Theor Appl Genet (2006) 112: 421–429
DOI 10.1007/s00122-005-0139-4

ORIGINAL PAPER

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Manifestation of heterosis during early maize (*Zea mays* L.) root development

Received: 22 June 2005 / Accepted: 23 October 2005 / Published online: 16 December 2005
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Abstract Heterosis is typically detected in adult hybrid plants as increased yield or vigor compared to their parental inbred lines. Only little is known about the manifestation of heterosis during early postembryonic development. Objective of this study was to identify heterotic traits during early maize root development. Four German inbred lines of the flint (UH002 and UH005) and dent (UH250 and UH301) pool and the 12 reciprocal hybrids generated from these inbred lines were subjected to a morphological and histological analysis during early root development. Primary root length and width were measured daily in a time course between 3 and 7 days after germination (DAG) and displayed average midparent heterosis (MPH) of 17–25% and 1–7%, respectively. Longitudinal size of cortical cells in primary roots was determined 5 DAG and displayed on average 24% MPH thus demonstrating that enlarged primary roots of hybrids can mainly be attributed to elongated cortical cells. The number of seminal roots determined 14 DAG showed on average 18% MPH. Lateral root density of all tested hybrids was determined 5 DAG. This root trait showed the highest degree of heterosis with an average MPH value of 51%. This study demonstrated that heterosis is already manifesting during the very early stages of root development a few days after germination. The young root system is therefore a suitable model for subsequent molecular studies of the early stages of heterosis manifestation during seedling development.

Communicated by D. A. Hoisington

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Introduction

The phenomenon of heterosis describes the superior performance of F_1 hybrids compared to their parental inbred lines (Falconer and Mackay 1996). Thus, heterosis can be described as a special instance of the general principles involved in inbreeding and outbreeding (Zirkle 1952). The ill effects of inbreeding of maize plants compared to crossing have already been observed by Darwin (1876), while the genetic basis of heterosis has been discussed for almost a century (Shull 1908; East 1908; Bruce 1910; Jones 1917). The term heterosis was proposed by Shull (1952) in a lecture he gave in Göttingen 1914. The degree of heterosis is controlled by the combination of a considerable number of genes (Lamkey and Edwards 1998; Stuber 1999). Adult plants show the highest degree of heterosis which is usually measured in terms of increased plant size, fruitfulness, speed of development and resistance to disease and insect pests or climatic rigor of any kind (Falconer and Mackay 1996). Traits like grain yield of maize show a high degree of heterosis up to 240% (Dudley et al. 1991). There are indications, however, that increased vigor is already manifested during embryo and early seedling development (Shull 1952). In several studies increased vigor of maize was measured in terms of dry or wet weight of seeds and embryos (Ashby 1930, 1932; Sprague 1936), as width, length and volume of whole embryos or embryo fractions including embryo axes, endosperm and germs (Murdoch 1940; Kempton and McLane 1942; Wang 1946), or by whole plants, leaves, ear length and ear diameter, number of kernel rows and shelled grains per plant (Ashby 1930; Lindstrom 1935). None of these studies analyzed early postembryonic seedling root development or applied histological or microscopic techniques to investigate heterosis on the cellular level. Interestingly, while there is some data available on het-

erotic traits during early seedling development in old studies of the 1930s and 1940s, to our knowledge no information is available on these traits for modern hybrids which have been greatly enhanced by extensive breeding efforts in the last decades.

In this work 4 maize inbred lines and 12 reciprocal hybrids were used to study manifestation of heterotic traits during early root development. Knowledge about the early stages of heterosis manifestation in the root system of maize will help to establish a model system that allows for molecular studies of heterosis already during early seedling development.

Materials and methods

Plant material and growth conditions

The maize inbred lines UH002 [National listing of plant varieties (NLPV), Accession no (AC): M7830, European flint] and UH005 (NLPV AC: M9379, European flint) from the flint pool, and the inbred lines UH250 (NLPV AC: M9005, Iowa Stiff Stalk Synthetic) and UH301 (NLPV AC: M8652, Iodent) from the dent pool and the 12 possible reciprocal hybrid combinations derived from these inbred lines were generated in the nursery of the University of Hohenheim near Eckartsweier (Germany) in the summer season of 2003. For each experiment pooled seeds from approximately 20 ears per genotype were analyzed under standardized conditions. Genetic distances between the different hybrids were determined with 53 SSR markers providing an even coverage of the maize genome. Distance measure was calculated with modified Roger's distance (MRD): UH002–UH005: 0.643; UH002–UH301: 0.791; UH002–UH250: 0.819; UH005–UH301: 0.772; UH005–UH250: 0.795; UH301–UH250: 0.714 (T. Schrag, preliminary unpublished results). Small MRD values indicate small genetic distances between two hybrids and vice versa.

Seeds were surface sterilized with 6% hypochlorite for 6 min, thoroughly rinsed in distilled water and germinated on moistened filter paper (20×70 cm Grade 603 N, Sartorius, Göttingen, Germany) which was rolled up with 20 seeds per filter paper in a phytochamber at 26°C, with a 16 h light, 8 h dark cycle and 60% humidity in twice distilled water according to Hetz et al. (1996). Seedlings used for Feulgen staining experiments were grown in the dark (at 26°C) in order to suppress anthocyanin production in the roots which would have interfered with the staining procedure. For further analyses primary roots were excised using a razor blade.

Determination of primary root length and width

Excised primary roots were documented with a HP Scanjet 7400c scanner. Primary root length and width of the scanned roots were determined via Image-Pro Express software (version 4.5.1.3., MediaCybernetics, Silver Spring, MD, USA).

