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Heterosis for water uptake by maize (*Zea mays* L.) roots under water deficit: responses at cellular, single-root and whole-root system levels

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Abstract: To examine the potential heterosis for water uptake by maize roots, the hydraulic properties of roots in the F_1 hybrid (Hudan 4) were compared with those of its inbred parents (♂ 478 and ♀ Tian 4) at cellular, single-root and whole-root system levels under well-watered and water-deficit conditions. The cell hydraulic conductivity (Lp_c) decreased under water deficit, but the Lp_c of the F_1 was higher than that of its inbred parents with or without stress from water deficit. Marked reductions in Lp_c were observed following Hg^{2+} treatment. The hydrostatic hydraulic conductivity of single roots (hydrostatic Lp_{sr}) varied among genotypes under the two water treatments, with the highest in the F_1 and the lowest in ♂ 478. Radial hydraulic conductivity (radial Lp_{sr}) and axial hydraulic conductance (L_{ax}) of the three genotypes varied similarly as Lp_{sr}. The variations in hydraulic parameters were related to root anatomy. Radial Lp_{sr} was negatively correlated with the ratio of cortex width to root diameter (R^2=–0.77, P<0.01), whereas L_{ax} was positively correlated with the diameter of the central xylem vessel (R^2=0.75, P<0.01) and the cross-sectional area of xylem vessels (R^2=0.93, P<0.01). Hydraulic conductivity (Lp_{wr}) and conductance (L_{wr}) of the whole-root system followed the same trend under the two water treatments, with the highest values in the F_1. The results demonstrated that heterosis for water uptake by roots of the F_1 occurred at cellular, single-root and whole-root system levels under well-watered and water-deficit conditions.

Keywords: heterosis; water uptake; hydraulic conductivity; water deficit; maize

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Low rainfall and water shortage are important environmental characteristics in arid and semi-arid areas, and they seriously restrain plant growth, development, and productivity. The uptake of water by crop plants is an important problem for modern agricultural systems (Blum, 2009). Water shortage is the major limiting factor in crop production. Intensive agriculture (e.g. wheat-maize double cropping) with limited water is practiced to meet the large demand for grains (Sun et al., 2010), especially in the North China Plain, the Loess Plateau and surrounding areas of China. An improvement in the ability of crop roots to take up water under conditions of water shortage is thus important.

Maize is an important cereal crop grown for food, feed and forage throughout the world. It is sensitive to water deficiency due to its high demand for water. Maize is also one of the earliest crops in which heterosis was applied for practical use. Heterosis in maize has been mainly studied recently for under-

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standing the characteristics of physiology and the underlying molecular regulatory mechanisms for increasing crop yield (Bruce et al., 2002; Tollenaar et al., 2004; Hoecker et al., 2006; Hochholdinger and Hoecker, 2007; Lee and Tollenaar, 2007). The effect of heterosis on water uptake by roots, though, has not been studied.

Root hydraulic conductivity (or conductance) is an important hydraulic parameter that can reflect the ability of roots to take up water and that can be studied at cellular, single-root and whole-root system levels. The flow of water through a single root can be divided into axial and radial flows. Based on the composite transport model of roots, water flows radially along two parallel and variable pathways (apoplastic and cell-to-cell pathways) (Steudle and Peterson, 1998). The demonstration of water uptake by single roots requires a study of the anatomical characteristics of roots (Cruz et al., 1992; Steudle and Peterson, 1998; Rieger and Litvin, 1999). Aquaporins (AQPs) in cell membranes is an important limiting factor for the cell-to-cell pathway and for cellular water uptake. At the cellular level, rapid and reversible regulation of water transport across membranes is related to the expression and activity of AQP mRNAs (Henzler et al., 1999; Clarkson et al., 2000; Hukin et al., 2002; Javot et al., 2003; Zhao et al., 2004; Ehlert et al., 2009), both of which can be regulated by metabolic and environmental factors (Zhang and Tyerman, 1999; Clarkson et al., 2000; Wan et al., 2004; Lee et al., 2005; Ye and Steudle, 2006; Maurel et al., 2010). Moreover, an individual cell can be considered a perfect osmometer (reflection coefficient=0), whereas a single root can only be considered a membrane system (reflection coefficient >0) consisting of several cell layers (Steudle and Peterson, 1998). The transport of water at the cellular level thus differs from that at the single-root level. Water flow into or from the root system can be affected by the spatial distribution and morphology of roots. Due to anatomical differences in root types or to the developmental phase of a root system (Knipfer and Fricke, 2011), and even along the length of an individual root (Melchior and Steudle, 1993), responses in the ability of water uptake at root-system and single-root levels are not linearly correlated (Mu et al., 2006). The extrapolation of root hydraulic conductivity of isolated cells or excised single roots to the whole-root system may be misleading (Doussan et al., 2006). The simultaneous study of water uptake at cellular, single-root and whole-root system levels is therefore necessary.

