REGULAR ARTICLE

Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance

Andreas Hund · Nathinee Ruta · Markus Liedgens

Abstract Deep rooting has been identified as strategy for desiccation avoidance in natural vegetation as well as in crops like rice and sorghum. The objectives of this study were to determine root morphology and water uptake of four inbred lines of tropical maize (*Zea mays* L.) differing in their adaptation to drought. The specific questions were i) if drought tolerance was related to the vertical distribution of the roots, ii) whether root distribution was adaptive or constitutive, and iii) whether it affected water extraction, water status, and water use efficiency (WUE) of the plant. In the main experiment, seedlings were grown to the V5 stage in growth columns (0.80 m high) under well-watered (WW) and water-stressed (WS) conditions. The depth above which 95 % of all roots were located (D_{95}) was used to estimate rooting depth. It was generally greater for CML444 and Ac7729/ TZSRW (P2) compared to SC-Malawi and Ac7643 (P1). The latter had more lateral roots, mainly in the upper part of the soil column. The increase in D_{95} was accompanied by increases in transpiration, shoot dry weight, stomatal conductance and relative water content without adverse effects on the WUE. Differences in the morphology were confirmed in the V8 stage in large boxes: CML444 with thicker (0.14 mm) and longer (0.32 m) crown roots compared to SC-Malawi. Deep rooting, drought sensitive P2 showed markedly reduced WUE, likely due to an inefficient photosynthesis. The data suggest that a combination of high WUE and sufficient water acquisition by a deep root system can increase drought tolerance.

Keywords Drought avoidance · Root length density · Root morphology · Stomatal conductance · Water use efficiency · Zea mays L

Abbreviations

ASI	anthesis-silking interval
CER	Leaf carbon exchange rate
Ci	intercellular CO_2 mole fraction
D_{95}	depth above which 95 % of all roots were located
dpi	dots per inch
DR	specific proportion of *deep roots*
g_s	stomatal conductance
Lat	lateral root
RLD	root length density
RLDD	root length in diameter-class distribution
RtSA	root surface area
RWC	relative water content
StDW	shoot dry weight

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V4, V5	vegetative stage, indicating the number of leaves with fully visible collars
Θ	Volumetric soil water content
WS	water stressed treatment (30% maximum water-holding capacity)
WUE	water use efficiency
WW	well watered treatment (100% maximum water-holding capacity)

Introduction

Drought tolerance and WUE of crop plants are increasingly important because aridity in many areas of the world severely limits yield. This problem is expected to become more severe (Petit et al. 1999). Plant growth is a function of a complex interplay between source and sink limitations of the two main organs of a plant, the root system and the shoot, establishing functional equilibrium (Brouwer 1983). Accordingly, given unlimited nutrients and water supply near the soil surface, the plant's growth strategy is straight forward: i) rapid canopy closure, which maximizes light interception, and ii) maintenance of a high rate of photosynthesis, achieved by high stomatal conductance (g_s). This was demonstrated by Fischer et al. (1998) for irrigated wheat where grain yield was positively correlated g_s ($r^2 = 0.94$) and g_s was heritable. Under such conditions the demand for resource allocation to the root system is assumed to be minimal and in line with today's irrigated high-input agricultural systems, for which soil resources are ample (O'Toole and Bland 1987). In drought-prone, rain fed environments the situation is far more complex, and genotypes must adapt to meet the plant's demand for water. Therefore, it is surprising that maize selected for drought tolerance maintained high rates of photosynthesis and transpiration at the risk of water depletion and death of the plants before the end of the crop cycle (Bruce et al. 2002). Is this possible because a highly efficient root system guarantees that the plant's demand for water is met?

Root characteristics which allow access to deep water at a minimum use of carbon, hold great promise for plant adaptation to drought-prone conditions. With regard to the depth of roots of natural vegetations in a given climatic zone (i.e. boreal, temperate, tropical),

arid and semi-arid vegetation systems tend to have deeper roots than vegetations in humid systems (Schenk and Jackson 2002). With regard to crops, deep roots in moist soil layers contribute substantially to crop performance (Passioura 1983). For wheat, Kiregaard et al. (2007) showed that an extra 10.5 mm of additional subsoil water used in the 1.35–1.85 m layer after anthesis increased grain yield by 0.62 t/ha. Landraces of upland rice, adapted to drought, exhibited substantially larger root systems with some large-diameter roots able to colonize the deep soil layers even in the presence of plough pans (Ekanayake et al. 1985). Good drought tolerance of rice is also positively related to ^{32}P uptake, an estimator of root length density, at depth (Reyniers et al. 1982) as well as water uptake (Mambani and Lal 1983a; 1983b; Puckridge and O'Toole 1981) from soil layers one meter deep. Some drought-tolerant genotypes of sorghum have deeper roots (Ludlow et al. 1990; Santamaria et al. 1990), higher yields and, nevertheless, use less water (Wright and Smith 1983): the reduced production of leaves allowed for the conservation of soil water during the vegetative stage, which could then be exploited during grain filling. In maize, reports about genetic differences in the rooting depth are scarce: Vamerali et al. (2003) reported such differences, but these were not relevant for crop yield.

Carbon expended in root respiration accounts for 8 to 50 % of daily net photosynthesis and depends on three major energy-requiring processes, i.e., root construction, root maintenance, and ion uptake (Lambers et al. 2002). To keep these costs low, an efficient root system must explore resource-rich zones at the expense of resource-poor zones. This strategy was also revealed in a selection experiment for drought tolerance in maize. After eight cycles of selection, Bolaños et al. (1993) reported a 33% reduction of the root biomass in the dry upper 50 cm of the soil.

To understand genotypic differences in water acquisition, a distinction must be made between the fine lateral roots, usually with diameters smaller than 0.8 mm (Cahn et al. 1989; McCully 1999), and their larger parental axile roots. In the fibrous root system of maize, axile roots emerge from the stem, guaranteeing a wide vertical and horizontal distribution of the root system, away from the plant basis, while lateral roots are of major importance for the efficient short-distance exploitation of water and nutrients

(Eissenstat 1992; McCully 1999). The importance of lateral roots of maize in the uptake of water is attributed to the fact that they make up about eight times the surface area of their parental axile root and take up about eight times as much water. Water uptake of a maize root, i.e. the axis and its associated laterals, is maximal at 30 to 60 cm from the main root tip and decreases to about 25 % of the maximum in older regions (Varney and Canny 1993). Differences in carbon allocation to different root types in maize are documented: laterals roots of field-grown maize plants accumulated more ^{14}C-labeled assimilates per unit weight than did subtending main roots (McCully and Canny 1985). In conclusion, in order to determine the potential costs and benefits of the root system in terms of water acquisition, it is crucial to determine the relative distribution of the different root types within the soil profile.