Determination of cortical cell length in primary roots

Measurement of primary root cortical cell size was performed with a confocal laser-scanning microscope (CLSM) (DM-IRBE, Leica, Wetzlar, Germany) using a TRITC wide filter. Longitudinal hand sections of primary roots were prepared with a razor blade and the specimens were subsequently stained for 5 min in 5 µg/ml propidium iodide followed by several washing steps in water (Oparka and Read 1994). Scanning of the root samples was done with the cut surface up covered with glycerin on a microscope slide.

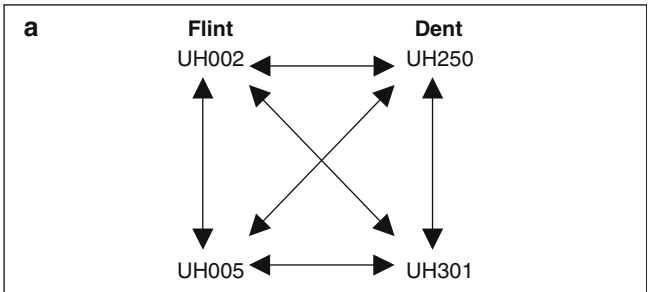
Determination of lateral root density

Lateral root primordia were detected 5 days after germination (DAG) via Feulgen staining. Primary roots were fixed over night in a solution of 70% ethanol and glacial acid in a 3:1 ratio. Subsequently, roots were washed several times with 70% ethanol, 10 min each, followed by a gradual rehydration in increasing ethanol concentrations in 10% increments, 5 min per step with two–three changes of water at the end. Hydrolysis was performed in 1 N HCl for 7 min at 60°C, and stopped by replacing HCl with 4°C water followed by two washing steps in water at room temperature. Staining was achieved for 1 h in the dark at room temperature with Schiff's Solution (Sigma, Taufkirchen, Germany) (De Tomasi 1936). After 1 h the roots were scanned (see above) in Schiff's solution and analyzed using Image-Pro Express software.

Statistical analyses

Linear models were fitted for the response variables root length, root width, cortical cell length, number of seminal roots, lateral root density, respectively. The models contain an intercept, a fixed effect for the genotype and a normally distributed, random error term. Midparent heterosis (MPH) can be calculated as a linear contrast of genotypes by $\mathrm{MPH} = F_1 - \frac{P_1+P_2}{2}$, where F_1 is the numerical value trait measurement in the hybrid and P_1 and P_2 are the measurements in the parents. Significance of this contrast was determined at a level α of 5% by a two-sided t test, i.e., it was accounted for both positive and negative heterosis. MPH is often given as percentage of the parental mean by $\mathrm{MPH}\% = \frac{\mathrm{MPH}}{P_M} \cdot 100$, where P_M is the parental mean given by $P_M = \frac{P_1+P_2}{2}$. Similary best parent heterosis (BPH) can be calculated by $\mathrm{BPH}\% = \frac{h-\mathrm{bp}}{\mathrm{bp}} \cdot 100$, where h is the numerical value trait measurement in the hybrid and bp is the numerical value trait measurement in the best parent.

The difference between hybrid and reciprocal hybrid in percentage based on the hybrid-reciprocal mean is



b

Inbred combinations:	Reciprocal hybrids:
Intra-pool:	
UH005, UH002	UH005 x UH002, UH002 x UH005
UH250, UH301	UH250 x UH301, UH301 x UH250
Inter-pool:	
UH002, UH250	UH002 x UH250, UH250 x UH002
UH002, UH301	UH002 x UH301, UH301 x UH002
UH005, UH250	UH005 x UH250, UH250 x UH005
UH005, UH301	UH005 x UH301, UH301 x UH005

Fig. 1 Experimental design. **a** Reciprocal crosses of the four inbred lines UH002, UH005 (flint pool), UH250 and UH301 (dent pool) result in **(b)** 12 hybrids that are arranged in two intra- and four inter-group pools

calculated by $\mathrm{HR} = \frac{h-r}{1/2(h+r)} \cdot 100$, where h is the numerical value trait measurement in the hybrid (F_1) and r the numerical value trait measurement in the reciprocal hybrid. Significance was again determined by a two-sided t test at a level $\alpha = 5\%$. The estimate is positive if the hybrid has a higher value than the reciprocal and negative if the reciprocal has a higher value than the hybrid.

Results

Experimental design

Goal of this work was to quantify various traits of the young maize root system with regard to heterosis. Analyses were performed with 12 hybrids generated by crosses of the inbred lines UH002, UH005 (flint pool), UH250 and UH301 (dent pool) (Fig. 1a) from the breeding program of the University of Hohenheim (UH). Hybrids and inbred lines were arranged in six pools each containing two reciprocal hybrids plus their parental inbred lines, thus, forming two intra-group pools (dent × dent and flint × flint combinations) and four inter-group pools (flint × dent combinations; Fig. 1b).

Primary root length displays consistent heterosis between 5 and 7 DAG

The primary root of maize becomes visible as a distinct morphological structure approximately 2 DAG and is consistently elongating during early development. Primary root length of the reciprocal hybrids was compared to the MPH and BPH values of the six parental hybrid combinations in a time course experiment between 3–7 DAG (Fig. 2, Table 1). Thus, 12 hybrids were analyzed at five points in time. Measurement of the root length of each genotype at each point in time was performed from at least 50 replicates grown in at least ten different paper rolls under standardized conditions (see Materials and methods). Measurements of 34 of 36 hybrid data points between 5 and 7 DAG displayed a significantly increased primary root length compared to the midparent values and 28 of 36 data points showed high parent heterosis (Fig. 2). Between 3 and 4 DAG 14 of the 24 data points showed MPH and, 9 of the 24 data points displayed BPH during these early developmental stages for this trait. Thus, primary root length is already a reliable heterotic trait early during root development. MPH reached up to 37% (UH250 × UH301) at 5 DAG, up to 62% (UH301 × UH250) at 6 DAG and up to 25% (UH250 × UH005) at 7 DAG. The highest MPH was achieved by the inter-group cross UH005 × UH250 at 3 DAG (134%). On average between 17 and 26% MPH was detected over all 12 hybrids between 3 and 7 DAG (3 DAG 25%; 4 DAG 18%; 5 DAG 23%; 6 DAG 26%; 7 DAG 17%). Interestingly, 22 of the 30 reciprocal hybrid combinations tested between 3 and 7 DAG displayed significant reciprocal effects concerning this trait (Table 1).

