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ORIGINAL PAPER

Mapping of QTLs for lateral and axile root growth of tropical maize

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Abstract Maize genotypes may adapt to dry environments by avoiding desiccation by means of a deeper root system or by maintaining growth and water extraction at low water potentials. The aim of this study was to determine the quantitative genetic control of root growth and root morphology in a population of 236 recombinant inbred lines (RILs) from the cross between CML444 (high-yielding) × SC-Malawi (low-yielding), which segregates for the response to drought stress at flowering. The RILs and the parental lines were grown on blotting paper in growth pouches until the two-leaf stage under non-stressed conditions; the parents were additionally exposed to desiccation stress induced by polyethylene glycol with a molecular weight of 8000 Dalton (PEG-8000). The lengths of axile and lateral roots were measured non-destructively at 2, 5, 7 and 9 days after germination, by scanning with an A4 scanner followed by digital image analysis. CML444 had a lower rate constant of lateral root elongation (k_{Lat}) than SC-Malawi, but the two genotypes did not differ in their response to desiccation. QTLs affecting root vigor, as depicted by increments in k_{Lat}, the elongation rate of axile roots (ER_{Ax}) and the number of axile roots (No_{Ax}) were identified in bins 2.04 and 2.05. QTLs for No_{Ax} and ER_{Ax} collocated with QTLs for yield parameters in bins 1.03–1.04 and 7.03–04. The correspondence of QTLs for axile root traits in bins 1.02–1.03 and 1.08 and QTLs for lateral roots traits in bins 2.04–2.07 in several mapping populations suggests the presence of genes controlling root growth in a wide range of genetic backgrounds.

Introduction

In order to cope with dry environmental conditions plants have evolved different strategies such as the avoidance of and tolerance to desiccation. Desiccation avoidance is associated with the minimization of water loss and the maximization of water uptake (Ludlow and Muchow 1990). Water uptake in drying soils can be improved by optimizing access to water-bearing soil layers. For the individual plant, drought will be most successfully avoided when changes in carbon allocation patterns result in the formation of a deep root system before the onset of a growth-limiting water shortage (Fischer and Turner 1978; Blum 1985; Campos et al. 2004). Improvements of root distribution in the soil (Price et al. 2002), root length density and root hydraulic conductivity (Steudle 2000) are important factors, which determine grain yield in drought-prone environments.

Several authors found genotypic variation for rooting depth in maize: Two drought-tolerant maize hybrids had 2.3–3.3 times more axile roots (referred to as primary roots) in deep moist soil layers than a drought-sensitive hybrid (Wan et al. 2000) when grown in soil pots. The maize hybrid Pioneer-3165, with a deeper root profile, wilted later under drought than Pioneer-3192 (Lorens et al. 1987). Hund et al. (2009a) reported greater rooting depth of the high-yielding subtropical line CML444 compared to the lower-yielding line SC-Malawi. This difference was associated with a greater extraction of water from deep soil layers in the case of CML444.

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Many traits that are related to the water status of the plant may be affected by deeper rooting in drought-prone environments. A drought-tolerant ideotype with improved morphology can be defined, according to Ribaut et al. (2008), as a plant with smaller tassels, smaller leaves above the ear, erect leaves, a larger stem diameter, stay-green and deeper rooting with less lateral branching and less root biomass. Avoiding water loss by smaller leaves in combination with an increased uptake of water and nitrogen due to deeper root systems enhances the avoidance of desiccation during drought periods. This in turn may allow the plant to maintain organ growth and functioning, leading to a narrow anthesis–silking interval (ASI) and to reduced senescence. A narrow ASI (i.e. the synchrony between male and female flowering time) has been an important trait for improving drought tolerance of maize (Edmeades et al. 1999). Desiccation stress during flowering slows down ear and silk growth and delays silk emergence (Fuad-Hassan et al. 2008), leading to reduced kernel set due to a wider ASI. Reduced or delayed plant senescence, which is also referred to as stay-green, is in parts a consequence of a sufficient uptake of N and water (Rajcan and Tollenaar 1999) and may be related to a deep root system with higher uptake rates (Gallais and Hirel 2004). Leaf longevity and the duration of grain filling are both extended in stay-green genotypes (Paponov et al. 2005), thereby increasing the weight per kernel (Wang et al. 1999). These examples illustrate how important the architecture of the root system might be for the response of maize to water-limited conditions in the field.

However, root morphology and architecture are usually not directly accessible for breeders. The obvious reason is that roots located in the soil cannot be assessed with current methodologies. QTL analysis in combination with proteome and transcriptome analysis have revealed insights into the genetic basis of root architecture affecting crop yield under different water regimes (Hochholdinger and Tuberosa 2009). The detection of quantitative trait loci (QTLs) for root traits offers marker-assisted selection (MAS) as an alternative approach to destructive root sampling. Marker-assisted backcross selection has been applied to increase root length and diameter in Indian upland rice varieties by the introgression of four unlinked QTLs (Steele et al. 2006). Shen et al. (2001) transferred Azucena alleles to IR64 in rice for deeper roots. Furthermore, Ribaut and Ragot (2007) used MAS to introgress favorable alleles for yield and flowering traits in five target regions in tropical maize. QTLs for root traits (Tuberosa et al. 2002 and references therein) as well as their response to drought (Lebreton et al. 1995), low phosphorous (Zhu et al. 2005a, b), low nitrogen availability (Gallais and Hirel 2004; Liu et al. 2008) and cold stress (Hund et al. 2004) have been identified. Tuberosa et al. (2002) and Liu et al. (2008) observed collocating QTLs between root morphology at the seedling stage, traits related to drought tolerance in the field and grain yield, which indicates the relevance of root morphology at the seedling stage for plant establishment and performance at later stages.

Preliminary experiments revealed that the tropical maize line CML444 produced more axile than lateral roots in growth pouches relative to SC-Malawi. It may be postulated, that this relative increase of axile roots led to deeper rooting of CML444 at later stages of development as shown by Hund et al. (2009a). Growth pouches, consisting of a piece of blotting paper covered with opaque plastic film, are suited to identify QTLs controlling root morphology at an early seedling stage. Upon validation of these QTLs with results obtained during later stages of development, the pouch system could be used as tool to predict the development of the root system of maize genotypes. Moreover, desiccation stress can easily be applied in growth pouches by varying concentrations of PEG-8000, an osmolyte that lowers the water potential of the substrate (Lawlor 1970; Money 1989; Oertli 1985; van der Weele et al. 2000, Nayyar and Gupta 2006; Sanguineti et al. 2006). This makes it possible to assess the response to desiccation in the pouch system, besides measuring constitutive differences in the development of the root system among genotypes.