Information about genetic differences in the root morphology and architecture of maize roots and their relationship to drought tolerance and water uptake is scarce. As for rooting depth, little is known about the morphological and architectural adaptation of the root system of crop plants to drought.

The aim of this study was, thus, to assess whether drought-sensitive and drought tolerant inbred lines, selected by the International Maize and Wheat Improvement Center (CIMMYT), differ in root morphology and root architecture. The investigated genotypes are the parents of two mapping populations. Their different degree of drought tolerance has been well documented under field conditions (Messmer 2006; Ribaut et al. 1996; Ribaut et al. 1997) and controlled conditions (Welcker et al. 2007). However, apart from root capacitance measures in the field (Messmer 2006), neither the populations nor their parents have yet been evaluated for root traits in combination with water uptake. Therefore, the specific questions were: i) are rooting depth and the vertical distribution of the root length density of lateral and axile roots related to water uptake, i.e. does form follow function? ii) Are root morphological differences constitutively expressed or, alternatively, are they due to a different ability to adapt to drought environments? iii) Do differences in the form and function of roots have consequences for shoot traits such as canopy size and stomatal conductance?

Materials and methods

Plant material

Four maize (*Zea mays* L.) inbred lines with known shoot responses to drought were studied; the parental lines of two mapping populations (recombinant inbred lines of Ac7643 (P1) × Ac7729/TZSRW (P2) and CML444×SC-Malawi). P1 was selected for a short anthesis-silking interval (ASI, Betrán et al. 2003) and it yields well under drought compared to P2, which has a long ASI (Ribaut et al. 1996). CLM444 was selected for its high grain yield under drought conditions in Southern Africa (Messmer 2006). It is among the most drought-tolerant germplasm available at CIMMYT. SC-Malawi was developed in the 1960s in Zimbabwe and has a moderate yield under water-limited conditions (Messmer 2006). It was widely used in crosses for developing public and private hybrids.

Experimental designs and growth conditions

Growth columns

All four inbred lines were tested in growth columns (80 cm high, 10.5 cm in diameter) filled with quartz sand. The experiment consisted of three runs, each set up as a factorial design with two water treatments, i.e. well-watered (WW) and water-stressed (WS), as well as the four genotypes listed above. Additionally, a non-planted control was included, which was used to assess the changes in vertical distribution of soil water due to soil evaporation and percolation during the experiment. All factorial combinations were assigned to plots of three complete randomized blocks in each of three runs. One plot consisted of one growth column containing one plant. Analysis of variance was computed using PROC MIXED (SAS 8.2). Block within run was considered random; all other factors were considered fixed.

The columns were filled with batches of quartz sand (0.08–0.2 mm diameter) mixed with a nutrient solution containing 0.2% (v/v) of the liquid fertilizer Wuxal (Aglukon Spezialdünger GmbH, Düsseldolf, Germany; composition per liter: 100 g N, 100 g P_2O_5, 75 g K_2O, 190 mg Fe, 162 mg Mn, 102 mg B, 81 mg

Cu, 61 mg Zn, 10 mg Mo). The amount of nutrient solution was adjusted to obtain 100% (WW) and 30 (WS) of the maximum water-holding capacity (0.228 m^3 m^{-3}) of the substrate. Evaporation was prevented by covering the columns with plastic foil.

Seeds were germinated in a soil-sand-vermiculite mixture for five days. Seedlings with emerged coleoptiles 10 mm long were transferred to the columns (day 0). Plants were then grown in a growth chamber (PGW36, Conviron, Winnipeg, Canada) at 28/24°C (day/night), 70/60% relative humidity (day/night) and a 12 h photo period at 600 µmol cm^{-2} s^{-1} light intensity. All the plants were harvested 30 days after transplanting when plants had reached the V5 and the V4 stage in WW and WS, respectively.

Growth containers

To verify rooting depth and diameter of the axile roots, CML444 and SC-Malawi were grown for 60 days to the V8 sage in large containers: wooden boxes with a square area of 0.96 m^2 (1.20×0.80 m) and a soil depth of 1.0 m were subdivided into four 0.30 m wide subunits separated by partitios. Polyethylene foil prevented water exchange between the subunits. The substrate was a mixture of sand (75%) and fine soil derived from the washing of sugar-beets before processing. The final texture of the substrate was 6% clay, 11% silt, and 83% sand. The soil organic mater was 3% and the pH 7.7. The substrate contained 53 mg kg^{-1} NO_3, 4.1 mg kg^{-1} P, 79.9 mg kg^{-1} K, 164.4 mg kg^{-1} Ca, and 13.3 mg kg^{-1} Mg.

Two water treatments were imposed: a well water treatment (WW) at field capacity receiving water to replenish the loss by evapotranspiration, and a water stressed treatment (WS) receiving half of the water of the WW treatment. The amount of water lost by evapotranspiration was estimated by measuring the weekly loss of water of eight control columns (0.105 m diameter, 1 m height) planted with each of the two tested genotypes. The water supply in the boxes was corrected for the difference in soil volume with regard to the soil columns. The experiment was arranged as four completely randomized blocks (containers), each block harboring a complete set of Genotype-by-water treatment combinations. The root system of one plant per plot (subunit) was harvested and analyzed. An analysis of covariance was computed in asreml-r

(Butler 2006) to estimate the effects of watering supply, genotype, and crown root tier on the axile root diameter and the length of the axile roots. These traits were considered as fixed effects while block and plot (one plant) within block were considered random effects. For the diameters, the order of the crown root tiers (tier 1–5) was entered as continuous covariate.