Primary root width displays less heterosis than primary root length

Similarly, primary root width was measured (Table 1) for the same seedlings that were used for the primary root length determination. Root width was determined in the most proximal and thus most differentiated part of the primary root next to the coleorhiza which defines the connection between kernel and root. While in 11 of 12 analyzed hybrids MPH was detected at 7 DAG during earlier stages of development, only 2–6 of the 12 hybrids displayed MPH with reference to primary root width (3 DAG: 8/12; 4 DAG: 2/12; 5 DAG: 7/12; 6 DAG: 6/12) (Table 1). Only 11 of the 60 measured time points revealed BPH for this trait (Fig. 1). Thus, primary root width does not display heterosis as consistently as primary root length. This could be explained by the fact that the contrasts between root width of the inbred lines and hybrids are smaller than the contrasts of root length and that root width increases to a much smaller degree between 3 and 7 DAG compared to the constantly, longitudinally growing primary root.

Between 5 and 7 DAG the maximum MPH values were 9% (UH250 × UH005) at 5 DAG, 14% (UH250 × UH301) at 6 DAG and 16% (UH250 × UH002) at 7 DAG. On average between 1 and 7% MPH was detected over all 12 hybrids between 3 and 7 DAG

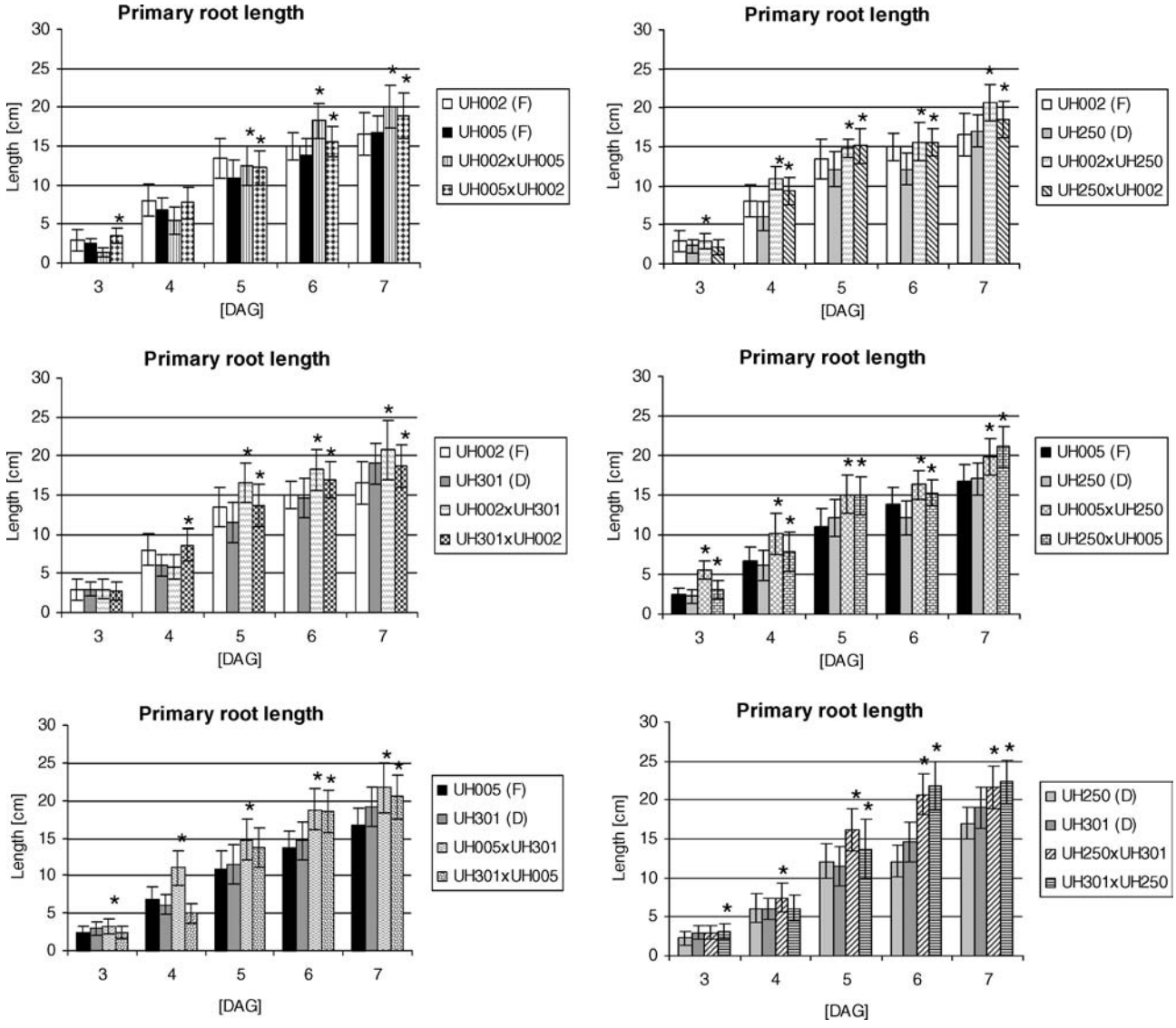


Fig. 2 Primary root length of 4 inbred lines and the 12 resulting reciprocal hybrids. *Each data point* represents at least 50 replicate measurements. *Asterisks* indicate that the hybrid differs significantly from the midparent value (*t* test; $\alpha = 0.05$). *Error bars* represent standard deviations per group. *D* dent, *F* flint, *DAG* days after germination

(3 DAG 5%; 4 DAG 1%; 5 DAG 2%; 6 DAG 4%; 7 DAG 7%). Thus the trait primary root width displayed less heterosis than the trait primary root length during the early stages of primary root development. Sixteen of the 30 reciprocal hybrid combinations tested between 3 and 7 DAG displayed significant reciprocal effects concerning primary root width (Table 1).