The objectives of this study were to (1) evaluate the root development of CML444 and SC-Malawi under well-watered and water-stressed conditions induced with PEG-8000, (2) identify QTLs for root traits at the juvenile stage and after successful identification, (3) compare these QTLs with QTLs previously identified in the reproductive stage under field conditions (Messmer 2006; Messmer et al. 2009).

Materials and methods

Plant material

Two tropical maize inbred lines and 236 recombinant inbred lines (RILs, F7:S6) were used. The parental line CML444 is much alike the ideotype of a drought tolerant maize line described by Ribaut et al. (2008). It has been developed at CIMMYT during the 1990s, selected for a short ASI, and is among the most drought-resistant CIMMYT germplasm. SC-Malawi is an old inbred line developed in Zimbabwe in the 1960s. Compared to CML444, SC-Malawi has a low yield under stress and non-stress conditions (Messmer et al. 2009).

Growth conditions

Seeds were surface sterilized with 2.5% NaOCl solution. Upon germination seeds with equal root lengths were transferred to the moistened blotting paper (Anchor Paper, St. Paul, MN, USA) which was placed in growth pouches (21 cm × 30 cm), as described by Hund et al. (2009b).

In the desiccation stress experiment, pouches were placed upright into plastic containers (27 cm wide × 37 cm long × 32 cm high) containing 4 l of a modified Hoagland solution (5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM KH_2PO_4), so that the lowest 2 cm of the pouches were immersed in the nutrient solution. At 3 days after germination (DAG) the nutrient solution was removed. The plants were then subjected to optimal water availability (0% PEG), a mild (15% PEG) or a severe desiccation stress (20% PEG) treatment. The severity of desiccation stress was quantified by measuring the predawn water potential (WP) on the mesocotyl 5 mm above the seed using a Scholander pressure chamber (Plant Water Status Console 3000, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA) according to Scholander et al. (1965). Plants grown under well-watered conditions had a WP of −0.14 MPa, while the application of desiccation stress resulted in a significantly lower WP of −0.40 (15% PEG) and −0.60 MPa (20% PEG). To allow the water contained in the pouch to get into equilibrium with the PEG solution, the pouches were immersed in PEG solution for 20 min on the first day of the PEG treatment. Pouches were subsequently immersed on a regular basis, to compensate for water losses. The daily immersions were limited to 5 min in order to avoid problems with the oxygen supply of the root system. All the same, the pouches were continuously moist.

In the QTL mapping experiment the pouches were placed in containers (132 cm long × 37 cm wide × 32 cm high) so that the lowest 2 cm of the pouches were immersed in 15 l of the modified Hoagland solution. The containers were placed in a growth chamber (PGW36 Conviron, Winnipeg, MB, Canada) at a temperature of 27°C at the seed level, a relative humidity of 70% and a photosynthetically active radiation of 400 μmol s^{-1} per m^2 at a photoperiod of 12 h. In order to minimize heating due to light radiation, the containers were covered with aluminum laminated styrofoam, leaving a 2 cm wide opening at the seedlings position. At harvest leaf area was measured using a Li-3000A (LI-COR Biosciences, Lincoln, NE, USA).

Root measurements

Roots growing on the surface of the germination blotter were measured non-destructively by scanning with a Hewlett Packard Scanjet 4670 "See Thru Scanner" (Hewlett-Packard, Palo Alto, CA, USA). The pouches were scanned 2, 5, 7 and 9 days after germination (DAG). The acquired 24-bit jpeg images were processed in Adobe Photoshop 7.0 in three steps (Adobe Systems Inc., San Jose, CA, USA) as described by Hund et al. (2009b). In the first step the saturation channel was used to obtain 8-bit images, with enhanced contrast between the roots and the background. In the second step a median filter, with a radius of three pixels, was used to remove image noise, which would have resulted in the detection of spurious roots in WinRHIZO 2003b (Regent instruments, Montreal, QC, Canada). In the third step, a threshold of 120 was applied to the tonal value to obtain binary images. These images were then analyzed by means of WinRHIZO. Seventy-two root diameter-width classes were defined, ranging from 42.33 μm (1 pixel) to 3.05 mm (72 pixels). The debris removal filter removed objects with an area smaller than 0.02 cm^2 and a length/width ratio below five. The lengths of axile and lateral roots were extracted from the root length-in-diameter class distribution obtained by WinRHIZO. Roots with diameters below 0.546 mm were treated as lateral roots, those with diameters above 0.546 mm as axile roots.

Axile and lateral roots seemed to elongate at constant rates up to the destructive harvest. Root elongation was modeled according to the elongation patterns (Fig. 1a, b) using either an exponential or a linear growth function for lateral and axile roots, respectively. Therefore, the increase in the axile root length was modeled according to:

$$x(t) = x(t_0) + \mathrm{ER}_{\mathrm{Ax}}t; \quad \mathrm{ER}_{\mathrm{Ax}} = \frac{x(t) - x(t_0)}{t} \tag{1}$$

where $x(t)$ is the root length at time t after germination, $x(t_0)$ is the root length at the first day of scanning (DAG 3) and $\mathrm{ER}_{\mathrm{Ax}}$ represents the daily elongation rate. The increase in the lateral root length was modeled as:

$$x(t) = x(t_0) \times e^{\mathrm{k}_{\mathrm{Lat}}t}; \quad \mathrm{k}_{\mathrm{Lat}} = \frac{\log(x(t)) - \log(x(t_0))}{t} \tag{2}$$

where $\mathrm{k}_{\mathrm{Lat}}$ is the rate constant for lateral root elongation. The mean R^2 of each fitted model (see Fig. 2) was extracted and used to identify plants with a bad model fit (R^2 below 0.8). These plots were evaluated separately and problematic images were eventually eliminated from the analysis (3 images based on low R^2 for $\mathrm{k}_{\mathrm{Lat}}$ and 1 based on a low R^2 value for $\mathrm{ER}_{\mathrm{Ax}}$). In order to obtain a measure of the increase of lateral root length per unit increase of axile root length, the ratio between $\mathrm{k}_{\mathrm{Lat}}$ and $\mathrm{ER}_{\mathrm{Ax}}$ was calculated ($\mathrm{k}_{\mathrm{Lat}}/\mathrm{ER}_{\mathrm{Ax}}$).