Data sampling

Shoot

In the column experiment, physiological measurements were made in the center of the last fully developed leaf. Leaf carbon exchange rate (CER), g_s, and the intercellular CO_2 mol fraction (Ci), were measured for a 2 cm^2 area of the blade using a portable, open-flow gas exchange system LI-6400 (LI-COR). At least 20 min before the measurement, the pots were rotated such as to expose the target leaf to full light intensity (600 µmol m^{-2} s^{-1}). The flow rate of air through the chamber and the sample-side infra-red gas analyzer of the LI-6400 was set at 300 µmol s^{-1}. To avoid strong fluctuations of CO_2 concentration, the intake air was taken from a 5 l buffer volume placed within the growth camber. CER, g_s, and Ci were calculated by the LI-6400's operating software, which follows the method of von Caemmerer and Farquhar (1981). The light intensity and temperature in the measuring chamber were set according to the conditions in the growth chamber. A mixture of blue (10) and red (90) LEDs was used as light source. g_s was recorded when the total coefficient of variation ($\Delta_{CO2}+\Delta_{H2O}+\Delta_{flow}$) was below 0.2%, which usually took about 2 min. The leaf area of all green leaves was measured with a leaf-area meter (LI-COR 3100, Lincoln, NE, USA); the dry weight of the shoot (StDW) was recorded after drying at 65 °C for 72 h.

The relative water content of a piece of leaf, 15 mm long, was estimated according to Weatherley (1950). The amount of transpired water (T) was calculated by subtracting the evaporated water (E_s), determined for the control tubes, from the total water loss per column. The WUE was calculated as WUE= TE/(1+E_s/T) where TE is the transpiration efficiency (StDW/T) (Richards 1991).

Analysis of roots from the columns experiment

After harvesting the shoot, the columns were separated into 10-cm segments, and the root samples were separated from the substrate, either by sieving (block one of each run) or by washing under running water (blocks two and three of each run). The sieving enabled the determination of the water content of the sand (see below). The number of crown roots was counted, and the root samples were stored at −20 °C until further processing.

Root samples from each segment were spread in a 1-cm layer of water in transparent trays and imaged with a flatbed scanner equipped with a top light (Epson, Expression 1640 XL, Epson America, Inc., USA) at a resolution of 23.6 pixel mm^{-1} (600 dpi). The lengths of the roots, classified by diameter classes (steps of 42 μm), were extracted by means of the image processing software WinRhizo Pro (Regent Instruments, Québec, Canada). When the length of the axile roots, i.e the main axes of the primary, seminal, and crown roots (Cahn et al. 1989), and their lateral roots is in the same order of magnitude, the root length in diameter-class distribution (RLDD) typically displays a bimodal pattern, i.e. one peak within the low and one peak within the higher diameter classes, reflecting the lateral and the axile roots, respectively (Hund et al. 2004). The trough between the two peaks can be used to define a threshold for discriminating between lateral and axile roots. For this purpose, we used the RLDD curves of the lower two section of the soil columns where the proportion of axile and lateral root length could be expected to be at the same order of magnitude. In order to detect the position of the trough none parametric local polynomial fits of second order and a neighborhood proportion of 30% (span, $\alpha=0.3$) were fitted to the RLDD using the R function loess(). (R Development Core Team 2008). The median diameters were calculated from the RLDDs of axile and lateral roots using linear interpolation. The root length density (RLD) was calculated as root length per unit soil volume.

To summarize the vertical distribution of the RLD we used the concept of Schenk and Jackson (2002), i.e. reporting the depth above which 95 % of the roots were located in the column (D$_{95}$). D$_{95}$ was determined by interpolating the depth profiles using a non parametric smoothing function. First, the cumulative root length within each soil layer was calculated and expressed as proportion of the total root length. Second, none parametric local polynomial fits of second order and a neighborhood proportion of 60% (span, $\alpha=0.6$) were fitted to these proportion using the R function loess(). Third, these fits were used to predict the proportion of the cumulative root length for each 1 mm depth increment using the function predict.loess(). From these predictions, the D$_{95}$ and the proportion of the root length below half D$_{95}$ (DR) were extracted. DR can be regarded as a vertical shape parameter of the root system independent of its overall root length and depth.

Linear regressions for the dependency of water use and water status on D$_{95}$ were calculated for all combinations of genotype and water supply using the R packages asreml (Butler 2006):

$$y_{ijk} = d + w_i + dw_i + r_j + b_{jk} + e_{ijk} \qquad (1)$$

where y_{ijk} is the trait values of the plots regressed on their D$_{95}$ values d, the effect of the water treatment w_i, and block b_{jk} within replication r_j. D$_{95}$ was centered at 45 cm, i.e. D$_{95}$ was subtracted by 45. Replications within blocks were considered random. The final formulation of the models resulted from backward selection based on the p-value with a probability threshold at the 0.05 level.

Analysis of roots from the containers experiment

At harvest, containers were opened on the long side, and the root system of one plant per plot was carefully removed from the soil. Root systems were stored at 4°C in 70 % EtOH until further processing. The internodes were successively numbered considering the mesocotyle as internode one. Accordingly, the first whorl of crown roots emerged from internode two. For each internode tier, a representative root was chosen and its proximal diameter of the main axis (5 cm from the shoot base) was measured with a caliper gauge and its overall length was measured on a cm-scale.

Vertical profile of water extraction

Soil samples from the columns (see above) were weighed immediately after harvest and again after oven-drying (105°C, 72 h) to obtain the absolute water content per section. The volumetric water

content (Θ) was then calculated as the ratio between the absolute water content and the volume of the section. The difference in water content between the start and the end of the experiment was calculated for the planted ($\Delta\Theta_{plant}$) and control columns ($\Delta\Theta_{control}$). The water extracted by the plants was then estimated for each planted column (block 1 of each run, harvested by sieving) by subtracting the $\Delta\Theta_{control}$ of the water treatment (mean of all three blocks within each run) from $\Delta\Theta_{plant}$.

Results

Water shortage affected shoot growth to a greater extent than root growth and had little effect on stomatal conductance

The lower water availability in the WS treatment resulted in a 65 % reduction in transpired water (Table 1). The plant traits most severely affected by water shortage were shoot growth, i.e. StDW (−75%) and leaf area (−68%), followed by root growth, i.e. root surface area (−51 %), and finally by the physiological parameters, CER (−18%), g_s (−27%), and WUE

(−29%) (Tables 1 and 2). The treatment did not affect the D_{95} or the relative water content. Surprisingly, DR was increased by water shortage.

