# Collocations of QTLs for Seedling Traits and Yield Components of Tropical Maize under Water Stress Conditions

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## ABSTRACT

Genetic variation in root morphology and its response to water deficit might be crucial for the adaptation of maize (*Zea mays* L.) to drought, but information about this is scarce. A set of 208 recombinant inbred lines (RILs) from the cross between Ac7643 (Parent 1) and Ac7729/TZSRW (Parent 2) was investigated in growth pouches to determine the traits of seedling roots and shoots. Water stress was induced by polyethylene glycol (PEG 8000). Quantitative trait loci (QTLs) were identified for seedling traits at the first leaf stage; their collocation was compared with published yield-related traits found in drought-stress experiments. Some seedling QTLs were specific to water stress, such as QTLs for root dry weight, shoot dry weight, and leaf area-to-root length ratio. Four root and shoot QTLs collocated in bin 2.02, indicating a relationship of this chromosome region to early vigor under water stress. Quantitative trait loci for ear number collocated with QTLs for the shoot-to-root dry weight ratio and leaf area-to-root length ratio. Quantitative trait loci for the anthesis-silking interval collocated with QTLs for the numbers of crown roots and seminal roots irrespective of water supply. Quantitative trait loci controlling the balance between early root and shoot development may provide useful information to enable the prediction of maize performance under field conditions.

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**Abbreviations:** ABA, abscisic acid; ASI, anthesis–silking interval; $DW_{Rt}$, root dry weight; $DW_{St}$, shoot dry weight; $DW_{StRt}$, ratio of dry weight of root to shoot; ENO, ear number; KNO, kernel number; LA, leaf area; LARL, leaf area-to-root length ratio; LOD, logarithmic odds ratio; $L_{Rt}$, root length; $No_{Cr}$, number of crown roots; $No_{Se}$, number of seminal roots; P1, Parent 1 (Ac7643); P2, Parent 2 (Ac7729/TZSRW); PEG, polyethylene glycol; QEI, QTL × environment interaction; QTLs, quantitative trait loci; RIL, recombinant inbred line; WS, water stressed; WW, well watered.

POOR SEEDLING ESTABLISHMENT under water shortage can reduce plant density and the grain yield of maize (*Zea mays* L.) (Edmeades et al., 1989). Under such conditions roots tend to grow deeper. Compared with the shoot, their growth may even improve in dry soil (Sharp and Davies, 1989; Weerathaworn et al., 1992a, 1992b). Under water deficit in the topsoil, seedlings with vigorous root growth are more likely to sustain growth and to be productive (Cutforth et al., 1986). As a consequence, drought stress shifts the shoot–to–root ratios of maize, based on both weight and surface area, in favor of the roots (Weerathaworn et al., 1992a, 1992b). Similar shifts were observed for young field-grown cotton (*Gossypium hirsutum* L.) plants (Pace et al., 1999).

Most genetic studies on water stress in maize under field conditions focused on the "above-ground" part of the plant, and targeted harvest index, grain yield, and secondary traits, such as the anthesis–silking interval (ASI) (for review see Ribaut et al., 2008). A large proportion of the variation observed in these traits may

be due to altered root morphology and architecture, as hypothesized by Hammer et al. (2009), based on a simulation study. According to Tuberosa et al. (2002b), root quantitative trait loci (QTLs) from unstressed seedlings in hydroponics were related to QTLs of field-grown maize under drought stress, such as leaf abscisic acid (ABA), ASI, and grain yield.

Roots are not easily accessible under field conditions, especially under drought conditions; as a consequence, phenotyping large numbers of roots is practically impossible under such situations. Ex situ investigations of seedlings, based on growth pouches containing blotting paper, were performed to study various topics, including plant nutrition (Liao et al., 2001; Yan et al., 2004) and water stress (Wang et al., 2005). Combined with digital image processing, growth pouches are excellent for phenotyping the roots of seedlings of large sets of genotypes (Hund et al., 2009a). They can also be used to evaluate the response of root growth to abiotic stresses, such as desiccation induced by polyethylene glycol (PEG).

To mimic low water potential conditions, it is important that an osmoticum does not interact with plant tissues but only lowers the water potential of the medium (Verslues et al., 1998). Polyethylene glycol, with a molecular weight >6000 Da, has been used for many years because its high molecular weight prevents it from penetrating the cell wall pores (Carpita et al., 1979). An unavoidable effect of PEG is the high viscosity of the solution, which limits the movement of oxygen and, consequently, can reduce primary root elongation and metabolism (Verslues et al., 1998).

A better understanding of the genetic basis of maize responses to drought from early to late stages may enable an in-depth understanding of complex mechanisms of drought tolerance of the whole plant. The present study was conducted at the early, still heterotrophic stage of maize seedlings with a segregating population of recombinant inbred lines (RILs). The population had already been studied extensively in the field under drought stress (Ribaut et al., 1996, 1997) and low nitrogen supply (Ribaut et al., 2007). The major objectives of this study were (i) to identify QTLs for root characteristics and shoot-to-root relationships in response to water stress, and (ii) to relate them to published QTLs for yield components and secondary traits as identified in the same mapping population.

## MATERIALS AND METHODS
### Plant Material
Seeds of 208 RILs were obtained from the International Maize and Wheat Improvement Center in Mexico; they were derived from the cross between Ac7643 (Parent 1 [P1]) and Ac7729/TZSRW (Parent 2 [P2]). Under drought, P1 has a short ASI and a relatively high yield, while P2 has a long ASI and relatively low yield (Ribaut et al., 1996).