Several studies have demonstrated variations in water uptake among different crop species or genotypes. Rieger and Litvin (1999) reported that anatomical differences may contribute to the variability in hydralic conductivity of whole-root systems in two woody and three herbaceous species. Zhao et al. (2005) showed that hydraulic conductivity in the whole-root systems of six wheat genotypes increased during the evolution of wheat and was significantly correlated with root characteristics. Matsuo et al. (2009) discussed the relationship between hydraulic conductance in whole-root systems and root anatomy in three rice genotypes. For many years, studies on root hydraulic behavior were conducted at the whole-root system level and were limited by difficulties in measurement. In recent years, new techniques such as cell and root pressure probes have advanced the associated research. Bramley et al. (2009) assessed the roles of morphology, anatomy and AQPs in determining contrasting behaviors of root hydraulics in one wheat and two lupin varieties at the cellular to organ levels with the aid of pressure-probe techniques. Few studies, however, have compared hybrid plants and their inbred parents.

To examine potential heterosis for water uptake by maize roots and to test the feasibility of pressure-probe techniques in studying genotypic differences in the parameters of root-water relations, the study compared the properties of root hydraulics in the F_1 maize hybrid (Hudan 4) and its inbred parents (♂ 478 and ♀ Tian 4) at cellular, single-root and whole-root system levels under well-watered and water-deficit conditions. Genotypic differences in AQP activity, anatomical characters and morphological parameters of roots were also examined.

1 Materials and methods

1.1 Plant materials

Seeds of three maize genotypes, 478 (♂), Tian 4 (♀)

and Hudan 4 (F_1 hybrid, 478 × Tian 4), were germinated on wet filter paper for 3 d at 25°C in the dark. Primary roots of germinated seedlings (5–6 cm in length) were transferred to plastic barrels (depth: 25 cm, diameter: 20 cm) filled with distilled water. The seedlings were then subjected to the following two water treatments for 7 d after a one-day period of adaptation: well-watered (Hoagland nutrient solution only) and water deficit (water deficit simulated by PEG-6000 in Hoagland nutrient solution, ψ_s = –0.2 MPa). Barrels were placed in a climatic chamber (ZPW-280B, China) under the following growth conditions: light intensity of 400 μmol/(m^2·s), day/night rhythm of 12 h/12 h, day/night temperatures of 27ºC/20ºC and relative humidity of 60%–70%. The solutions in the barrels were ventilated with an air pump for 1 h 3–4 times per day and replaced every 2 d during the period of observation. The experiment was a completely randomized design (*CRD*) with 4 replicates.

1.2 Cell pressure probe measurements

The cell pressure probe employed in this study was the same as that used by Ye and Steudle (2006) and Wan et al. (2004). An excised root segment was fixed by magnetic bars to a metallic sledge arranged at an angle of 45°. The root segment was placed on a layer of tissue paper to maintain moisture. A nutrient solution, the same as that used for the hydroponic culture of seedlings, ran along the root segment in a circulating system. An oil-filled glass capillary was attached to a probe with a tip diameter ranging from 7 to 10 μm. Cortical cells from the fourth to the sixth layer and about 20 mm from the root apex were punctured with the probe. A meniscus formed between the cellular sap and the oil in the oil-filled microcapillary of the probe. Cellular turgor was rebuilt by gently pushing the meniscus to a position close to the surface of the root. When the turgor pressure stabilized, parameters of the cellular hydraulics were determined. Relaxations of hydrostatic pressure were induced by using the micrometer screw of the probe to rapidly move the meniscus and by maintaining the new position until a steady pressure was re-established. To avoid mechanical inhibition of the AQPs, the peak pressure changes were kept below 0.1 MPa (Wan et al., 2004). Curves of pressure versus time (relaxations) were recorded to evaluate the hydrostatic half-time ($t_{1/2}$) of water flow across the cell membrane, which was inversely proportional to the hydraulic conductivity of the cell membrane. The hydraulic conductivity (Lp_c) of the cell was calculated by:

$$Lp_c = \frac{V_c}{A_c}\frac{\ln 2}{t_{1/2}\ (\varepsilon + \pi^i)} \approx \frac{d}{4}\frac{\ln 2}{t_{1/2}(\varepsilon + \pi^i)}. \qquad (1)$$

Where, Lp_c is the cell hydraulic conductivity (m/(s·MPa)), V_c is the cell volume (m^3), A_c is the cell surface area (m^2), d is the cell diameter (m), $t_{1/2}$ is the half-time of water exchange (s), ε (MPa) is the volumetric elastic modulus of the cell wall and π^i (MPa) is the cell osmotic pressure.

To determine the activity levels of the AQPs during water flow across the roots, the Lp_c was measured after a 20-min treatment with 50 μmol/L $HgCl_2$ and thereafter 20 min following treatment with 50 μmol/L mercaptoethanol.