The root length in diameter-class distribution was used to distinguish axile from lateral roots

The RLDD (Fig. 1) of the entire root system is dominated mostly by small-diameter roots. In contrast, the RLDD in the lower 60 to 80 cm sections of the growth columns, where the number of lateral roots was lower, revealed the expected bimodal distribution. The location of the trough separating lateral and axile roots depended on the water treatment and the genotype: it was at 700 and 870 μm for WS and WW, respectively (Fig. 1c; vertical lines) and at 569 and 868 μm for SC-Malawi and CML444 (Fig. 1d; vertical lines), respectively.

Most of the plant roots were in the upper half of the columns; water at harvest was still available in the lower half

Water distribution in the control columns under WS was more uniform because of the greater loss in the

Table 1 Summary statistics (ANOVA significance levels and means) of the main effect of water treatment (Tr) and genotype (Gen) and the treatment-by-genotype interaction (Tr:Gen) on shoot traits

		StDW		Leaf area		CER		g_s		Ci		RWC		WUE		T	
		g		cm^2		$mmol\ m^{-2}s^{-1}$		$mmol\ m^{-2}s^{-1}$		$\mu mol\ mol^{-1}$		$\%$		$g\ l^{-1}$		ml	
ANOVA	Tr		***		***		*		***		***		ns		***		***
	Gen		**		†		*		***		†[a]		***		***		*
	GenxTr		†		ns		*		*		ns		**		*		ns
Tr	Gen																
WW	CML	1.90	AB[b]	467	A	18.0	A	60.0	A	304	AB	86.7	B	2.66	B	567	A
	Mal.	1.50	B	402	A	16.5	A	51.2	AB	248	B	87.9	AB	2.45	BC	451	B
	P1	2.18	A	482	A	14.9	AB	40.9	B	249	B	86.8	B	3.09	A	555	A
	P2	1.67	B	398	A	11.6	B	48.0	B	412	A	89.8	A	2.27	C	576	A
WS	CML	0.48	A	145	A	16.4	A	44.3	A	130	A	90.3	A	1.91	A	199	A
	Mal.	0.42	A	133	A	8.8	B	25.5	B	152	A	85.5	B	1.89	AB	171	A
	P1	0.48	A	153	A	11.0	B	32.4	B	233	A	87.1	B	1.99	A	186	A
	P2	0.40	A	124	A	13.7	AB	43.5	A	232	A	90.3	A	1.65	B	194	A
	Red.[c]	0.75		0.68		0.18		0.27		0.38		−0.01		0.29		0.65	

The treatments were well watered (WW) and water-stress (WS). Traits are shoot dry weight (StDW), leaf area, leaf carbon exchange rate (CER), stomatal conductance (g_s), internal CO_2 concentration (Ci), leaf relative water content (RWC), water use efficiency (WUE) and the total amount of transpired water (T)

[a] Significant at $P<0.1$

[b] Genotypes or treatments with the same letter are not significantly different ($P<0.05$)

[c] Reduction of trait value in the WS treatment compared to the WW treatment

Table 2 Summary statistics (ANOVA significance levels and means) of the main effect of water treatment (Tr) and genotype (Gen) and the treatment-by-genotype interaction (Tr:Gen) on root traits

		RtSA		AxL		AxM		LatL		LatM		LatL/AxL		Cr		LA/RA		D$_{95}$		DR	
		cm^2		cm		mm		cm		mm		cm cm^{-1}		#		m^2 m^{-2}		cm		%	
ANOVA	Tr	***		***		***		***		***		***		***		***		ns		***	
	Gen	ns		***		***		***		***		***		***		***		***		*	
	Gen:Tr	ns		ns		**		ns		†		ns		**		**		ns		ns	
Tr	Gen																				
WW	CML	1222	B	1055	A	1.21	A	7330	C	0.322	B	6.84	B	14.3	C	0.394	A	51.6	A	45.7	A
	Mal	1353	AB	842	B	1.09	B	10904	AB	0.276	D	13.33	A	25.0	A	0.312	C	44.1	B	45.2	A
	P1	1562	A	1037	AB	1.09	B	11389	A	0.297	C	11.89	A	21.4	B	0.352	B	44.5	B	40.3	B
	P2	1515	A	1185	A	1.04	C	9475	B	0.343	A	8.19	B	12.7	C	0.302	C	48.4	AB	43.5	AB
WS	CML	688	A	733	A	1.09	A	4753	A	0.259	B	6.35	C	7.8	C	0.216	A	51.2	A	48.9	AB
	Mal	628	A	518	B	0.90	D	6051	A	0.225	C	11.54	A	15.7	A	0.222	A	40.9	B	48.9	AB
	P1	726	A	662	AB	1.02	B	6141	A	0.227	C	8.46	B	10.6	B	0.205	AB	42.8	B	45.7	B
	P2	732	A	805	A	0.95	C	5031	A	0.296	A	5.66	C	7.6	C	0.177	B	46.5	AB	51.8	A
	Red.	0.51		0.34		0.11		0.44		0.19		0.20		0.43		0.40		0.04		−0.12	

Traits are root surface area (RtSA), axile root (Ax) and lateral root (Lat) length (L), and median diameter (M), the ratio between the length of lateral and axile roots (LatL/AxL), the number of crown roots (Cr), the leaf area-to-root surface area ratio (LA/RA), the depth above which 95 % of all roots were located (D$_{95}$) and the plant specific proportion of deep roots (DR). For more information see Table 1

top sections of the WW columns (Fig. 2); e.g. in the top 10 cm, 90 % of the water supplied at the start of the experiment was lost resulting in the same water content (0.035 ml^3 cm^{-3}) as in WS.