Longitudinal size of root cortex cells shows heterosis

The majority of the root parenchyma in maize is composed of cortex cells. In the transverse direction the maize root cortex consists of 8–15 cell layers (Hochholdinger et al. 2004a). Longitudinal elongation of roots is therefore reflected either by the size or by the number of cortex cells. CLSM of propidium iodide-stained specimens is an approach to detect and subsequently determine the size of cells inside the root parenchyma (Fig. 3a). CLSM was performed with three inbred lines UH005, UH250 and UH301 and their six resulting reciprocal hybrids at 5 DAG (Fig. 3b, Table 1). Each data point represents the measurement of at least 45 cells (on average 114 cells) from at least 10 different primary roots. Cortical cell length of hybrids was compared to the MPH of the parental inbred lines. For five of the six analyzed hybrids MPH and for four of the six hybrids BPH was detected. On average longitudinal cortical cell size displayed 24% MPH with a maximum of 47% (UH301 × UH005). Significant reciprocal effects were only observed for one of the three analyzed hybrid combinations concerning this trait (Table 1).

Table 1 Midparent heterosis (MPH) values (in percentage) of various root parameters in 12 hybrids generated from UH inbred lines

	Primary root length					Primary root width					Lateral root density	Cortical cell size	Seminal root no.
	3 DAG	4 DAG	5 DAG	6 DAG	7 DAG	3 DAG	4 DAG	5 DAG	6 DAG	7 DAG	5 DAG	5 DAG	14 DAG
UH002 × UH005	−52.2 (4.9)[b]	−27.0 (3.3)[b]	13.8[a] (3.2)	**27.5[a] (2.7)[b]**	**20.5[a] (2.3)[b]**	5.8[a] (1.6)	−1.7 (1.5)	5.0[a] (1.7)	9.1[a] (1.6)[b]	10.7[a] (1.5)	43.2[a] (0.7)[b]	ND	14.5[a] (0.8)
UH005 × UH002	27.8[a] (5.0)[b]	**3.7 (3.7)[b]**	16.1[a] (2.7)	8.3[a] (2.7)[b]	**13.3[a] (2.4)[b]**	5.8[a] (1.3)	0.0 (1.5)	2.9[a] (1.4)	1.6 (1.7)[b]	7.8[a] (1.6)	102.5[a] (0.9)[b]	ND	33.0[a] (0.7)
UH002 × UH250	13.5[a] (6.1)[b]	**54.2[a] (4.8)[b]**	**16.2[a] (3.4)**	14.6[a] (2.8)	**22.3[a] (2.5)[b]**	1.5 (1.5)	−0.8 (1.4)[b]	−1.6 (1.6)[b]	1.3 (1.5)	1.6 (1.5)[b]	20.7[a] (1.1)	ND	−17.0 (0.7)
UH250 × UH002	−14.8 (6.8)[b]	**32.0[a] (4.4)[b]**	**18.6[a] (2.9)**	14.4[a] (2.8)	**10.1[a] (2.2)[b]**	**5.0[a] (1.8)**	**6.5[a] (1.5)[b]**	**5.5[a] (1.4)[b]**	2.9 (1.6)	15.7[a] (1.5)[b]	38.8[a] (1.1)	ND	−11.5 (1.0)
UH002 × UH301	2.4 (4.8)	−18.3 (3.5)[b]	**33.1[a] (3.2)[b]**	**23.0[a] (2.6)[b]**	**16.7[a] (2.2)[b]**	1.4 (1.3)	0.9 (1.3)[b]	−3.3 (1.3)	−2.6 (1.4)	−1.9 (1.4)[b]	130.0[a] (1.0)[b]	ND	−2.6 (0.7)
UH301 × UH002	−7.1 (4.8)	22.4[a] (4.2)[b]	6.6[a] (2.6)[b]	0.9 (2.3)[b]	0.8 (2.0)[b]	1.6 (1.4)	−4.4 (1.3)[b]	−3.8 (1.2)	−3.4 (1.4)	2.2 (1.3)[b]	53.8[a] (0.8)[b]	ND	−8.6 (0.7)
UH005 × UH250	**134.3[a] (10.5)[b]**	**56.8[a] (5.2)[b]**	**30.3[a] (3.5)**	**27.0[a] (2.8)[b]**	**17.2[a] (2.2)[b]**	3.7[a] (1.5)[b]	2.4 (1.5)[b]	−3.6 (1.5)[b]	4.2[a] (1.5)[b]	7.2[a] (1.5)[b]	14.4[a] (0.7)	20.5[a] (0.4)[b]	43.3[a] (1.0)
UH250 × UH005	**27.4[a] (7.3)[b]**	**21.8[a] (4.5)[b]**	**29.0[a] (3.2)**	**18.7[a] (2.8)[b]**	**24.9[a] (2.2)[b]**	7.5[a] (1.7)[b]	**10.9[a] (1.5)[b]**	9.4[a] (1.4)[b]	**9.9[a] (1.6)[b]**	11.7[a] (1.4)[b]	28.8[a] (0.8)	44.9[a] (1.0)[b]	59.6[a] (1.0)
UH005 × UH301	17.2[a] (5.0)[b]	**70.7[a] (4.9)[b]**	**31.4[a] (3.4)[b]**	**33.4[a] (2.5)**	**21.2[a] (2.1)[b]**	3.5[a] (1.3)	−0.3 (1.2)	−1.5 (1.3)[b]	7.4[a] (1.4)	8.6[a] (1.4)	10.0[a] (0.5)	22.4[a] (0.8)	12.0[a] (0.8)
UH301 × UH005	−12.1 (4.9)[b]	−22.6 (4.7)[b]	**22.0[a] (3.3)[b]**	**30.6[a] (2.6)**	**14.4[a] (2.6)[b]**	5.1[a] (1.4)	−1.5 (1.7)	7.5[a] (1.4)[b]	5.6[a] (1.5)	8.0[a] (1.8)	−11.1 (0.4)	47.4[a] (0.8)	14.9[a] (0.8)
UH250 × UH301	13.9[a] (6.5)	**22.3[a] (5.1)[b]**	**37.2[a] (3.3)[b]**	**49.9[a] (2.9)[b]**	**19.5[a] (2.3)**	**12.2[a] (1.5)[b]**	−0.7 (1.4)	**7.6[a] (1.3)[b]**	**13.5[a] (1.4)[b]**	12.9[a] (1.6)[b]	110.0[a] (1.0)[b]	1.1 (0.6)	54.7[a] (2.3)
UH301 × UH250	21.0[a] (6.6)	0.3 (4.6)[b]	**16.1[a] (3.2)[b]**	**62.4[a] (3.1)[b]**	**23.8[a] (2.3)**	1.1 (1.5)[b]	−0.6 (1.7)	4.7[a] (1.4)[b]	0.4 (1.3)[b]	2.9[a] (1.4)[b]	66.9[a] (0.8)[b]	9.0[a] (0.2)	20.1[a] (1.3)