1.3 Root pressure-probe measurements

An excised segment of a single primary root (5–15 cm) was tightly connected to the root pressure probe using a silicone rubber seal. Because the fluid in the root pressure probe (silicone oil and water) was nearly incompressible, root pressure built up over 1–3 h and was measurable with a pressure transducer. During the hydrostatic experiment, root pressure was changed by pushing water into or out of the cut end of the root segment with the aid of a metallic rod. During the osmotic experiment, bathing media modified by Hoagland nutrient solution contained a NaCl solution of 100 mmol/L, in which osmotic water flow was induced. Both experiments resulted in root pressure relaxations via water movement into or out of the root. The half-time of water exchange of single roots ($T_{1/2}$) and the elastic coefficient of the measuring system (β) were measured using a root pressure probe. Data were used to calculate hydrostatic or osmotic hydraulic conductivity of single roots (Lp_{sr}) of the genotypes (Steudle and Frensch, 1989; Steudle, 1993). The surface area of the root segment (A) was also measured to evaluate Lp_{sr}. Because the xylem vessels of the root tip (about 15–20 mm from the apex) were immature, the axial hydraulic resistance was much larger than the radial hydraulic resistance, and the hydraulic conduc-

tivity of a single root was considered to be the radial hydraulic conductivity of the single-root segment (Frensch and Steudle, 1989; Melchior and Steudle, 1993). The hydraulic conductivity of single roots (Lp_{sr}) was calculated by:

$$Lp_{sr} = \frac{\ln 2}{T_{1/2} \times \beta \times A}. \quad (2)$$

Where, Lp_{sr} is the hydraulic conductivity of a single root (m/(s·MPa)), $T_{1/2}$ is the half-time of water exchange (s), β is the elastic coefficient of the measuring system (MPa/m^3) and A is the effective surface area of the root segment (m^2).

The root segment was subsequently cut with a razor blade from a position where the root segment was attached to the silicone rubber seal. The remaining material of the root segment within the seal was about 18 mm in length. The root pressure immediately dropped as soon as the xylem vessels were cut. The hydraulic conductance of the root segment was calculated when the root pressure stabilized at a new level. Because the root segment was sealed by the rubber seal, radial water could not move into or out of the root segment, and the hydraulic conductance calculated in the cutting experiment was the axial hydraulic conductance per unit root length (L_{ax}) (Frensch and Steudle, 1989; Melchior and Steudle, 1993):

$$L_{ax} = \frac{\ln 2 \times l}{T_{1/2} \times \beta}. \quad (3)$$

Where, L_{ax} is the axial hydraulic conductance of a single root (m^3/(s·MPa)), $T_{1/2}$ is the half-time of water exchange (s), β is the elastic coefficient of the measuring system (MPa/m^3) and l is the length of the root segment in the silicone rubber seal (m).

1.4 Measurements of hydraulic parameters of the whole-root system

The hydraulic conductivity of the whole-root system (Lp_{wr}) was measured using pressure chambers following Mu et al. (2006). Root systems detached from maize seedlings were inserted into the pressure chamber filled with growth solution, with the stems penetrating the rubber seal of the metallic lid. The balance pressure (P_0, MPa), called the ex-pressure, was determined when the sap initially exuded. Pressure was increased from P_0 (MPa) to P_0+0.5 (MPa) at intervals of 0.1 MPa. Under each pressure, the exuded sap was collected for 5 min when the flow rate stabilized (about 5 min). The collections were repeated at least three times at 1-min intervals, and the exuded sap was collected with an attached water-absorbent paper, and the weight was determined with an analytical balance (0.01 mg).

The flow rate Jv (m/s) was calculated by:

$$Jv = V / (SA \times t). \quad (4)$$

Where, V is the volume of absorbed water (m^3), t is the time of water absorption (s) and SA is the surface area of the whole-root system (m^2) determined by an Epson Perfection V700 scanistor (Seiko Epson, Japan) and analyzed with a WinRHIZO root image analysis system (Regent Instrument, Canada).

The hydraulic conductivity, Lp_{wr} (m/(s·MPa)), of the whole-root system was determined from the slope of the regression line by plotting Jv against pressure, i.e.:

$$Lp_{wr} = Jv/\Delta P, \quad (5)$$

The hydraulic conductance, L_{wr} (m^3/(s·MPa)), of the whole-root system was calculated as:

$$L_{wr} = Lp_{wr} \times SA. \quad (6)$$

1.5 Measurements of root anatomical parameters

Free-hand cross and longitudinal sections were taken at 60–80 mm from the apex of the primary root and stained with 0.5% (w/v) Toluidine blue O at room temperature for 2 min (Ranathunge et al., 2005). The root diameter (RD), cortex width (CW), diameter of central xylem vessel (VD), the sum of cross-sectional areas of the xylem vessels in the root (VA) and the diameters and lengths of cortical cells were measured. Sections were observed and photographed using an optical microscope (OLYMPUS JNOEC xs-212-201, Japan). Associated anatomical parameters were measured using NIKON ACT-2U software.

1.6 Measurements of proline and malondialdehyde (MDA)

The levels of proline and MDA in roots of the three maize genotypes were determined following Choudhary et al. (2007).