The plants were harvested ten DAG. The dry weight of the shoots (SDW) and roots (RDW) was determined after drying for 72 h at 65°C. The length of the primary axile root ($\mathrm{L}_{\mathrm{PrAx}}$) was measured digitally in images acquired nine DAG using the ruler tool in Photoshop. The number of axile roots ($\mathrm{No}_{\mathrm{Ax}}$) was counted manually on nine DAG.

Fig. 1 Mean values of lateral root length (**a**) and axile root length (**b**) of the CML444 × SC-Malawi population non-invasively measured 2, 5, 7 and 9 days after germination. *Error bars* represent 1 SE

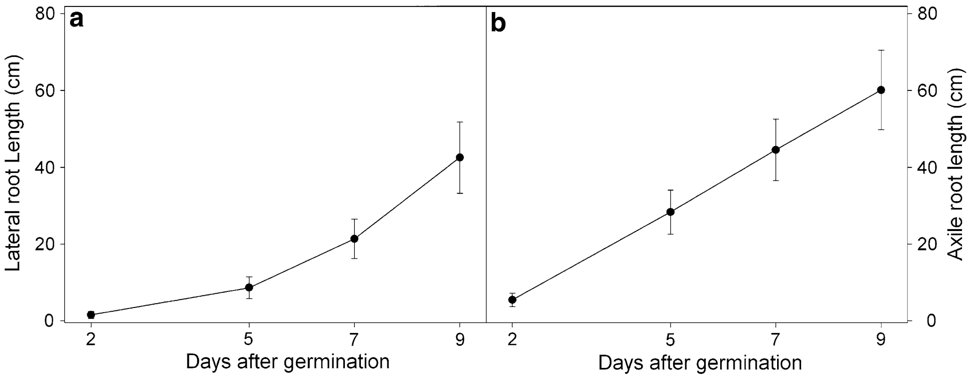
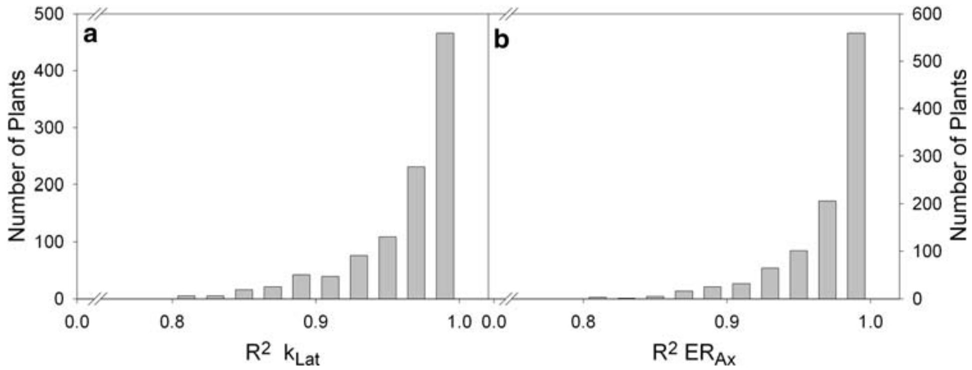


Fig. 2 Frequency distribution of the coefficients of determination (R^2) of model 1 (**a**) and model 2 (**b**), fitted to the development of each individual plant, to estimate the elongations rates of lateral (k_{Lat}) and axile (E_{RAx}) roots, respectively



Experimental design and statistics

Desiccation stress experiment

The desiccation stress experiment was carried out with the parental lines only. These were evaluated in six consecutive growth chamber runs (r_k, $k = 1,\ldots,6$), each containing two desiccation stress treatments (15 and 20% PEG) and a non-stressed control treatment (without PEG). Each treatment contained one plant of either line, resulting in a total of six plants per genotype and treatment. The phenotypic data was analyzed with the linear mixed-effect model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + r_k + (r\beta)_{kj} + \varepsilon_{ijk} \qquad (3)$$

where Y_{ijk} is the trait value of genotype i in treatment j within growth chamber run k, $\alpha\beta$ is the genotype-by-treatment interaction, $r\beta$ the growth chamber run-by-treatment interaction, and ε the residual error. All effects were fixed, except for r, $r\beta$ and ε.

Analysis of variance was performed using the GLM procedure in NCSS (Number Cruncher System, Kayesville, UT, USA). Comparisons among treatment levels and among genotypes were carried out using the Tukey–Kramer multiple comparison test.

QTL mapping experiment

The experiment was designed as an alpha lattice (0,1) with six independent replications (r_j), 240 genotypes (g_i; 236 RILs and 2 × 2 parents) and 12 plots per incomplete block (p_{jkl}). The 20 incomplete blocks were distributed in five growth containers (b_{jk}; 132 cm × 32 cm 5 cm) in a growth chamber. The effects of the replication were considered to be fixed, while genotypes, containers and incomplete blocks were random.

Accordingly, the mixed linear model was:

$$Y_{ijkl} = \mu + r_j + g_i + b_{jk} + p_{jkl} + \varepsilon_{ijkl} \qquad (4)$$

where Y_{ijkl} is the effect of genotype i in growth chamber run j, growth container k and incomplete block l, and ε is the residual error.

Analysis of variance was carried out with the ASReml-R package (Butler 2006), and the best linear unbiased predictors (BLUPs) were extracted and used as the input values for QTL mapping. The broad-sense heritability of each trait was calculated as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{1}{j}\sigma_\varepsilon^2} \qquad (5)$$

where σ_g^2 is the genetic variance, σ_ε^2 the residual error variance and j the number of replications.

Phenotypic Pearson's correlations between pairs of traits were estimated using the function cor.test() in R (R Development Core Team 2004). Significant correlations with $0.2 \leq r^2 \leq 0.5$, $0.5 < r^2 \leq 0.8$, $0.8 < r^2$, were defined as weak, moderate and strong, respectively.