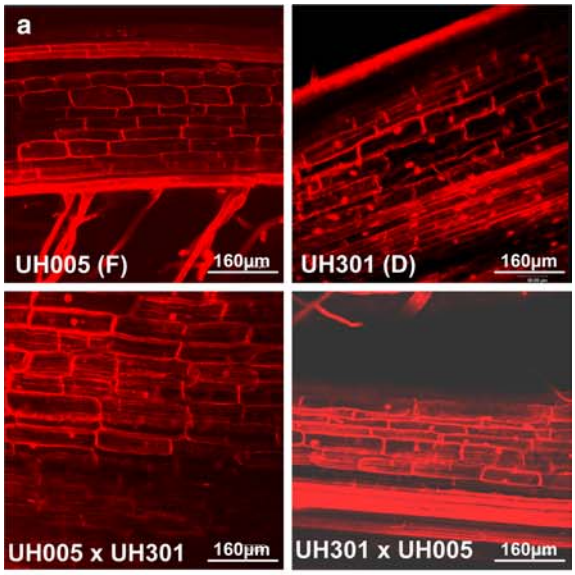
[a]Values that are significantly higher than the midparent value (positive heterosis); *t* test; $\alpha = 0.05$. Standard error in brackets
Values in bold: values displaying best parent heterosis (BPH)
[b]Significant reciprocal effects

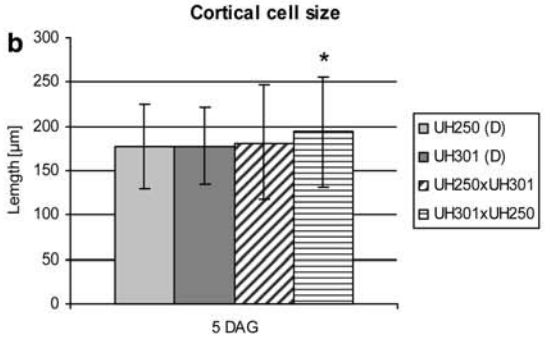
Number of seminal roots as marker for heterosis

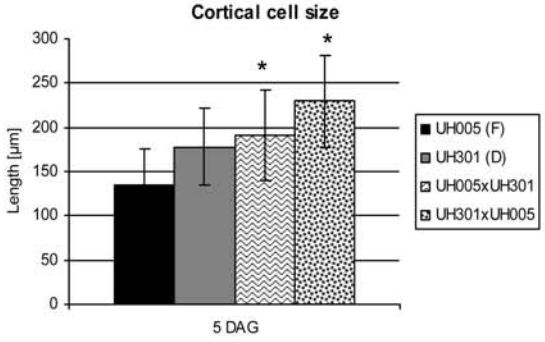
Seminal root primordia are formed during late embryogenesis between 22 and 40 days after pollination (Hochholdinger et al. 2004b). Postembryonically, seminal roots emerge within the first week after germination at the scutellar node. The number of seminal roots of at least 50 plants per genotype was determined 14 DAG (Fig. 4) for the four parental inbred lines and the resulting 12 reciprocal hybrids. Eight out of 12 analyzed hybrids showed significant MPH concerning the number of seminal roots with an average MPH of 18% and a maximum MPH of 60% (UH250 × UH005), while only two hybrids displayed BPH for this trait. For none of the six hybrid combinations reciprocal effects were detected concerning the number of seminal roots (Table 1).