1.7 Statistical analysis

Data analysis was performed using SPSS13.0 software

for Windows (Chicago, USA). Two-way analyses of variance (ANOVAs) were performed, and treatment means were compared using Duncan's multiple range test.

2 Results

2.1 Hydraulic parameters of cells

The turgor of cortical root cells of the three maize genotypes was about 0.6 MPa, with no genotypic differences in the well-watered treatment (Table 1). In contrast, the turgor of the F_1 and both parents decreased under water deficit, but the turgor ranking was in the order F_1 > ♀ Tian 4 > ♂ 478. These results indicated that the F_1 was best able to maintain root-cell turgor under water deficit.

In the well-watered treatment, the Lp_c was highest (P<0.05) in the F_1 and lowest in ♂ 478 (Table 1). The Lp_c of the three genotypes substantially decreased in the water-deficit treatment, and the Lp_c ranking was in the order F_1 > ♀ Tian 4 > ♂ 478. The Lp_c was greatly reduced following treatment with the AQP inhibitor, $HgCl_2$, and the Lp_c ranking was also in the order F_1 > ♀ Tian 4 > ♂ 478. Mercaptoethanol partly reversed the lowering effect of $HgCl_2$, but the final Lp_c was less than the original value prior to $HgCl_2$ treatment. Also, the Lp_c of the F_1 after treatment with $HgCl_2$ and mercaptoethanol was larger than that of either ♀ Tian 4 or ♂ 478 in the two water treatments. These results suggested a heterosis for the ability of the F_1 to take up water at the cellular level.

Table 1 Root cortical cell turgor pressure and cell hydraulic conductivity (Lp_c) of F_1 hybrids in comparison with the parental inbred lines

Water condition	Turgor (MPa)	Lp_c (10^{-6} m/(s·MPa))		
		Control	$HgCl_2$	Mercaptoethanol
Well-watered treatment				
F_1	0.620^{a†}	4.032^{a†}	1.287^{a†}	3.665^{a†}
♀	0.624^{a†}	3.362^{b†}	1.085^{b†}	2.897^{b†}
♂	0.599^{a†}	2.510^{c†}	0.730^{c†}	2.013^{c†}
Water-deficit treatment				
F_1	0.423^{a}	1.984^{a}	0.994^{a}	1.563^{a}
♀	0.374^{b}	1.610^{b}	0.765^{b}	1.154^{b}
♂	0.302^{c}	0.989^{c}	0.418^{c}	0.690^{c}
F-value				
Genotype	20.485**	167.172**	1,554.322**	61.694**
Water level	717.344**	987.326**	1,357.359**	344.002**
Genotype×water level	9.697**	7.317**	0.889^{ns}	5.869**

Note: For each water treatment, means in the same column bearing the same letter are not significantly different (P>0.05). For each genotype, † indicates significant differences (P<0.05) between the values in the well-watered and water-deficit treatments. Factors influencing these traits are expressed as F-values; * and ** indicate significant difference at P<0.05 and P<0.01, respectively; ns, not significant; n=10.

2.2 Hydraulic parameters of single roots

Water flow across a single root is mediated by two driving forces, hydrostatic and osmotic pressure. Under a gradient of hydrostatic pressure, the radial Lp_{sr} and the hydraulic L_{ax} of the three maize genotypes decreased in the water-deficit treatment (Table 2). The radial Lp_{sr} and the L_{ax} values in the F_1 were signifcantly higher (P<0.05) than those in both parents in the two water treatments. Under a gradient of osmotic pressure, the osmotic Lp_{sr} decreased under water deficit. Again, the osmotic Lp_{sr} in the F_1 was the highest (F_1 > ♀ > ♂) across the water conditions. The osmotic

Table 2 Single-root hydrostatic hydraulic conductivity (hydrostatic Lp_{sr}), axial hydraulic conductance (L_{ax}) and osmotic hydraulic conductivity (osmotic Lp_{sr}) of F_1 hybrids in comparison with the parental inbred lines

Water condition	Hydrostatic Lp_{sr} (radial Lp_{sr}, 10^{-7} m/(s·MPa))	L_{ax} (10^{-11} m^3/(s·MPa))	Osmotic Lp_{sr} (10^{-8} m/(s·MPa))
Well-watered treatment			
F_1	20.52^{a†}	6.29^{a†}	6.78^{a†}
♀	12.83^{b†}	2.64^{b†}	2.51^{b†}
♂	8.35^{c†}	2.54^{b†}	1.98^{c†}
Water-deficit treatment			
F_1	9.26^{a}	4.49^{a}	1.82^{a}
♀	5.62^{b}	1.64^{b}	1.33^{b}
♂	3.06^{c}	1.38^{b}	0.64^{c}
F-value			
Genotype	570.206**	311.074**	469.130**
Water level	1,250.685**	109.401**	874.005**
Gentype×water level	61.845**	3.771*	212.962**

Note: For each water condition, means in the same column bearing the same letter are not significantly different (P>0.05). For each genotype, † indicates significant differences (P<0.05) between the values in the well-watered and water-deficit treatments. Factors influencing these traits are expressed as F-values; * and ** indicate significant difference at P<0.05 and P<0.01, respectively; n=4.