QTL analysis

QTLs were identified by composite interval mapping (CIM) using QTL cartographer 1.17 (Basten et al. 2002) based on genetic information from the linkage map published in Messmer et al. (2009). Co-factors were selected by forward and backward regression with the in and out values set at 0.05 and 0.1, respectively. In zmapqtl a walking speed of 2 cM was selected. The number of background parameters was set to 5. QTLs were estimated on the basis of the BLUPs and a LOD threshold of 2.6. Assuming that each chromosome arm segregates independently, the corresponding experimental Type-*I* error probability (α') was approximately 0.05.

QTLs for different traits collocated when their LOD-support intervals overlapped, which were defined as the intervals on the chromosome in which the LOD at the peak decreased by half. A collocation was considered to be positive when the additive effects had the same algebraic sign and negative when they had opposed algebraic signs.

Results

Desiccation stress

The desiccation treatment significantly affected k_{Lat}, ER_{Ax}, RDW, WP and leaf area. CML444 and SC-Malawi significantly differed for k_{Lat}, WP and leaf area (Table 1). No significant desiccation-by-genotype interactions were identified. In response to the highest desiccation level, k_{Lat} and ER_{Ax} were reduced by 20 and 32%. By contrast, L_{PrAx} and No_{Ax} were not altered in response to desiccation. In accordance with reductions in root elongation, RDW was reduced by 19 and 50% at the moderate and the severe desiccation stress level, respectively. With respect to shoot traits both WP (−65%; −74%) and leaf area (−44%; −55%) were reduced at the 15 and 20% PEG treatment, respectively. The application of desiccation stress resulted in similar responses for both CML444 and SC-Malawi, none being more tolerant to the PEG-induced desiccation stress. However, irrespective of the PEG treatment the genotypes differed for k_{Lat}, WP and leaf area. The obtained data strongly suggest that SC-Malawi formed more lateral roots (data not shown) resulting in higher k_{Lat} (+20%) than CML444. SC-Malawi also formed a larger leaf area (+25%). Since the genotypes did not differentially respond to the desiccation treatment, we concluded that constitutive differences in root morphology were of greater importance than adaptive mechanisms in response to desiccation stress. The evaluation of the RILs was therefore carried out under control conditions only.

QTL experiment

Differences in root traits

Within the RIL population the axile roots grew linearly (Fig. 1b), while lateral roots grew exponentially (Fig. 1a). The coefficients of determination (R^2) of the model fitted for each RIL individually ranged from 0.78 to 0.99 for the linear ER_{Ax} and from 0.69 to 0.99 for the exponential k_{Lat}, with average values of 0.96 and 0.97 per RIL (Fig. 2).

The results from the QTL experiment were similar to those obtained in the desiccation stress experiment: CML444 formed a root system with a lower proportion of lateral roots than SC-Malawi, indicated by a lower k_{Lat}/ER_{Ax} (Table 2). This low ratio was a result of both a significantly lower k_{Lat} (−12%) and a non-significant but markedly higher ER_{Ax} (+24%) of CML444.

The growth constant of the lateral roots, which was determined in the RIL population, had an interquartile range (IQR) between 0.363 and 0.413 cm day^{-1} and a heritability of 0.73, whereas ER_{Ax} had an IQR between 6.72–9.40 cm day^{-1} and a heritability of 0.8. As a result, the ratio between k_{Lat} and ER_{Ax} had an IQR between 0.0416 and 0.0569. The heritability of this ratio was 0.83. Similar heritabilities were obtained for RDW (0.87) and leaf area (0.84).

There was a weak positive correlation between HKW and each of the following traits: No_{Ax}, ER_{Ax}, the length of the axile root (L_{Ax}) and leaf area, indicating that the growth of the seedling was affected by seed reserves (Table 3). With regard to root morphological traits, we observed a typical covariance among the hierarchically dependent root traits. Since ER_{Ax} estimates the overall growth rate of all the axile roots, a strong positive correlation was found with the number of axile roots; since the lateral roots emerged from the mother axile root, k_{Lat} showed a weak positive correlation with ER_{Ax}. Similarly, the ratio between k_{Lat} and ER_{Ax} showed a strong negative correlation. ER_{Ax} showed a strong positive correlation with RDW, while the contribution of k_{Lat} was not significant. This finding is confirmed by a weak negative correlation between the ratio of k_{Lat} and ER_{Ax} with RDW. Furthermore, a moderate negative correlation was found between leaf area and the ratio of k_{Lat} to ER_{Ax}.

Table 1 Analysis of variance for root and shoot traits of CML444 and SC-Malawi as affected by desiccation stress induced by polyethylene glycol (PEG)

ANOVA	No_{Ax}	ER_{Ax} (cm day^{-1})	L_{PrAx} (cm)	k_{Lat} (cm day^{-1})	$k_{Lat}ER_{Ax}$	RDW (mg)	WP (-MPa)	LA (cm^2)
Significance level								
Treatment	ns	**	ns	*	ns	*	***	***
Genotype	ns	ns	ns	*	ns	ns	***	*
Treatment × genotype	ns	ns	ns	ns	ns	ns	ns	ns
Treatment (% PEG)								
0	4.8	6.26 a	181	0.478 a	0.08	63.3 a	0.139 a	27.8 a
15	4.1	5.99	210	0.463	0.08	51.2	0.397 b	15.6 b
20	3.5	4.46 b	206	0.414 b	0.13	38 b	0.606 c	12.1 b
Genotype								
CML444	4.1	5.77	204	0.426 a	0.07	45.3	0.55 a	15.9 a
SC-Malawi	4.2	5.36	193	0.478 b	0.12	56.4	0.31 b	21.1 b

Significance level and mean values for each treatment are displayed for the number of axile roots (No_{Ax}), the elongation rate of axile roots (ER_{Ax}), the length of the primary axile root (L_{PrAx}), the rate constant of lateral root elongation (k_{Lat}), the ratio between k_{Lat} and ER_{Ax} (k_{Lat}/ER_{Ax}), the root dry weight (RDW), water potential (WP) and leaf area (LA). Values followed by the same letter are not significantly different at $P = 0.05$. Since no significant genotype-by-treatment interaction was observed mean values for genotypes across treatments are not shown