Lateral root density as a marker for heterosis

Although Ashby mentioned already in 1932 that the most striking manifestation of hybrid vigor in seedlings was the number of lateral roots, to our knowledge no quantitative data on this trait is available. Lateral root initiation starts approximately 4 DAG and lateral root primordia become visible around 5 DAG (Fig. 5). Lateral root formation is a variable trait and maize has no predictable pattern of lateral root formation. Typically, lateral roots emerge in a sequential pattern close to the root tip. Thus, the youngest lateral roots are close to the root tip, while older lateral roots are found in the proximal part of the root. Since lateral roots are proliferated constantly during plant development, many lateral roots are already initiated but not visible from outside. Therefore, counting the visible lateral roots is not an accurate approach to detect lateral roots of all developmental stages along the primary root. Feulgen staining is a technique that stains meristematically active tissue by forming a purple precipitate in these cells (De Tomasi 1936). Lateral root primordia stained with the Feulgen technique are visible from outside as purple dots and can be easily counted. After having demonstrated that 5 DAG primary roots reliably display heterosis with respect to root length (Fig. 2) the number of lateral root primordia was determined at this early developmental stage. Lateral root density was calculated as number of lateral root initial sites per cm of primary root. At least 15 roots were stained with Schiff's reagent (Feulgen staining) per genotype. Eleven of the 12 analyzed hybrids displayed MPH concerning the density of lateral root primordia (Table 1) with an average MPH of 51% and a maximum MPH of 130% (UH002 × UH301). Eight of 12 analyzed hybrids displayed BPH for this trait (Table 1) Thus, lateral root density was the trait that displayed the highest degree of MPH in this study. Three of the six analyzed hybrid combinations displayed significant reciprocal effects concerning this trait (Table 1).







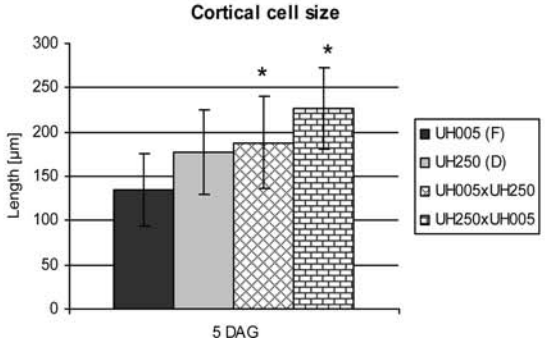


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Fig. 3 **a** Longitudinal expansion of cortex cells of the inbred lines UH005 and UH301 and the reciprocal hybrids generated from these inbred lines documented with a confocal laser-scanning microscope (CLSM). **b** Three inbred lines (UH005, UH250 and UH301) and the corresponding six reciprocal hybrids have been analyzed via confocal laser scanning microscopy (CLSM) to quantify cortical cell length. At least 45 cells (on average 114 cells) in 10 different roots have been analyzed for each genotype. *Asterisks* indicate that the hybrid differs significantly from the midparent value (*t* test; $\alpha = 0.05$). *Error bars* represent standard deviations per group. *D* dent, *F* flint, *DAG* days after germination

Discussion

Heterosis is an agronomically important phenomenon which is typically studied in adult differentiated plants where traits important for crop improvement like vigor or yield are eminent (Shull 1952). Although heterosis was discovered almost a century ago (Shull 1908; East 1908) the molecular basis of this phenomenon remains elusive. A major problem when studying heterosis in adult plants is that heterosis is already manifested at this developmental stage. Thus, gene expression differences that might be detected between adult inbred and hybrid plants might not reflect differences in heterosis-related gene expression but secondary effects caused by morphological differences that developed between the different genotypes during development. It has been demonstrated recently that morphological differences of the primary root of two maize genotypes can significantly affect their proteome composition (Hochholdinger et al. 2004c). In this study the proteome of wild-type primary roots was compared with the primary root proteome of the mutant *lrt1* that does not initiate lateral roots. Thus, in order to identify genes that are actually related to heterosis, early developmental stages need to be studied. It was already stated by Whaley (1952) that "the early postembryonic part of the growth cycle during which the important differences seem to be developed has been neglected". The only heterosis reference related to root development is by Ashby (1932) indicating that "the most striking manifestation of hybrid vigor is the number of lateral roots", however, without presenting any quantitative data. To our knowledge no studies on the manifestation of heterosis in modern maize hybrids during early seedling development are available. The rationale of this study was therefore to identify traits that display heterosis very early in seedling development and that can be used for subsequent molecular analyses to study the molecular events during the manifestation of heterosis. The young maize primary root provides several advantages relevant for this study. First, the primary root becomes visible as a distinct morphological structure already 2 DAG (Hochholdinger et al. 2004a). Moreover, the primary root has a