Lp_{sr} of the three genotypes was 30–50 times less than the hydrostatic Lp_{sr}. The differences between hydrostatic and osmotic Lp_{sr} can be explained by the root composite transport model, with different parallel-pathway switching for radial water movement exhibiting different Lp_{sr}. For a certain root, switching and offsetting between the pathways in water movement always occurs to ensure the plant stays alive under adverse conditions. Our results indicated a heterosis for the ability of single roots of the F_1 to take up water under gradients of hydraulic and osmotic pressure.

2.3 Hydraulic parameters of the whole-root system

In both well-watered and water-deficit treatments, Lp_{wr} of the F_1 was the highest and that of ♂ 478 the lowest, with significant differences among the three genotypes (P<0.05, Table 3). Moreover, the Lp_{wr} of each genotype substantially decreased under water deficit. The L_{wr} of the three genotypes varied similarly with the Lp_{wr}. These results are indicative of heterosis for the ability of the whole-root system of the F_1 to take up water.

2.4 Root anatomical parameters

Whether under well-watered or water-deficit conditions, RD varied in the order F_1 > ♀Tian 4 ≥ ♂478 and CW, VD and VA varied similarly (Table 4). Our calculations showed that the CW/RD ratio in the two water treatments followed the order F_1 < ♀ Tian 4 ≤ ♂ 478, and water deficit increased these values. The RD, CW, VD and VA of the three maize genotypes decreased under water deficit, whereas the CW/RD ratio increased.

Table 3 Root system hydraulic conductivity (Lp_{wr}), hydraulic conductance (L_{wr}) and surface area (SA) of F_1 hybrids in comparison with the parental inbred lines

Water condition	Lp_{wr} (10^{-7} m/(s·MPa))	L_{wr} (10^{-10} m^3/(s·MPa))	SA (cm^2)
Well-watered treatment			
F_1	$5.84^{a\dagger}$	$11.11^{a\dagger}$	18.98^{b}
♀	$4.48^{b\dagger}$	$6.58^{b\dagger}$	14.68^{c}
♂	$2.45^{c\dagger}$	$5.05^{c\dagger}$	$20.57^{a\dagger}$
Water-deficit treatment			
F_1	4.04^{a}	8.79^{a}	$21.76^{a\dagger}$
♀	1.95^{b}	3.26^{b}	$16.71^{b\dagger}$
♂	1.35^{c}	2.19^{c}	16.11^{b}
F-value			
Genotype	465.231^{**}	178.934^{**}	42.825^{**}
Water level	491.892^{**}	96.406^{**}	0.079^{ns}
Genotype×water level	25.678^{**}	1.007^{ns}	30.820^{**}

Note: For each water condition, means in the same column bearing the same letter are not significantly different (P>0.05). For each genotype, † indicates significant differences (P<0.05) between the values in the well-watered and water-deficit treatments. Factors influencing these traits are expressed as F-values; ** indicates significant difference at P<0.01; ns, not significant; n=4.

Table 4 Root diameter (RD), cortex width (CW), ratio of cortex width to root diameter (CW/RD), diameter of central xylem vessel (VD) and cross-sectional area of xylem vessels (VA) of F_1 hybrids in comparison with the parental inbred lines

Water condition	RD (μm)	CW (μm)	CW/RD	VD (μm)	VA (10^4 μm^2)
Well-watered treatment					
F_1	$992.87^{a\dagger}$	$260.50^{a\dagger}$	0.2624^{b}	$80.625^{a\dagger}$	$3.910^{a\dagger}$
♀	$944.43^{b\dagger}$	$259.15^{b\dagger}$	0.2744^{a}	$75.300^{b\dagger}$	2.592^{b}
♂	$939.30^{b\dagger}$	$258.28^{b\dagger}$	0.2745^{a}	$70.775^{c\dagger}$	$2.504^{b\dagger}$
Water-deficit treatment					
F_1	939.00^{a}	250.28^{a}	$0.2665^{c\dagger}$	71.775^{a}	3.152^{a}
♀	896.33^{b}	248.55^{b}	$0.2776^{b\dagger}$	66.150^{b}	2.474^{b}
♂	835.05^{c}	246.70^{c}	$0.2954^{a\dagger}$	59.175^{c}	2.083^{c}
F-value					
Genotype	407.074^{**}	22.883^{**}	0.2253^{**}	30.672^{**}	80.060^{**}
Water level	922.666^{**}	951.937^{**}	0.1344^{**}	71.080^{**}	26.040^{**}
Genotype×water level	62.093^{**}	1.346^{ns}	0.4925^{**}	0.554^{ns}	4.758^{*}

Note: For each water condition, means in the same column bearing the same letter are not significantly different (P>0.05). For each genotype, † indicates significant differences (P<0.05) between the values in the well-watered and water-deficit treatments. Factors influencing these traits are expressed as F-values; * and ** indicate significant difference at P<0.05 and P<0.01, respectively; ns, not significant; n=4.