The vertical profile of the RLD differed from that of the water distribution in the control columns at harvest: it was highest between 10 and 30 cm (3.16 in WW and 1.60 cm cm^{-3} in WS) and then rapidly
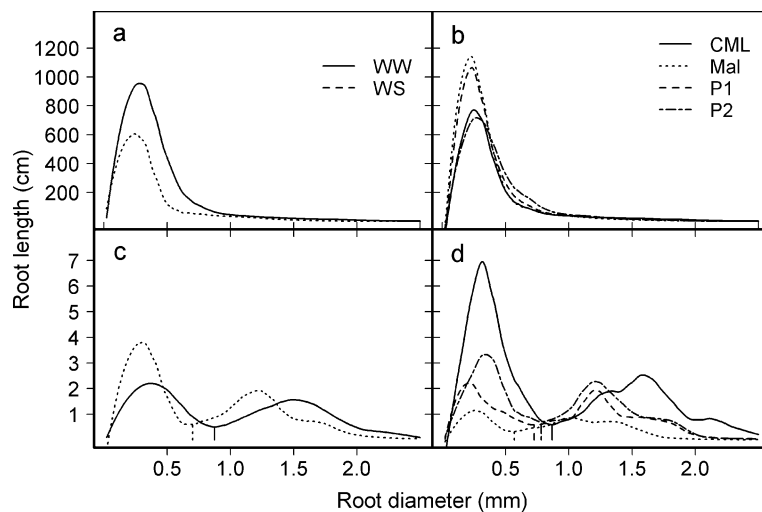
Fig. 1 Root length in diameter-class distribution of the whole root sytem (**a**, **b**) and of roots sampled between 0.6 and 0.8 m soil depth (**c**, **d**). Root length in each diameter class was either averaged across the well-watered (WW) and water-stressed (WS) treatment (**a**, **c**) or across genotypes (**b**, **d**). Root diameter was sampled at one pixel (42.3 µm) intervals and lines show the mean of LOESS fits. Vertical lines in c and d indicate the trough between the peaks considered as belonging to the lateral roots and those belonging to the axile roots. Each data point within a diameter class is an average of 36 (**a**, **c**) or 18 (**b**, **d**) samples
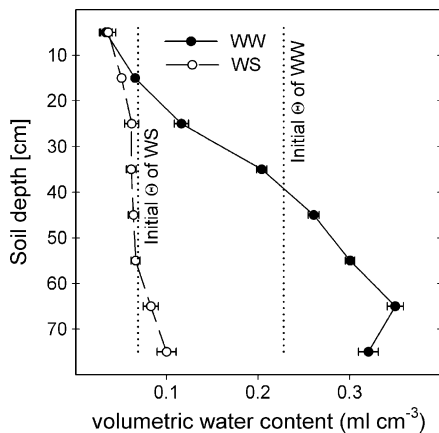
Fig. 2 Volumetric water content of the unplanted control columns at harvest dependent on column depths. The water regimes had an initial water content of 100 % (WW, 0.228 m^3 m^{-3}) or 30 % (WS) of the maximum water-holding capacity throughout the profile (dotted lines). Each plotted point represents an average of nine samples. Error bars represent 95% confidence intervals

decreased in both treatments to values equal or smaller than 0.2 cm cm^{-3} in the column sections below 50 cm (Fig. 4). To access the water at depth we consider two components as important: i) D_{95} as proxy measure for the absolute depth reached by the root system and ii) DR as measure for the vertical distribution of the roots given their absolute rooting depth. Figure 3 illustrates how the parameters were determined. Since Schenk and Jackson (2002) used D_{50} (the depth reached by 50% of the roots) and D_{95} to summarize depth profiles, we also calculated D_{50} and compared it with D_{95} and DR. D_{50} of individual plants was reasonably strong correlated with their D_{95} (r=0.79), suggesting that the two measures were expressing essentially the same, namely the proliferation of roots at greater depth. By contrast, DR was moderately correlated with D_{50} (r=0.55) but not with D_{95} (r=0.06). This indicates that DR was not affected by the rooting depth. To use the example given in Fig. 3, CML444 and SC-Malawi differed strongly in D_{50} and D_{95} but not in DR since the relative distribution of their roots was very similar.

D_{95} was positively correlated with plant transpiration

We used linear regressions (Table 3) to estimate the effect of rooting depth, expressed as D_{95}, on the water use and water status of the plants. Transpiration and WUE were used as integrative measures of plant-water consumption and efficiency over the time

course of the experiment, while g_s and leaf RWC reflect the plant water status at harvest. Importantly, the overall root length did not affect D_{95} (data not shown) and, therefore, D_{95} was not simply an effect of the size of the root system (c.f. Fig. 4).

Transpiration increased linearly with D_{95}, with a slope of 17.6 and 5.1 ml cm^{-1}, reflecting a relative increase of 3.4 and 2.9% cm^{-1} compared to the intercept of WW and WS, respectively (Table 3). Note that the intercept reflects a D_{95} of 45 cm. StDW was affected by D_{95} with 59 mg cm^{-1} and 13 mg cm^{-1}, reflecting an increase of 4.3 and 3.1% cm^{-1} under WW and WS, respectively. Thus, the effects of a greater D_{95} were larger under WW conditions than under WS and the plants profited relative more by deeper rooting when sufficient water was available.

There was no interaction between g_s and D_{95}. When this interaction term was dropped from the model, g_s increased with D_{95} by 0.66 mmol m^{-2} s^{-1} cm^{-1} (data not shown), reflecting a mean increase of 1.6% cm^{-1}. Leaf RWC was affected by D_{95} by −0.03 and 0.26% cm^{-1}, reflecting a small change of −0.03 and 0.3% for WW and WS, respectively while WUE was not influenced by D_{95}.
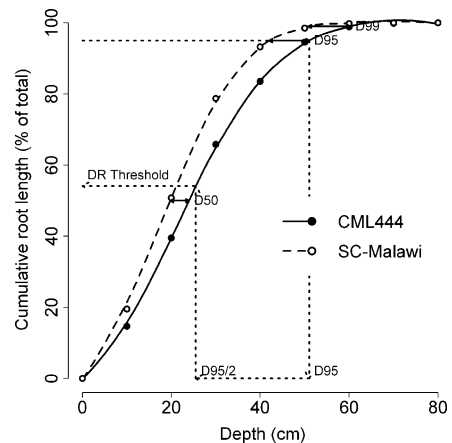
Fig. 3 Cumulative root length within the growth column for the genotypes CML444 and SC-Malawi. Symbols indicate mean values of 18 plants (WW and WS) while lines indicate the mean of LOESS fits. Solid arrows indicate genotypic differences (Δs) in D_{50}, D_{95}, and D_{99}. Dotted arrows indicate how the threshold for the specific proportion of deep roots (DR) was determined using the mean values CML444 as an example. DR measures the proportion of roots below half of the depth reached by 95% of the roots (D_{95}/2). Note that the DR threshold for SC-Malawi is about the same as the one for CML444

