory studies or under greenhouse conditions with plants cultivated in pots or other small containers. Under such conditions, temperature variations throughout 24 h can be considerable; especially when plants are grown under relatively high light intensities. Then, topsoil pot temperatures can exceed air temperature by 10°C owing to the radiative heat input and the negligible thermal buffering capacity of small pots [11], adding up to the numerous perils of pot experiments [40]. Minuscule root systems of plants such as Arabidopsis thaliana would, under such circumstances, have an enormous advantage if their growth temperature optimum was wider than usual. Clear heterosis effects in young Arabidopsis seedlings bred from accesses of different geographical origins have been demonstrated [41]. It will be important to elucidate whether short-term fitness advantages in laboratory-based pot experiments can be correlated with the performance under field conditions. In the field, crops have to perform well under changing conditions of abiotic stress throughout the season.

There are several explanations for how marginal over-dominance in the case of klat in the UH005 × UH250 cross might have been controlled genetically. First, hybridization may lead to hemizygous complementation of many genes with small effects [42]. This complementation may also involve loci that have different temperature optima, thereby providing one explanation for marginal overdominance. Second, gene expression in maize hybrids most frequently follows the mid parent expression of the two parental alleles, meaning that one allele is preferably expressed [43]. In response to abiotic stress, these two alleles can be regulated differentially [31] or their gene products can be broken down at different threshold temperatures, as shown for rubisco activase [25]. Consequently, the enzyme with the lower temperature optimum would deteriorate first in a hybrid, leaving only its counterpart to function. We hypothesize that it is possible to combine inbred lines with optimal response pathways to low and high temperatures in order to produce a superior hybrid that would be tolerant to a range of abiotic stresses. Owing to the observed parent-of-origin effects, it may be important to select the appropriate maternal line.

Dent inbred lines as a mother increased the maximum elongation rate. Hoecker et al. [14] studied the same set of genotypes and did not find similar effects for the length of the primary root. However, there was a significant heterosis effect on cortical cell size and a tendency that the dent inbred lines and the hybrids with dent inbreds as a mother had longer cortical cells. Such effects could be explained by genomic imprinting or by maternal genetic effects. Imprinting effects have been suggested for germination of maize [44]. High temperature during seed drying (43°C) decreased germination of some reciprocal hybrids but had no effect on others [44]. Certainly, more experimental data will be necessary to elucidate which are the causal mechanisms of these parent-of-origin effects.

(a) Conclusion and outlook

Classical research on temperature tolerance focuses on either cold or heat stress. However, it is becoming increasingly evident that breeding research has to focus on the whole range of possible temperatures to which the plant may be exposed. The linkage between chilling tolerance and heat sensitivity in very early maize material [4] demonstrates this need. With advantages in statistical computing, nonlinear models allow characterization of genotypes based on model parameters. The major advantage of this approach is that such experiments can be carried out without a marked increase in the biological replications. For example, a classical approach with two treatments would estimate two parameters for each genotype, i.e. the intercept and the slope. The model used here requires only one additional mathematical parameter but allows conclusions for more relevant biological parameters or crop traits.

The presented set of genotypes did not differ markedly with respect to temperature response. However, it could be shown at least for the growth of lateral roots of one cross, that the concept of marginal overdominance for
temperature response may be exploitable. Appropriate combinations of maternal and paternal lines may lead to hybrids with a wider optimum range, an intermediate optimum temperature and an unaltered maximal root growth potential compared with both parental lines. Proper registration and application of this heterosis effect can lead to successful hybrid lines that can perform well in a wider range of temperature conditions, thereby allowing for improved whole-plant performance under abiotic stress conditions. Further research will have to elucidate to what extent the effect is accessible for breeding and which traits are the most promising to connect hybrid performance under controlled conditions with hybrid performance under productive conditions in the field or greenhouse.

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