2.5 Physiological parameters of the root system

To evaluate the effect of osmoregulation on the activities of water uptake and on the lipid peroxidation of the membrane, we determined the levels of proline and MDA (Table 5). The results indicated a significant difference in free proline and MDA under well-watered and water-deficit conditions. The level of proline in the F_1 was higher, while the level of MDA was lower than those of the parental genotypes. The levels of free proline and MDA increased under water deficit.

Table 5 Levels of free proline and MDA in roots of F_1 hybrids in comparison with the parental inbred lines

Water condition	Proline (μg/g)	MDA (mmol/g)
Well-watered treatment		
F_1	2.11^{a}	5.082^{b}
♀	1.39^{b}	7.312^{a}
♂	1.25^{b}	8.047^{a}
Water-deficit treatment		
F_1	$3.70^{a\dagger}$	$7.498^{c\dagger}$
♀	$1.92^{c\dagger}$	$22.487^{b\dagger}$
♂	$2.23^{b\dagger}$	$28.632^{a\dagger}$
F-values		
Genotype	90.654^{**}	213.938^{**}
Water level	150.853^{**}	674.452^{**}
Genotype×water level	13.184^{**}	120.819^{**}

Note: For each water condition, means in the same column bearing the same letter are not significantly different ($P>0.05$). For each genotype, † indicates significant differences ($P<0.05$) between the values in the well-watered and water-deficit treatments. Factors influencing these traits are expressed as *F*-values; ** indicates significant difference at $P<0.01$; $n=4$.

3 Discussion

3.1 AQPs and root water uptake ability

In this study, the heterosis in water uptake by maize roots was demonstrated at different levels. For the same treatment, Lp_c was highest and Lp_{wr} was lowest in the F_1. Lp_c was positively and significantly correlated with Lp_{sr} and Lp_{wr} (Fig. 1). These hydraulic traits at all three scales were consistent regardless of genotype and water condition. The Lp_c of the F_1 was higher than that of either parent under well-watered and water-deficit conditions (Table 1). Our previous studies (Wu et al., 2006a, b) found that the relative levels of *PIP1;1* mRNA in the well-watered treatment and of *PIP1;1* and *PIP2;5* in the water-deficit treatment of the F_1 plants were the highest. The growth conditions of the present and the previous studies were the same, suggesting that high levels of transcription of *PIP*s may contribute to the high Lp_c. *PIP*s can regulate root hydraulic resistance (Maurel et al., 2010), and our results have shown consistent levels of transcription of *PIP1;1* and *PIP2;5* and consistent root hydraulic traits in the maize genotypes. More research is required, however, to clearly show the role of *PIP*s in maize heterosis, for example in measurements of protein abundant.

Under conditions of water deficit, the levels of the transcription products of *PIP1;1* and *PIP2;5* were upregulated in the roots of the F_1 and ♀ Tian 4 (Wu et al., 2006a, b). We suggest that the upregulation in the density of AQPs in the cell membranes of maize roots

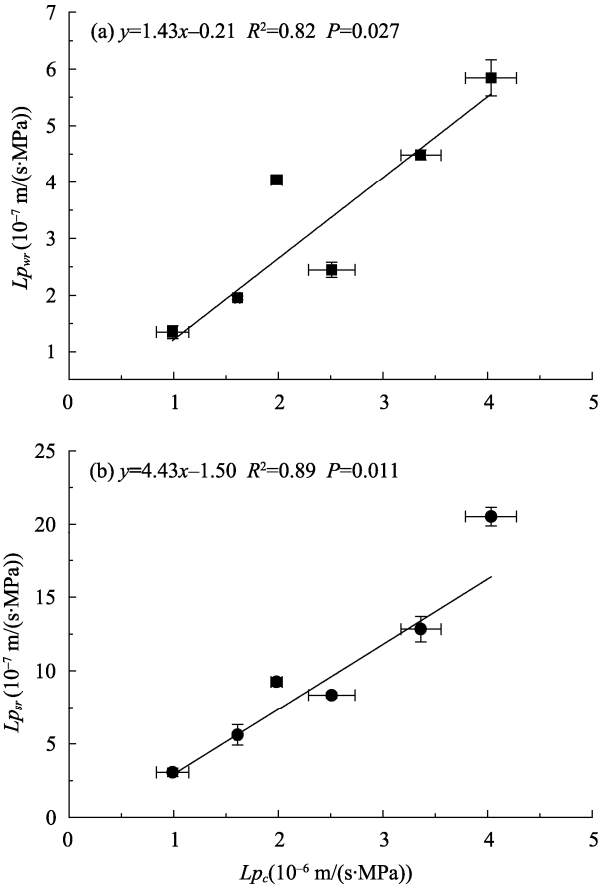

Fig. 1 Correlations between Lp_c and Lp_{wr} (a) or radial Lp_{sr} (b). Each point is an average for a treatment. Error bars represent the standard error; $n=6$.

may enhance water flow through the cell-to-cell pathway. This scenario supports the composite transport model of roots in which water flow switches between apoplastic and cell-to-cell pathways to maintain the water status of a plant (Steudle and Peterson, 1998). The Lp_c of the three genotypes, however, substantially decreased under conditions of water deficit (Table 1). Together, these results suggest that the substantial decreases in Lp_c were possibly caused by the decreases in AQP activity under conditions of water deficit, and the upregulation of AQP density had a compensatory effect on the inhibition of AQP activity.