Table 3 Traits related to water use and water status in response to rooting depth (D_{95}) and water treatment

Estimates	Trans	StDW	g_s	RWC	WUE
	ml	mg	mmol m^{-2} s^{-1}	%	mg l^{-1}
Intercept	***	***	***	***	***
D_{95}	***	***	*	*	ns
Treatment	***	***	***	ns	***
D_{95}:Tr.	***	***	ns	**	ns
Int. WW	520.7	1746	50.44	87.81	2578
Slp. WW:D_{95} (cm)	17.6	59	0.84	−0.03	31
Int. WS	172.8	405	34.98	87.35	1812
Slp. WS:D_{95} (cm)	5.1	13	0.47	0.26	13

The estimates of the intercept (Int.; centered at 45 cm) and the response to D_{95} (Slp.) for the well watered (WW) and water stressed (WS) treatment were calculated as linear combination of the coefficients of the fixed effects of equation 1. Significances indicated by the stars are given for the intercept, the main effects of D_{95} and water treatment, and their interaction (D_{95}:Tr.). Target traits were the total amount of transpired water (Trans), shoot dry weight (StDW), stomatal conductance at harvest (g_s), leaf relative water content (RWC) and water-use efficiency (WUE)

The amount of water taken up from or released into the soil profile depended on root type, local root length density, and water content

Water uptake was defined as a change in the water extraction until harvest of the planted columns in relation to the unplanted control. In general, water uptake per unit root length exponentially increased with depth (Table 4) as water availability increased while RLD decreased (c.f Fig. 2 and Fig. 4, respectively). The relationship between RLD and total water extraction varied dependent on the water treatment. Water uptake was affected by root length in the zone between 20 and 70 cm for both treatments. Under WS, it was affected even in the lowest 80 cm section (Table 4). There, it reached the highest uptake rate of 762 µl cm^{-1}. The negative relationship between the RLD and extracted water in the upper-

most 10 cm of the WW treatment suggests water release from the roots.

The drought-tolerant inbred line CML444 profited most from deep rooting

While water availability affected canopy size and overall root length, it did not affect D_{95}. However, the genotypes differed with regard to the distribution of their RLD. CML444 proliferated more roots below 50 cm compared to P1 and SC-Malawi (Fig. 4), and it proliferated more axile roots below 70 cm compared to all other genotypes (Fig. 5). The increased RLD of CML444 below 50 cm coincided with greater water uptake, which exceeded the values observed for P2 and SC-Malawi (Fig. 6). Considering D_{95}, CML444 and P2 reached constitutively greater depths than P1 and SC-Malawi (Table 2), and this coincided with

Fig. 4 Root length density by harvest at different soil depths as influenced by the water regime (**a**) and genotypes (**b**). Each plotted point represents the average of 32 (**a**) and 18 (**b**) samples. Genotypes with the same letter within a soil layer are not significantly different ($P>0.5$)
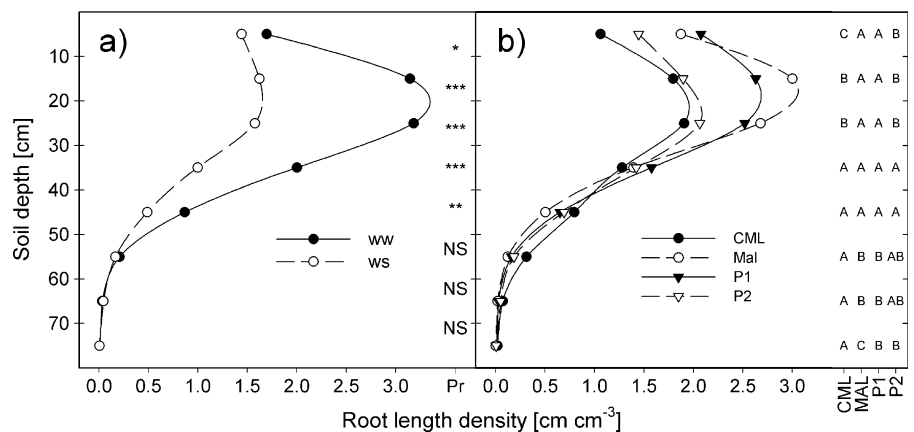
Table 4 Cumulative water uptake by harvest as a function of the root length, calculated for well watered and water stressed conditions at eight column sections

Column section	WW		WS	
cm	$\mu l \; cm^{-1}$		$\mu l \; cm^{-1}$	
5	−3.0	*	0.8	ns
15	−0.2	ns	2.1	ns
25	8.7	**	9.2	**
35	14.6	**	28.7	***
45	30.3	***	31.1	***
55	105.1	**	28.7	***
65	165.1	*	101.8	***
75	−923.1	ns	762.3	***

a decreased root length in the upper 50 cm of the column. However, there was not much change in the relative distribution of the roots among genotypes. When only the main effect of the genotypes for RD was considered (data not shown), CML444 and SC-Malawi and P2 had the same DR (47 %) indicating a similar vertical distribution of the root length and only P1 had significant lower DR (43 %). Considering axile roots only, CML444 produced more axile roots than SC-Malawi almost throughout the entire soil column (Fig. 5). CML444 and SC-Malawi also contrasted for the median diameters of their axile roots: CML444 with the largest diameters of axile roots, CS-Malawi with strongest decrease of it's axile root diameter by 0.19 mm under WS vs. WW (Table 2). Thus, CML444 maintains a higher absolute RLD lower in the profile (Fig. 4), improving drought avoidance.(Fig. 6).

Larger axile root diameters and lengths of CML444 compared to SC-Malawi were confirmed at a later stage

To verify the differences in root diameter and axile root length, CML444 and SC-Malawi were grown to the V8 stage in 1 m deep containers with a more natural substrate. About 17 axile roots per plant had developed by the V8 stage. This number was neither affected by genotypes nor by the water treatment or their interaction (data not shown). The embryonic root system (primary and seminal roots) was often strongly deteriorated, mostly with missing root tips and, therefore, not considered further. The mean diameter of the crown roots was affected by the water treatment, the internode tier, and the genotype, but there were no interactions among these factors. WW increased the root diameters by 0.195±0.08 mm compared to WS (data not shown). Root diameter increased linearly with increasing tier number: averaged over the two genotypes the slope was 0.226± 0.032 mm per tier and the diameters ranged between 0.68 and 1.58 mm for tier two and six, respectively (Fig. 7a). The diameters of the crown roots of CML444 exceeded those of SC-Malawi by 0.144± 0.08 mm, illustrated by the difference between the parallel regression lines in Fig. 7a. The length of axile roots was not affected by the water treatment: it ranged between 60 and 70 cm for the three oldest tiers and decreased as tiers got younger (Fig. 7b). Averaged over all tiers, axile roots of CML444 were about 32 cm longer ($P=0.03$).