Table 2 Summary statistics of traits measured for the parental inbred lines (means) and their segregating RIL offspring

Trait	CML444	Malawi	Sig	Pop. Mean	First Quartile	Third Quartile	h^{2} [a]
L_{Ax} (cm)	54.5	42.9	ns	63.2	52.2	72.9	0.81
No_{Ax}	5.17	4.09	ns	5.08	4.51	5.54	0.69
ER_{Ax} (cm day^{-1})	7.33	5.60	ns	8.11	6.72	9.40	0.8
L_{PrAx} (cm)	21.1	22.8	ns	23.1	21.0	34.3	0.81
L_{Lat} (cm)	28.5	38.9	ns	43.4	31.6	51.2	0.88
k_{Lat} (cm day^{-1})	0.37	0.42	*	0.387	0.363	0.413	0.73
$k_{Lat}ER_{Ax}$	0.0509	0.0756	*	0.0500	0.0416	0.0569	0.83
RDW (mg)	54.8	29.2	ns	42.5	37.2	46.4	0.87
LA (cm^2)	16.4	17.9	ns	21.29	18.65	23.7	0.84
HKW (g)	25.0	25.0	na	24.4	20.6	28.0	na

Traits were axile root length (L_{Ax}), the number of axile roots (No_{Ax}), the elongation rate of axile roots (ER_{Ax}), the length of the primary root (L_{PrAx}), the rate constant of lateral root elongation (k_{Lat}), the ratio between k_{Lat} and ER_{Ax} (k_{Lat}/ER_{Ax}), root dry weight (RDW), leaf area (LA) and hundred kernel weight (HKW)

na not available

* Significant differences ($P = 0.05$) between parents

[a] Mean-based heritability

Detected QTLs

All in all 12 QTLs were identified for four different root traits (Table 4): one for L_{PrAx}, three for k_{Lat} (all of them with negative additivity), four for both ER_{Ax} and No_{Ax} and five for HKW. No QTL was detected for k_{Lat}/ER_{Ax}.

The genomic region in bins 2.03–2.05 affected general root vigor. Four QTLs for No_{Ax}, HKW, ER_{Ax} and k_{Lat} positively collocated in bins 2.03–2.05. The allele of SC-Malawi increased the trait values for No_{Ax}, HKW, ER_{Ax} and k_{Lat} by 0.19, 1.36 g, 0.48 and 0.01 cm day^{-1}, respectively. The fact that there was a QTL for HKW in this region indicates that root vigor was affected by seed reserves. Therefore, the QTL for root vigor may in fact be a seed vigor QTL, which affects root growth.

In addition to the QTL for root vigor, three QTLs were identified for ER_{Ax} (Table 4). The presence of the CML444 allele altered ER_{Ax} by 0.54 cm day^{-1} (bin 1.03), 0.55 cm day^{-1} (bin 1.08) and −0.54 cm day^{-1} (bin 7.03). Each of the QTLs in these three regions explained about 7.6% of the phenotypic variance (PVE). Most QTLs identified for ER_{Ax} collocated with QTLs for No_{Ax}, reinforcing the strong correlation between these traits.

QTLs only altering k_{Lat} were identified in bins 1.01 and 8.01. Values for k_{Lat} were increased by alleles derived from SC-Malawi. Trait values for k_{Lat} were altered by −0.0093

Table 3 Pearson's phenotypic correlation coefficients among traits measured in the RIL population: hundred kernel weight (HKW), leaf area (LA), root dry weight (RDW), the constant of lateral root elongation (k_{Lat}), the elongation rate of axile roots (ER_{Ax}), the number of axile roots (No_{Ax}) and the ratio between k_{Lat} and ER_{Ax} (k_{Lat}/ER_{Ax}) and the length of the primary root (L_{PrAx})

	LA	RDW	k_{Lat}	ER_{Ax}	No_{Ax}	L_{Lat}	L_{Ax}	k_{Lat}/ER_{Ax}	L_{PrAx}
HKW	0.53***	0.21*	0.02*	0.42***	0.39***	0.23**	0.42***	0.14***	0.00, ns
LA		0.51***	0.12, ns	0.51***	0.47***	0.43***	0.61***	−0.56***	0.15***
RDW			0.14, ns	0.52**	0.42***	0.40***	0.56***	−0.45***	0.15***
k_{Lat}				0.38***	0.21**	0.68***	0.38***	0.08. ns	0.07***
ER_{Ax}					0.82***	0.53***	0.99***	−0.85***	0.18***
No_{Ax}						0.34***	0.81***	−0.77***	0.12, ns
L_{Lat}							0.60***	−0.19***	0.28***
$k_{Lat}ER_{Ax}$									0.11***

Correlation coefficients followed by *, **, and *** are significant at $P = 0.05$, 0.01, and 0.001, respectively

Table 4 QTLs (LOD > 2.6) involved in the expression of the number of axile roots (No_{Ax}), the elongation rate of axile roots (ER_{Ax}), the length of the primary root (L_{PrAx}), the rate constant of lateral root elongation (k_{Lat}), the ratio between k_{Lat} and ER_{Ax} ($k_{Lat}ER_{Ax}$), root dry weight (RDW), leaf area (LA) and hundred kernel weight (HKW)