AQP activity has frequently been tested using Hg^{2+}, which can bind to the SH groups of AQP cysteine residues exposed to the membrane surface. This process causes reversible changes in the structure of AQPs (Wan et al., 2004). When $HgCl_2$ was added to the bathing solution of the cell pressure probe in the well-watered treatment, the three genotypes showed substantial decreases in the Lp_c (68% to 71%) (Table 1). The inhibition of Lp_c by $HgCl_2$ was only partly recovered when $HgCl_2$ was replaced with the reducing agent mercaptoethanol. Water deficit induced a significant decrease in the Lp_c (50% to 60%) of maize root cortical cells. The effect of water deficit (50% to 60%) was smaller than that of Hg^{2+} (68% to 71%). When the AQPs were closed under water deficit, an extra effect of $HgCl_2$ was found. This effect indicated that AQPs were partly closed under water deficit in our experiment and might be completely closed under a more severe deficit.

3.2 Anatomical mechanisms for differences in water uptake

Root anatomy played an important role in the behavior of water uptake by roots. Correlation analysis of hydrostatic Lp_{sr} and root anatomical parameters (Fig. 2) showed that radial Lp_{sr} was positively correlated with CW (R^2=0.81, P<0.01) and negatively correlated with the CW/RD ratio (R^2= –0.77, P<0.01). Steudle and Frensch (1996) suggested that water flow through the radial pathway was inversely proportional to the length of the path or the number of cell layers. Rieger and Litvin (1999) studied two woody and three herbaceous species with different root anatomical features and found that root hydraulic conductivity was negatively correlated with root cortex width (R^2= –0.74). These results suggest the contribution of cortex cells to radical hydraulic resistance, which was also confirmed by our findings. The hybrid F_1 with the lowest CW/RD ratio had the lowest radial hydraulic resistance to water flow and the highest radial Lp_{sr}. Under conditions of water deficit, the RD and CW decreased, but the CW/RD ratio increased, creating a lower radial Lp_{sr} to reduce water consumption. In addition, we calculated and compared values of VD and VA (Table 4). The results showed that the axial movement of water mainly depended on the root xylem vessels. The L_{ax} was positively correlated with VD (R^2=0.75, P<0.01) and VA (R^2=0.93, P<0.01). These results were consistent with previous findings. Cruz et al. (1992) concluded that xylem vessels with smaller diameters reduced the L_{ax} under conditions of water deficit. The smaller diameters of xylem vessels may favor the slow utilization of water by roots to enhance water-use efficiency. Among the three genotypes, the hybrid F_1 had the smallest CW/RD ratio and largest VD and VA (Table 4). The F_1 thus had the lowest radial and axial hydraulic resistance to water movement, suggesting heterosis for water uptake by single roots.

3.3 Physiological explanation of differences in water uptake

The L_{wr}, which integrates Lp_{wr} and the root surface, represents the capacity for water uptake of the whole-root system (Maurel et al., 2010). The Lp_{wr} resulted from the dynamic integration of water flow through all single roots and cells. Both the Lp_{wr} and L_{wr} may be affected by factors regulating water movement at cellular and single-root levels, such as by AQPs and root anatomy. Clarkson et al. (2000) demonstrated that daily fluctuation of Lp_{wr} was in accordance with the diurnal cycle in the abundance of AQP mRNAs in the roots of *Lotus japonicus*. Previous reports indicated that Lp_{wr} can be affected by root morphology. Zhao et al. (2005) found that Lp_{wr} was negatively correlated with root surface area and length. In our study, the downregulation of Lp_{wr} in the three genotypes might be a protective reaction to prevent a possible plant-to-soil backflow of water under water deficit (Doussan et al., 2006; Maurel et al., 2010). The