Fig. 5 Root length density of large-diameter "axile" roots by harvest at different soil depths in dependence on water regime (**a**) and genotype (**b**). For more information see Fig. 4
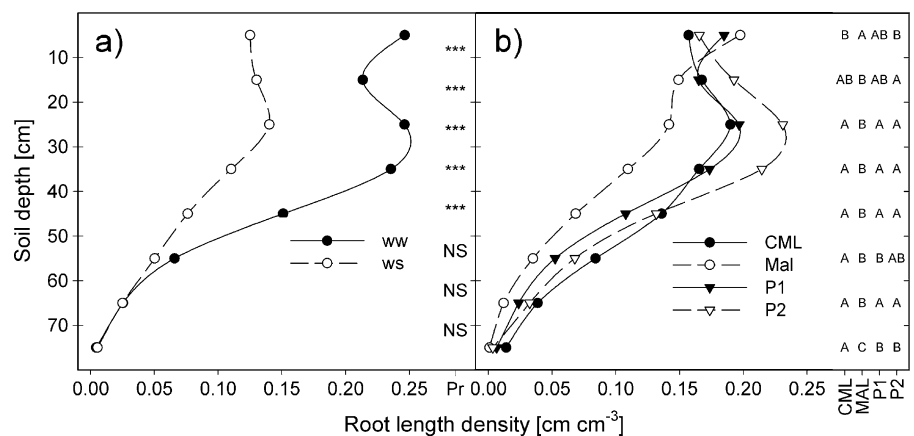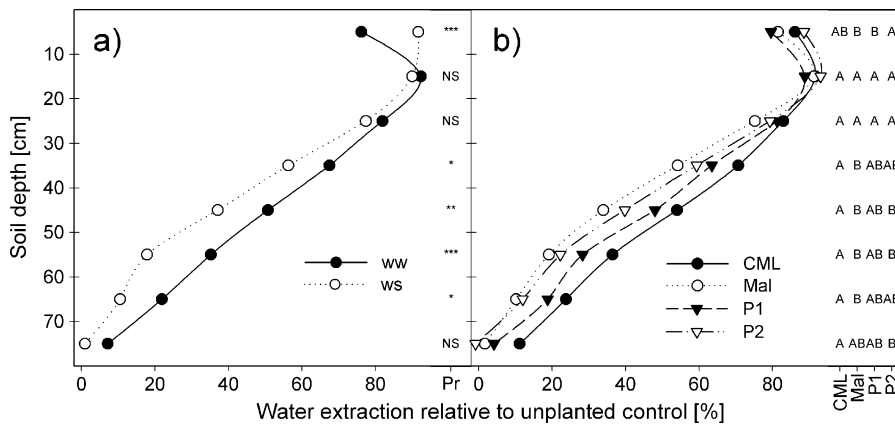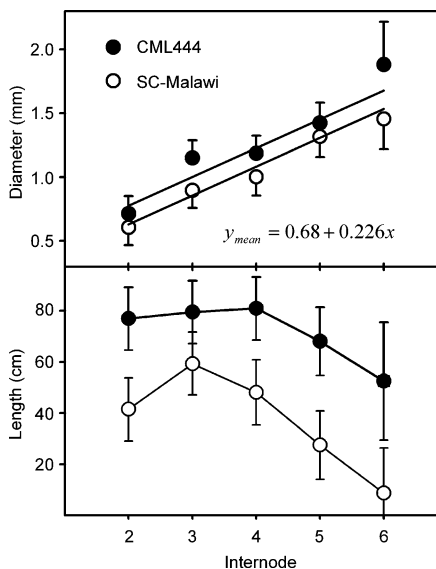
Fig. 6 Water extraction by harvest at different soil depths as influenced by the water regime (**a**) and genotype (**b**). The water extraction is expressed as a percentage of the available water in the unplanted control columns (c.f. Fig. 2). Each plotted point represents the average of 12 (**a**) or 6 (**b**) samples. Genotypes with the same letter within a soil layer are not significantly different (P>0.5)

P1 had the highest WUE

We used physiological traits to assess whether factors other than root morphology explained differences between the "drought-tolerant" genotypes (CML444 and P1) and the "drought-sensitive" genotypes (SC-Malawi and P2). The drought-tolerant inbred line P1 had similar root morphology as SC-Malawi with a

Fig. 7 Axile root diameters and lengths of CML444 and SC-Malawi as a function of their internode of origin. Only the first 5 crown root internodes are given. The linear regression between the mean diameters of both genotypes (y_{mean}) and the internode (x) is given. The intercept was set at internode 2. Individual regression lines are shown for each genotype. Each point represents an average of 7 plants with standard errors

large proportion of roots in the upper 30 cm of the soil profile but otherwise about the same root lengths at lower section as the drought sensitive genotype P2. However, P1 had the highest and P2 the lowest WUE in both water treatments (Table 2). The lower WUE of P2 under WW conditions might be explained by limitations of the primary photochemistry or the dark reaction: P2 showed higher C_i values and a tendency to higher g_s values compared to P1 but tended to have a lower CER. Thus, the decrease in CER was not due to limitations by stomatal aperture and a corresponding lower internal CO_2 concentration in the leaf. Therefore, differences in growth and drought tolerance between P1 and P2 are, at least partly, explained by physiological rather than root morphological differences. Finally, in the WW treatment both tolerant lines, CML444 and P1, had higher leaf-to-root area ratios than the drought-sensitive genotypes and could support a relatively larger canopy with a smaller root system.