Trait	Chr	Marker	Mark	Bin	Peak	Interval	LOD	ADD	PVE %
No_{Ax} (cm)	1	3	umc1041	1.01	45	23–53	4.89	0.24	8.93
No_{Ax} (cm)	1	17	umc128	1.08	219	214–231	2.61	0.17	4.31
No_{Ax} (cm)	2	5	csu40	2.03	100	88–114	3.16	−0.19	5.49
No_{Ax} (cm)	7	10	bnl14.07	7.04	106	97–129	4.97	−0.26	10.1
ER_{Ax} (cm day^{-1})	1	9	bnlg439	1.03	108	98–130	4.23	0.54	7.39
ER_{Ax} (cm day^{-1})	1	17	umc128	1.08	219	214–231	4.53	0.55	7.81
ER_{Ax} (cm day^{-1})	2	7	umc8 g	2.05	114	100–121	3.43	−0.48	5.83
ER_{Ax} (cm day^{-1})	7	9	bnlg1805	7.03	93	81–110	3.26	−0.54	7.46
L_{PrAx} (cm)	8	4	umc103a	8.02	63	51–78	2.72	−0.84	5.57
k_{Lat} (cm day^{-1})	1	1	phi056	1.01	10	0–20	2.97	−0.0093	5.15
k_{Lat} (cm day^{-1})	2	7	umc8 g	2.05	116	100–123	3.81	−0.011	7.21
k_{Lat} (cm day^{-1})	8	1	npi114a	8.01	0	0–14	4.24	−0.011	7.36
LA (cm^2)	5	17	umc104b	5.08	244	228–244	3.06	1.01	6.09
LA (cm^2)	6	6	umc65a	6.04	65	55–72	3.80	−1.10	7.24
HKW (g)	1	24	bnlg2331	1.11	347	330–360	2.93	1.16	4.87
HKW (g)	2	6	umc135	2.04	102	90–114	3.31	−1.36	6.42
HKW (g)	2	12	csu154a	2.07	162	155–167	2.67	−1.12	4.71
HKW (g)	6	6	umc65a	6.04	56	43–68	4.18	−1.38	7.14
HKW (g)	7	2	umc1066	7.01	13	1–25	6.45	1.91	12.7

Chromosome number (Chr), marker number on the chromosome (Marker), marker name (Mark), chromosome segment (Bin), position of the peak in cM (Peak), 0.5*LOD support interval (Interval), LOD score at the peak (LOD), additive genetic effect of the CML444 allele on trait expression (ADD) and percentage of phenotypic variation explained by an individual QTL (PVE)

and 0.0111 cm day^{-1}, respectively for both QTLs at PVE of 5.1 and 7.4%.

Collocation of QTLs for root traits with QTLs identified in the reproductive stage in the field

In order to get an idea of possible associations between the genetic control of seedling root traits and morpho-physiological traits measured in the same RIL population in several field experiments involving well-watered conditions and drought stress at flowering, the QTLs of the present study were compared with QTLs reported by Messmer (2006). Three genomic regions were identified on chromosomes 1, 2 and 7. The LOD support interval of the QTL for ER_{Ax} in bin 1.03 (near bnlg439) overlapped with those of QTLs in the most outstanding region identified by Messmer (2006) (Fig. 3). The latter comprises QTLs for grain yield and plant height in different environments, which suggested the presence of major genes regulating carbon-partitioning mechanisms, as well as QTLs for leaf greenness (SPAD-value) and

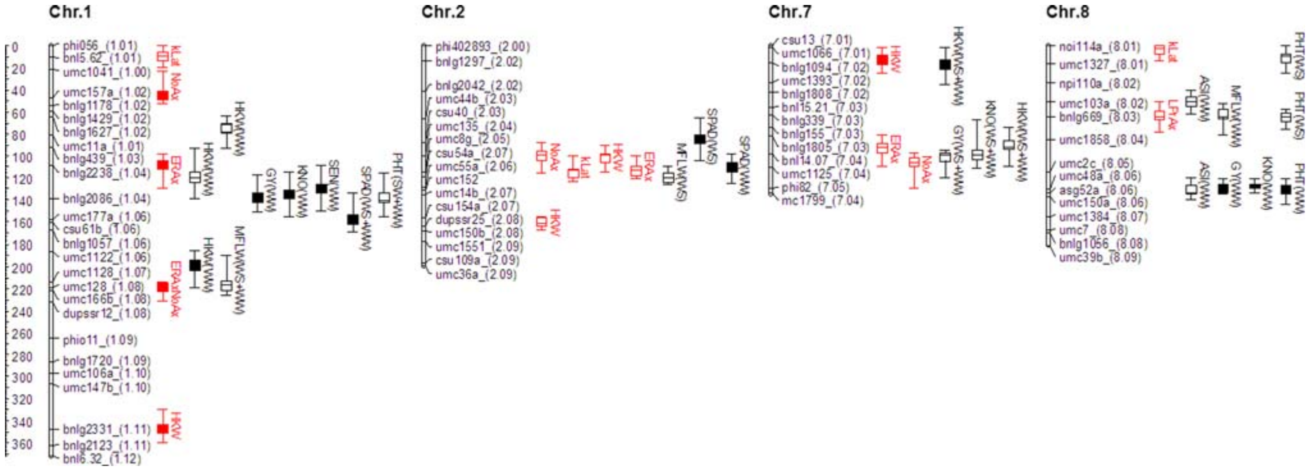


Fig. 3 Collocations of QTLs for seedling traits (*red boxplots*) with those detected by Messmer (2006) at the reproductive stage (*black boxplots*) under water stress (*WS*), well watered (*WW*) or both (WW + WS). Seedling traits were the rate constant of lateral root elongation (k_{Lat}), elongation rate of axile roots (E_{RAx}), the number of axile roots (No_{Ax}), the length of the primary root(L_{PrAx}) and the hundred kernel weight (*HKW*). Traits at the reproductive stage were the time to male flowering (*MFLW*), plant height (*PHT*), relative chlorophyll content of the ear leaf and/or the second leaf from the tassel (*SPAD*), visual senescence scoring (*SEN*), kernel number (*KNO*), hundred kernel weight (*HKW*) and grain yield (*GY*). The *boxes* indicate intervals of ±4 cM around the peak of the QTL. *Whiskers* indicate the LOD support intervals. *Filled* and *empty symbols* represent positive and negative additive effects (CML444 allele)

a visual score of plant senescence, mostly under well-watered conditions. The alleles of CML444 contributed to an increase in $\mathrm{ER}_{\mathrm{Ax}}$, to higher leaf greenness and to either higher grain yield (under well-watered conditions) or a reduced plant height (under drought stress). The cluster of QTLs for root vigor on chromosome 2 (bin 2.03–2.05) collocated with a cluster of QTLs controlling the greenness of the ear leaf and the second leaf from the tassel at flowering in six field experiments with different levels of water availability. While the allele of CML444 decreased the phenotypic value of all concerned root traits in the present study (Table 4), it was associated with higher leaf greenness at flowering (Messmer 2006). The third cluster of collocating QTLs was located on chromosome 7 (bin 7.03–7.04), where the support intervals of QTLs for $\mathrm{No}_{\mathrm{Ax}}$ and $\mathrm{ER}_{\mathrm{Ax}}$ overlapped with those of QTLs for grain yield, kernel number and hundred kernel fresh weight, which were identified in a combined QTL analysis across seven experiments. The allele of CML444 at these QTLs decreased the phenotypic values of $\mathrm{No}_{\mathrm{Ax}}$ and $\mathrm{ER}_{\mathrm{Ax}}$ (Table 4) as well as those of the yield parameters (Messmer et al. 2009).