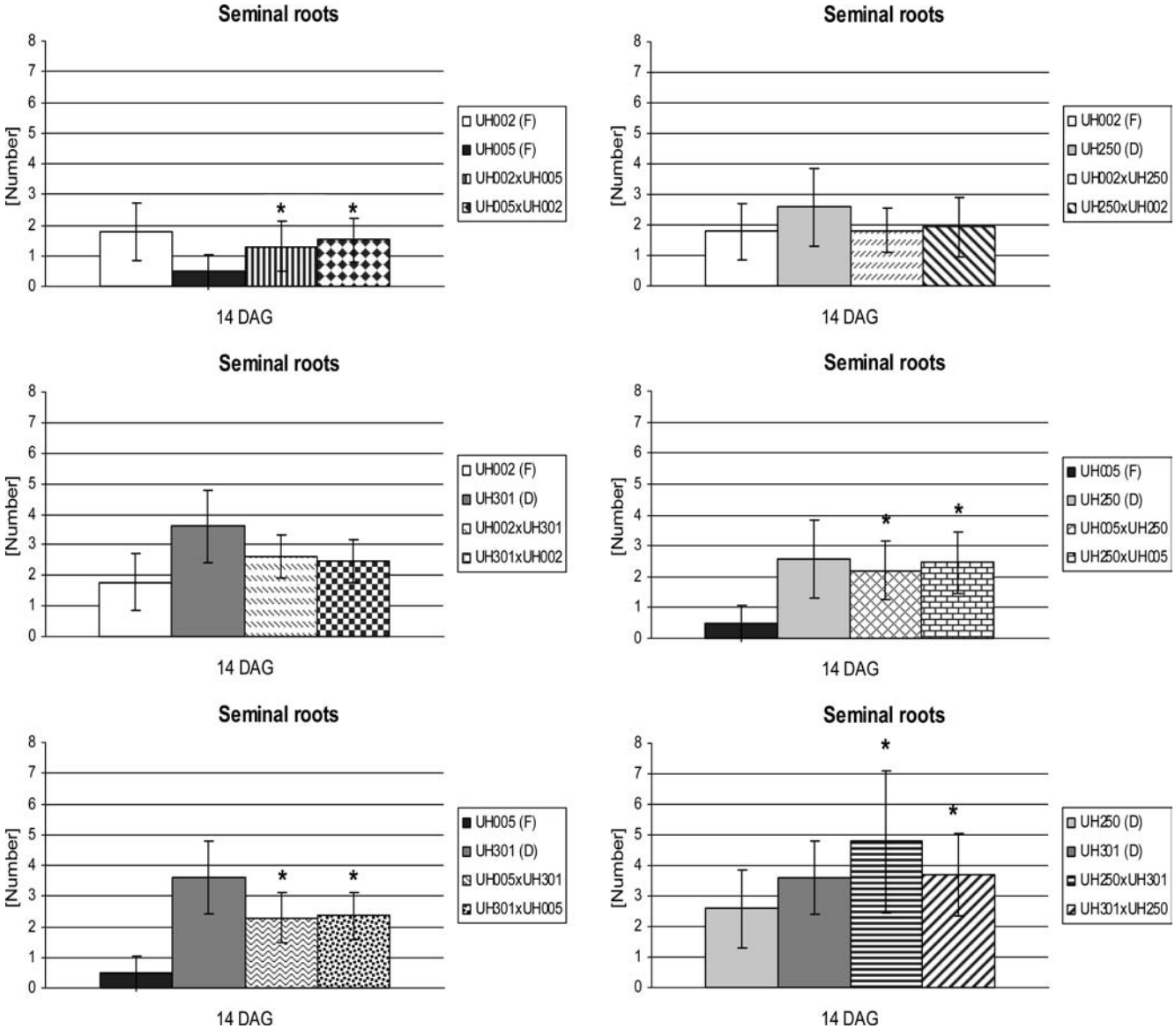

Fig. 4 Number of seminal roots of 4 inbred lines and the 12 resulting reciprocal hybrids 14 DAG. *Each data point* represents at least 50 replicate measurements. *Asterisks* indicate that the hybrid differs significantly from the midparent value (*t* test; $\alpha = 0.05$). *Error bars* represent standard deviations per group. *D* dent, *F* flint

very simple and defined anatomical structure with only little variability, thus representing a very stable morphological system (Hochholdinger et al. 2004a). Finally, the maize primary root has a defined sequence of developmental zones in the longitudinal direction (Ishikawa and Evans 1995) including (beginning from the root tip) the meristematic zone followed by the elongation and the differentiation zone. Root hairs are phenotypical markers that define the differentiated part of the primary roots already 3–5 DAG (Hochholdinger et al. 2004a). While there are a few old studies available on the manifestation of heterosis during embryo development (Ashby 1932; Murdoch 1940; Kempton and McLane 1942; Wang 1942) postembryonic seedling development has only been measured as total seedling dry weight (Sprague 1936; Kempton and McLane 1942) in these early studies.

The 12 maize hybrids investigated in this work represented all possible combinations that could be derived from two inbred lines of the flint pool (UH002 and UH005) and two inbred lines from the dent pool (UH250 and UH301). All traits that were investigated showed MPH to different degrees. Primary root length was the trait that showed the most consistent heterosis. MPH for this trait was in the range between 17 and 26% per time point. MPH concerning root width was on average much smaller and ranged only between 1 and 7% per time point. In addition, MPH was also less consistent concerning primary root width compared to primary root length. Only 22 of 36 hybrid data points