Discussion

Genotypic differences in rooting depth and water uptake

We observed a decrease in root diameters under WS. This is well known in maize (Sharp et al. 1988; Taylor and Ratliff 1969) and a result of the restriction of lateral expansion of both the stele and the cortex in the apical 5 mm zone (Liang et al. 1997). Here, the observed differences in root diameters among geno-

types are more interesting. Generally, thicker roots can have two advantages: i) increased root lengths or growth rates and ii) increased water transport. Concerning the first point, thicker roots tended to increase root length density in deeper soil layers in rice (Azhiri-Sigari et al. 2000; Kato et al. 2006). Here we demonstrated that the relationship between root diameter and rooting depth may also be valid for maize. Concerning the second point, it can be assumed that the water transport in xylem vessels follows the Hagen-Poiseuille equation, i.e. is proportional to the forth power of the radius of the root (c.f. Doussan et al. 1998) and there is a positive correlation between root diameters and xylem diameters in lateral roots (Varney et al. 1991). If this hold true also for axile roots, CML444 may transport more water from deep soil layers. This would be in contrast to Passioura's concept of saving water by increasing the resistance of xylem diameters (Passioura 1972). In a breeding program for low xylem diameters of the seminal roots in wheat, Richards and Passioura (1989) could show that yields could be improved by 8% in the driest environment without significant yield penalty under wetter environments. Nevertheless, roots can only take up water when they reach it and this may be achieved by increased axile diameter. These findings are the same as those for rice (Ekanayake et al. 1985) but otherwise the direct relationship between deep rooting and water extraction from deep soil layers is hardly documented. Puckridge and O'Toole (1981) found that the deep-rooting rice cultivar Kinandang Patong extracted more water from a depth of 40 to 70 cm than the shallow rooting cultivars IR20 and IR36. Sharp and Davies (1985) found that the deeper roots of un-watered maize plants exhibited very high rates of soil water depletion per unit root length compared to the well-watered control. Some reports indicate that deep rooting is a drought avoidance strategy in maize: Wan et al. (2000) reported that there were 2.3 to 3.3 times more axile roots (referred to as primary roots) in the deep moist soil layers of two drought-tolerant maize hybrids compared to a drought-sensitive hybrid, enabling a higher rate of water absorption. Vamerali et al. (2003) reported differences in rooting depth of two commercial hybrids. Lorens et al. (1987) reported a deeper root profile for maize hybrid Pioneer-3165 wilting later under drought compared to Pioneer-3192. None of these studies, however, related rooting depth to actual water uptake.

Genotypic differences in lateral rooting and the vertical distribution of roots

Three basic parameters can be used to summarize the distribution of the RLD in the growth columns, the overall root length, D_{95} and DR. We found that D_{95} differences can not be attributed to a grater overall root length. In fact, a decreased RLD in the upper part of the column, seemed to be accompanied by an increase in rooting depth. This may be viewed as a relative increase of the proportion of roots at greater depths which was not necessarily the case since only P1 differed in DR. There was rather a proportional decrease in D-values (e.g. D_{50}, D_{95}…) throughout the soil column, as illustrated for CML444 and SC-Malawi in Fig. 3. To express it in other words, the root system of CML444 compared to that of SC-Malawi, was decreased in its overall root length and stretched to reach greater depth without changing its relative shape. Many studies support the observation that not only a deeper root system but also a reduced RLD in the topsoil is correlated with drought tolerance. Results of selection experiments with maize indicate that root systems with reduced development of crown (adventitious) and lateral roots (Bruce et al. 2002), a smaller amount of roots in the top 50 cm of the soil profile (Bolaños et al. 1993), as well as reduced water extraction from the topsoil (Campos et al. 2004), are better adapted to drought conditions. Hybrid Pioneer-3192, which was wilting early under drought, compared to Pioneer-3165, had more roots in the topsoil and less roots in deeper layers (Lorens et al. 1987). Stamp et al. (1997) reported that, compared to a northern European flint line (Z7), a tropical inbred line from Thailand (Penjalinan) proliferated only about half as much roots in the upper half but more than twice as much roots in the lower half of a 40 m deep soil column. The result may be interpreted as an adaptation of the tropical Penjalinan to avoid dry topsoil. Up to now little evidence was presented for the association of drought tolerance with an increased RLD in superficial soil layers: Wan et al. (2000) found higher RLD of two drought-tolerant elite hybrids in the upper part of the soil compared to a drought-sensitive hybrid.

How may plants benefit from a decreased root density in the upper part of the soil? There are two hypotheses: i) avoidance of stress signaling of roots affected by soil surface drying, and ii) reduction or

optimization of carbon allocation. Concerning hypothesis one, Giuliani et al. (2005) reported a QTL (root-ABA1) constitutively affecting root architecture and stomatal conductance in near-isogenic hybrids differing at this locus. The authors argued that an increase of the root density of the (+/+) hybrid in the dryer superficial soil layers resulted in an increased flux of xylem ABA towards the leaf and, thus, in a lower g_s. This assumption is based on the observation that plants grown in drying soil can exhibit high, undisturbed turgor and yet show greatly reduced growth rates (Davies and Zhang 1991). Increased ABA due to more roots in a dry topsoil might reduce stomatal conductance, decreasing plant productivity but conserving soil water.

The second hypothesis, i.e. optimized carbon allocation, is based on the fact that lateral roots are considered expensive in terms of respiration (Nielsen et al. 1994) and C allocation (McCully and Canny 1985). Their reduction in a soil layer prone to water deficit might reduce the carbon costs of the root system and increase the efficiency of uptake and capture of water and nutrients from greater soil depth. It has to be noted that the selection for an efficient root architecture is complicated by the fact that it also must balance between the differing vertical distribution of essential soil resources, e.g. deep water vs. shallow phosphorus availability (Ho et al. 2005). Therefore, further studies are needed in order to define ideotypes with specific developmental patterns of their root architecture, adapted to the spatiotemporal distribution of water and nutrients in their target environment.

Conclusion

The data highlights the complexity of drought tolerance and its separation into multiple components. A deeper root system correlated with the ability to take up more water from deep layers, leading to sustained stomata opening under water-limited conditions. As shown for CML444 compared to SC-Malawi, a root system with fewer lateral roots in the topsoil but with thicker, deep axile roots seems more suitable for drought avoidance. Furthermore, as demonstrated for P1 and P2, high WUE is certainly as important with this regard. Knowledge about the genomic location controlling these traits will facilitate

marker assisted selection for a better adaptation to drought-prone environments with sufficient water sources in deep soil layers.

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