Discussion

Parental lines show similar responses to PEG-induced desiccation

For both genotypes the desiccation treatment caused a reduction in shoot and root growth except for the growth of the primary axile root ($\mathrm{L}_{\mathrm{PrAx}}$). Apart from the lack of differences in response to desiccation among the parents, the stress treatment caused symptoms similar to those observed in vermiculite substrate with a low water potential; i.e. decreased LWP and LA (Sharp and Davies 1985) and decreased root elongation rates (Sharp et al. 2004). However, care must be taken when interpreting the results from desiccation experiments using osmolytes such as PEG. Although PEG with a high molecular weight was used in many studies to simulate the effect of drought on the root system (Lawlor 1970; Money 1989; van der Weele et al. 2000; Nayyar and Gupta 2006) and to differentiate among genotypes (Sanguineti et al. 2006), it should be kept in mind that drought stress in the field does not affect the soil water potential alone. For example, drought goes along with changes in the mechanical resistance and soil-grown plants respond to drought in a complex way. However, PEG may be used for a first and rapid screening for desiccation tolerance.

The parents did not differ in their response to PEG and in their desiccation tolerance, but they differed strongly in root morphology, irrespective of the desiccation treatment. This observation is in line with other studies, which emphasized that constitutive rather than adaptive differences are key factors for drought adaptation of the root system in cereals: Hund et al. (2009a, for maize) and Kato et al. (2006, for rice) reported constitutive differences among genotypes for total root length and the amount of roots at depths below 50 cm. Moreover a constitutive QTL controlling leaf ABA (*root-ABA1*) content under water-stressed and well watered conditions with effects on root architecture (number, angles, branching, diameter and weight) (Giuliani et al. 2005) ultimately affecting root lodging and grain yield (Landi et al. 2007) was identified in bins 2.04.

Differences in k_{Lat} and ER_{Ax} may be explained by differences in the organization of the embryonic root system

We reported differences in root growth rates, rather than differences in root length at a given time to avoid errors due to differences in germination as pointed out by Hund et al. (2009b). At the seedling stage the variation in the length of the lateral roots that is due to differences in germination can be large. Initially, the entity of lateral roots per genotype elongates exponentially unless the first lateral roots cease growing. The growth constant k_{Lat} serves as an integrative measure for the potential final length and linear density of the lateral roots (Hund et al. 2009b). However, the separation into axile and lateral roots disregards the organization of the embryonic root system. Primary roots show considerable genetic variation with respect to the length of individual lateral roots. Depending on the genotype, the lengths of the longest lateral roots may vary between 2 and 17 cm at the V2 stage (Hund et al. 2007), while those of the seminal roots are usually short and in the range of 3 cm (McCully 1999; Hund et al. 2007). Hund et al. (2007) classified genotypes according to the organization of the embryonic root systems as homogeneous (similar primary and seminal roots) and heterogeneous (lateral roots of the primary root generally longer than the lateral roots of the seminal roots). A similar morphology was also demonstrated for two RILs used in the present study. The RILs differed for k_{Lat} because of differences in the final length and linear density of the primary lateral roots (Hund et al. 2009b). Apart from a direct genetic control of lateral root initiation and growth, more seed reserves may explain the more intense branching of the primary root as pointed out by Enns et al. (2006). These authors observed that the length and linear density of the population of primary lateral roots (branch roots) of about the first 8 cm proximal to the seed strongly depended on seed reserves. Indeed, the correlations between HKW and root traits as well as leaf area indicate the influence of the seed weight on early vigor. However, lateral roots and the k_{Lat}/ER_{Ax} ratio were hardly correlated ($r < 0.2$) with HKW, which indicates that root morphology as such was little affected by seed reserves. Nevertheless it should be considered that the collocating QTLs controlling HKW, axile and lateral root traits (bins 2.04–2.05) indicate a certain genetic correspondence.

The relationship between axile and lateral roots may be indicative for rooting depth

CML444 formed a root system with constitutively less lateral roots and in relation to the entire root length more axile roots than SC-Malawi, as indicated by a higher ER_{Ax} (ns), L_{PrAx} (ns) and a lower k_{Lat}. Root elongation depends on carbon availability within the plant (Pritchard and Rogers 2000). Assuming an ample nutrient supply in the soil, the growth of axile roots may be increased in a root system with fewer lateral roots, leading to deeper rooting. This hypothesis is supported by findings of Hund et al. (2009a) who showed that CML444 had a root system with fewer lateral roots in the topsoil but with thicker, deep-reaching axile roots, compared to SC-Malawi. As a consequence CML444 took up more water than SC-Malawi (Hund et al. 2009a). There are other studies supporting the hypothesis that the relationship between axile and lateral roots may be a critical trigger for the resource allocation within the root system leading to increased rooting depths. Maize genotypes with a reduced development of crown (adventitious) and lateral roots at the seedling stage (Bruce et al. 2002) and with a smaller amount of roots in the top 50 cm of the soil profile (Bolaños et al. 1993) extract less water from the topsoil (Campos et al. 2004) and are better adapted to drought conditions. This is in accordance with the model discussed by Giuliani et al. (2005) showing that a QTL for leaf-ABA content constitutively controlled root architecture irrespective of water availability. This gives rise to the important question whether the root morphology in growth pouches can be predictive for the performance under drought at later stages of development.