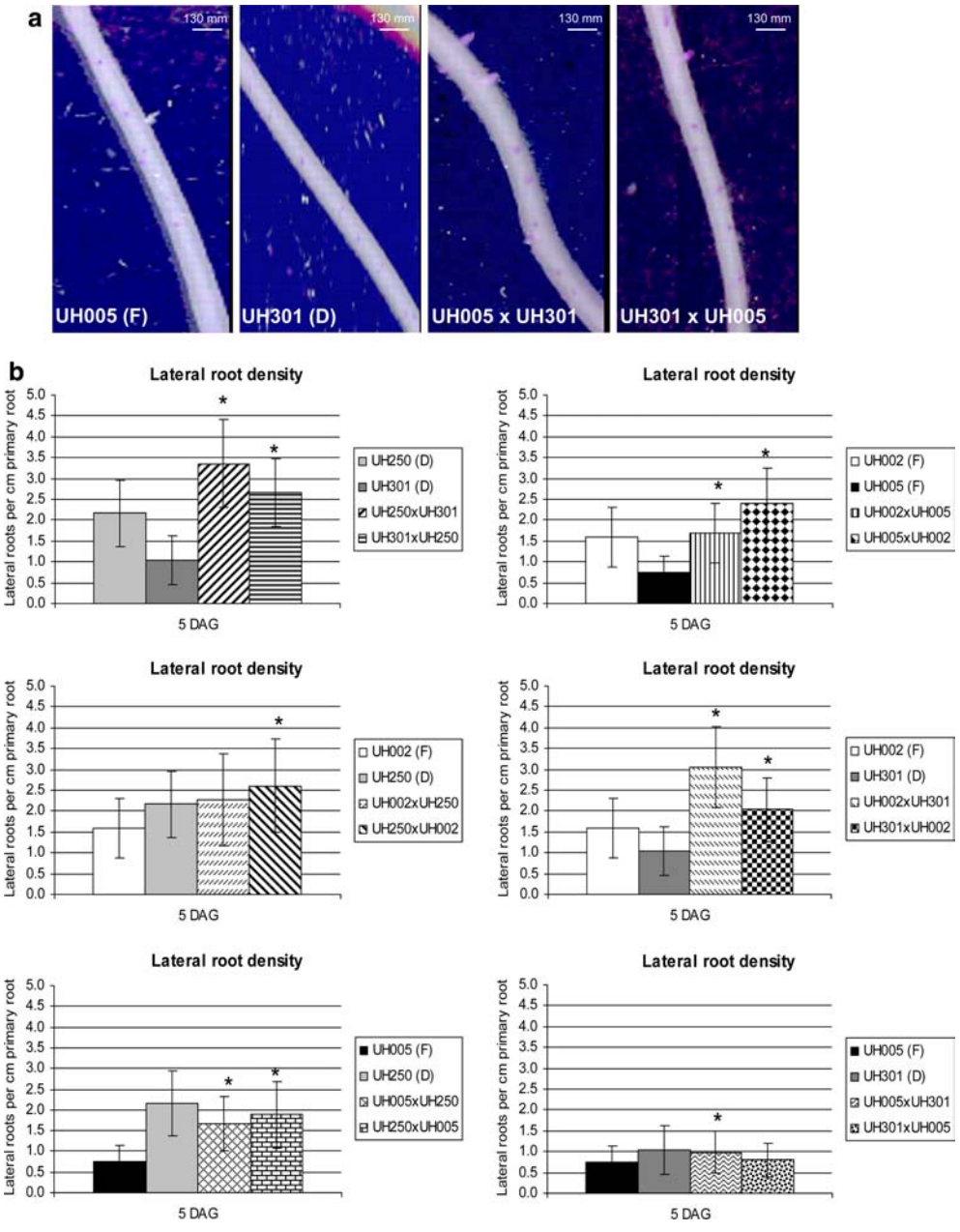

Fig. 5 **a** Detection of lateral root primordia (*purple dots*) in 5 DAG primary roots of the inbred lines UH005 and UH301, and their reciprocal hybrids after Feulgen staining of meristematically active cells. **b** Lateral root density per centimeter of primary root was determined of 4 inbred lines and the 12 resulting reciprocal hybrids. *Each data point* represents at least 50 replicate measurements. *Asterisks* indicate that the hybrid differs significantly from the midparent value (*t* test; $\alpha = 0.05$). *Error bars* represent standard deviations per group. *D* dent, *F* flint, *DAG* days after germination

between 5 and 7 DAG showed significant MPH with reference to primary root width, while 34 of 36 data points displayed significant MPH concerning primary root length. Root elongation reflects the most effective way to increase the absorbing surface of the roots. Increasing the diameter of the roots would be less effective for the plant because this would also correlate with an increase of the root volume. This strategy is reflected by the fact that root diameter in hybrids increases only slightly between 0 and 16% compared to their parental inbred lines in the period between 3 and 7 DAG while root length increases by 600–700% in the analyzed genotypes during this time period. Measurement of cortical cell length demonstrated that elongated primary root length is associated with increased elongation of the cortical cells. Cortical cell length was on average higher for all six measured hybrids compared to their parental inbred lines, although only five of six hybrids showed a statistically significant difference. On average the six hybrids showed a MPH of 24% which is similar to the primary root length MPH of the six hybrids 5 DAG which was 22%. Although cell division rates in the primary root apex were not measured in this study, this data indicates that longitudinal cell size of differentiated and thus maximally elongated cortex cells are major factors responsible for elongated primary root length of hybrids compared to their parental inbred lines. Moreover, the number of seminal roots displayed MPH. On average, hybrids formed 18% more seminal roots than their parental inbred lines. This supports the

importance of seminal roots during the early development. Seminal roots are a major component of water and nutrient uptake during the first weeks of seedling development. The increased number of seminal roots might in part explain the superior performance of hybrid seedlings over inbred seedlings. Finally, lateral root density of inbred lines and hybrids was compared. With one exception all analyzed hybrids showed a higher density of lateral roots (lateral roots/centimeter of primary root). In accordance with Ashby's (1932) unquantified observation lateral root density was also the trait that displayed the highest degree of heterosis in our study of modern hybrids. On average of all 12 hybrids this trait displayed a MPH of 51% while in one instance MPH of 130% was observed. Interestingly, for some traits considerable reciprocal effects were detected. For the trait primary root length 73% of the analyzed hybrids displayed such effects. There have been several reports where differential phenotypic expression was observed between reciprocal hybrids in maize for various germination and kernel traits (summarized in Kollipara et al. 2002). These differences can be attributed in diploid tissue to epigenetic phenomena such as genomic imprinting or cytoplasmatic effects. Genomic imprinting refers to a reversible epigenetic modification of loci resulting in differential expression of genes depending on the parent of origin (Kollipara et al. 2002). Thus, such epigenetic effects appear to play an important role during early maize primary root development.

In summary, this study analyzed several root traits during early seedling development in four inbred lines and 12 hybrids for their heterotic performance. All traits showed heterosis at different degrees. While primary root length between 5 and 7 DAG was the most consistent heterotic trait, the density of lateral root primordia showed the highest degree of heterosis. This study of the young maize root system as a model for heterosis will be the basis of a detailed molecular analysis of gene expression in the young maize root system during the early events of heterosis manifestation.

Acknowledgement We thank Dr. A. Melchinger (University of Hohenheim) and his coworkers for providing seeds of the inbred lines and hybrids used in this study, Dr. T. Schrag (University of Hohenheim) for sharing unpublished results ahead of publication, Dr. R. Schröder and Dr. R. Reuter for advice and access to their CLSM, and S. Kugel and D. Middendorf for excellent technical assistance. This project was supported by the Deutsche Forschungsgemeinschaft (DFG) grants HO 1149/6 and PI 377/5 within the framework program "heterosis in plants".

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