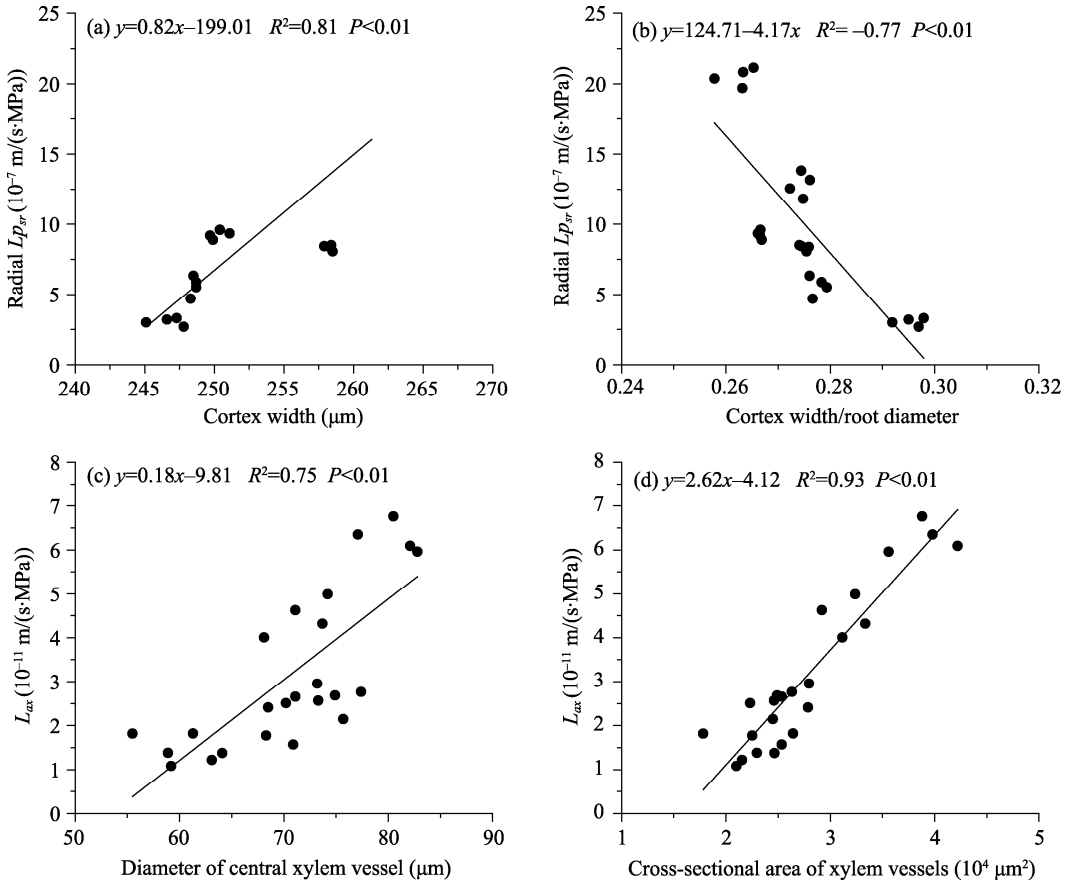

Fig. 2 Correlations between radial Lp_{sr} and cortex width (a), radial Lp_{sr} and cortex width/root diameter (b), L_{ax} and diameter of the central xylem vessel (c) and L_{ax} and cross-sectional area of xylem vessels (d). Each point represents an individual root segment; n=24.

increases in root surface area in the F_1 and ♀ represented a mechanism of adaptation to water deficit. The adaptive reaction also compensated for the decreases in Lp_{wr}. Lp_{wr}, however, was not correlated with root surface area. Root morphology may thus not contribute to the heterosis of Lp_{wr}.

Proline plays a vital role in osmoregulation (Ahmad and Hellebust, 1988; Choudhary et al., 2007). The production of free proline in plant tissues can be induced under stress to protect plants against damage by reactive oxygen species, but its concentration is relatively lower under normal circumstances. MDA is a cytotoxic product of lipid peroxidation and an indicator of free-radical production and consequent tissue damage (Choudhary et al., 2007). Our results showed that at the whole-root system level, the L_{wr} and Lp_{wr} of the F_1 were higher than those of the inbred parents in the two water treatments. The heterosis for water uptake by the root system of maize may be related to the high levels of free proline and the low levels of MDA in roots of the F_1, which should favor the maintenance of membrane stability and the avoidance of damage, especially under conditions of water deficit.

4 Conclusions

Plant growth depends on an optimum balance between water uptake in the roots and water losses through the shoots. Low plant productivity mainly results from the loss of water balance of plants in arid and semi-arid regions. Regulation and control on water uptake by plant roots is an important approach to raise plant productivity. This work demonstrated the presence of heterosis for water uptake ability in F_1 roots at cellular, single-root and whole-root system levels under well-watered and water-deficit conditions. The heterosis for water uptake in the F_1 was comprehensively affected by heterosis for AQPs, anatomy, root morphology and

osmoregulation. Heterosis for water uptake ability should improve plant survival in adverse situations, particularly under conditions of water deficit. These results provided some insight into the mechanism of heterosis for water uptake by maize roots and should be useful for breeding or selecting new maize genotypes with improved abilities for water uptake by roots and for drought resistance.

This work is the first attempt to apply cell and root pressure probes to determine the parameters of water relations among genotypes of the same species. The results showed that pressure probe techniques can be used for studying genotypic differences in water uptake by plant roots.

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