QTLs for seedling root growth are associated with QTLs for grain yield in the field

The allelic effects at QTLs in the two genomic regions where collocating QTLs for seedling root traits and yield parameters in the field were observed are in agreement with the hypothesis that the growth of axile roots (at the seedling stage) is positively associated with rooting depth and, potentially, with drought resistance in the field (at flowering). The increase in the elongation of axile roots provoked by the CML444 allele at the respective QTL in bin 1.03–04 coincided with a positive effect on grain yield in an optimal growing environment. Under suboptimal conditions in the field, when the carbon allocation pattern within the plant seemed altered and the overall carbon assimilation was reduced, the CML444 allele provoked a reduction in plant growth. It is likely that the CML444 allele and the collocating QTL for ER_{Ax} reduced the elongation rate of axile roots in the field. This would explain why the QTL effect on grain yield disappeared in these situations. Moreover

Table 5 Comparison of root trait QTLs found in this study with those published in literature

Bin	Marker	Pioneer	Trait	Population	Reference
1.02	umc157a	29.8	No_{Ax}	CML444 × SC-Malawi	
1.02	bnlg1014	20.7	rtcs		Hochholdinger and Feix (1998) and Taromina et al. (2007)
1.02	bnlg1429	34.5	rtcs		
1.03	bnlg439	61	ER_{Ax};No_{Ax}	CML444 × SC-Malawi	
1.03	bnlg2238	69	ER_{Ax};No_{Ax}	CML444 × SC-Malawi	
1.03	bnlg2086	101	ER_{Ax};No_{Ax}	CML444 × SC-Malawi	
1.03	bnlg176	51	Length and weight of the primary root	Lo964 × Lo1016	Tuberosa et al. (2002)
1.03	umc11a	53	Leaf abscisic acid content	Os420 × IABO78	Tuberosa et al. (1998)
1.03	umc11a	53	Primary root weight, seminal axile root length	Lo964 × Lo1016	
1.03	asg45	74			Hund et al. (2004)
1.03	bnlg1866	60	Axile root length	Z3 × 87–1	
1.03	bnlg2180	64			Liu et al. (2008)
1.08	umc128	187	ER_{Ax};No_{Ax}	CML444 × SC-Malawi	
1.08	umc166b	167	ER_{Ax};No_{Ax}	CML444 × SC-Malawi	
1.08	dupssr12	160	ER_{Ax};No_{Ax}	CML444 × SC-Malawi	
1.08	gsy282	160	Whole plant nitrogen uptake efficiency	Io × F2	
1.08	umc83a	174	Whole plant nitrogen uptake efficiency	Io × F2	Gallais and Hirel (2004)
1.08	php20644	137	Length and weight of the primary root	Lo964 × Lo1016	Tuberosa et al. (2002)
1.08	umc128	187	Leaf abscisic acid content	Os420 × IABO78	Tuberosa et al. (1998)

Position of potential candidate genes and QTLs in relation to the QTLs detected in the present study and to common SSR markers from the CML444 × SC-Malawi population and from the Pioneer composite 1999 map

and as outlined below, this genomic region seems to be generally important for the genetic control of both shoot and root development.

The corresponding signs of the additive effects at the collocating QTLs on chromosome seven further suggest that the genetic control of root growth may be linked with the reproductive behavior in the plant material studied. However, the QTL effect on grain yield and kernel number has to be interpreted with care, as it was not significant in the analyses of individual experiments (data not shown).

Several genomic regions control the same root traits in other genetic backgrounds

We identified a QTL for No_{Ax} in the vicinity of the anchor marker *umc157a* (at 29.8 cM on the 1999 Pioneer composite map). The *rtcs* mutant (Hetz et al. 1996) lacking crown and seminal roots maps to the same region (Table 5). The *rtcs* locus has been cloned and is relevant for the activation of auxin-responsive genes involved in the initiation of root formation (Taromina et al. 2007). It is therefore possible that the QTL detected for No_{Ax} is a QTL involved in hormone physiology determining the number of axile roots.

The QTL identified for ER_{Ax} in bin 1.03 collocated with QTLs identified in the Lo964 × Lo1016 population for the weight of the seminal roots of hydroponically-grown seedlings and for grain yield in the field (Tuberosa et al. 2002; Hochholdinger and Tuberosa 2009) and the number and length of the seminal roots (Hund et al. 2004). Thus, in two independent populations and in several QTL studies, seminal root numbers and length were controlled by this locus. The QTL identified for ER_{Ax} in bin 1.08 collocated with QTLs for leaf ABA-content (Tuberosa et al. 1998), the weight of the seminal roots (Tuberosa et al. 2002), N-uptake (Gallais and Hirel 2004) and the average axile root length under high and low N (Liu et al. 2008). Overall these collocations indicate that the QTLs identified for ER_{Ax} in bins 1.03 and 1.08 are very important for axile root growth and underline the importance of a well developed root system for sufficient uptake of water and N, and for yield formation. Furthermore the QTL for k_{Lat} in bin 2.05 identified in the present study, collocated with a QTL for the number and length of lateral roots under conditions of high and low phosphorous in the IBM population (Zhu et al. 2005b). In summary, QTLs for root mass and axile root growth were found at the same position in several populations. Therefore it may be anticipated that the identified QTLs revealed a common genetic basis of (axile) root growth, N-acquisition and ABA-homeostasis.

Although growth rates of axile and lateral roots were affected by several QTLs, these explained only a small proportion of the observed phenotypic variance. None of the seven QTLs identified for ER_{Ax} and k_{Lat} explained more than 7% PVE. Therefore, it is likely that ER_{Ax} and k_{Lat} are controlled by many other loci, probably with lower PVE values, which were not identified in the present study.

Conclusion

Both lines, CML444 and SC-Malawi responded equally to desiccation stress induced by PEG-8000. CML444 formed a root system with less lateral roots and more axile roots in proportion to the entire root system than SC-Malawi, irrespective of the PEG concentration. These differences indicate that the superior resistance of CML444 to drought stress at flowering might be partially due to such an architecture of the root system, which helps avoiding desiccation. The collocation of QTLs for axile root traits in bins 1.03–1.04 and 7.03–7.04 with QTLs for yield components is in favor of this hypothesis. Considering the breeding history of the two parental lines, the differences in their early root morphology may partly be the results of selection for drought tolerance at flowering.

The correspondence of QTLs for axile root traits in bins 1.02–1.03 and 1.08 and QTLs for lateral root traits in bins 2.04–2.07 in several mapping populations suggests the presence of genes in controlling root growth in a wide range of genetic backgrounds.

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