## Growth Conditions
The experiments were performed from January 2006 to February 2007 at the Institute of Plant Science of the Swiss Federal Institute of Technology in Zurich using growth pouches as described by Hund et al. (2009b). Seeds were germinated in the dark at 27°C; healthy seedlings with a primary root length of about 1 cm were transferred to growth pouches. A pouch contained blue germination blotting paper, 24 by 29.5 cm (Anchor Paper, St. Paul, MN) as the substrate for the growing roots, both sides of the pouch were covered with black polyethylene sheets (Walser AG, Buerglen, Switzerland) to prevent light from reaching the roots. Seedling shoots (one per pouch) were placed upright in the middle top of the pouch so that the roots grew downward. Twelve pouches were hung in plastic containers (growth boxes), 27 by 37 by 32 cm. During the first 3 d after germination (DAG), all the plants were grown with the lower edge of the pouch submerged to a depth of about 2 cm in the basic medium solution of 0.23% (v/v) Wuxal (Aglukon Spezialdünger GmbH, Düsseldorf, Germany). Per liter, Wuxal contains 100 g N, 43 g P, 62.5 g K, 190 mg Fe, 162 mg Mn, 102 mg B, 81 mg Cu, 61 mg Zn, and 10 mg Mo. The growth boxes were covered with laminated polystyrene (Spaarpor Klaus Eckhardt GmbH, Neunkirchen, Germany) to ensure that light radiation did not lead to an increase in temperature of the pouches. After 3 d, all the pouches were submerged daily for 5 min in the basic medium solution (well watered [WW]) or in the basic medium containing 20% (w/v) PEG 8000 (Sigma Aldrich GmbH, Steinheim, Germany), hereafter referred to as water stressed (WS). The conditions in the growth chamber were 25/22°C (day/night), 70/60% relative humidity (day/night), and a 12-h photoperiod with a photosynthetically active radiation of 400 μmol $m^{-2}$ $s^{-1}$. The plants were harvested when all had reached the first leaf stage, typically 7 and 9 DAG for WW and WS, respectively.

## Root Measurements
At harvest, the root system was scanned with a Hewlett-Packard Scanjet 4670 See-Thru Vertical Scanner (Hewlett-Packard, Palo Alto, CA). Images were acquired with a resolution of 23.7 dots $mm^{-1}$ (600 dpi) and stored in a JPEG format of the highest quality. The acquired 24-bit JPEG images were subsequently processed in Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA) in three steps, as described by Hund et al. (2009a). 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 (Version 2003b, Regent Instruments, Montreal, 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 WinRHIZO to obtain the root length ($L_{Rt}$) of each genotype. The debris filter of WinRHIZO was set to remove objects with an area <0.02 $cm^2$ and a length/width ratio <5. In addition to the digital analysis, the numbers of crown roots ($No_{Cr}$) and seminal roots ($No_{Se}$) were counted and the root dry weight ($DW_{Rt}$) was determined after drying in an oven at 65°C for 72 h.

## Shoot Measurements

One plant per growth box was sampled to measure the predawn leaf water potential according to the method proposed by Scholander et al. (1965). The potential of freshly cut shoots was measured in the dark at the end of the 12-h night in a pressure chamber (Plant Water Status Console 3000, Soil Moisture Equipment Corp., Santa Barbara, CA). The leaf area (LA) was measured with a LI-3000A area meter (LICOR, Inc., Lincoln, NE), and the shoot dry weight ($DW_{St}$) was determined after drying in an oven at 65°C for 72 h. The shoot-to-root dry weight ratio ($DW_{StRt}$) and leaf area-to-root length ratio (LARL) were then calculated.

## Experimental Design and Statistics

An $\alpha$ lattice design was used with six independent runs, that is, replications. Each replication consisted of 18 incomplete blocks distributed in two walk-in growth chambers. One incomplete block was represented by a pair of growth containers, and one was assigned to the WW treatment and the other to the WS treatment, each containing the same set of 12 genotypes. The 208 RILs and their two parental lines (each repeated four times) represented a total of 216 experimental units in each water treatment × run combination. Treatment and replication effects were considered to be fixed, while incomplete blocks nested within growth chambers and replications were considered to be random. Analysis of variance was done with the R package ASREML (Butler et al., 2007), and the best linear unbiased predictors for each genotype, extracted for each treatment, were used for the QTL mapping. Based on the mean, the heritability for each treatment was calculated:

$$h^2 = \frac{\sigma^2_g}{\sigma^2_g + \frac{1}{b}\sigma^2_e}$$

where $\sigma^2_g$ is the genetic variance, $\sigma^2_e$ is the error variance, and $b$ is the number of replications. Phenotypic correlations among traits in both WW and WS treatments were calculated as simple Pearson's correlation coefficients using the "cor" command with the option of "pairwise.complete.obs" in R (R Development Core Team, 2008).

## QTL Analysis

Quantitative trait loci were identified with the restriction fragment length polymorphism (RFLP) linkage map published by Fracheboud et al. (2002). The map consisted of 132 RFLP markers with a total distance of 2250 cM and an average distance between markers of 17.1 cM. Quantitative trait loci were detected by composite interval mapping using QTL Cartographer 1.17 Model 6 (Basten et al., 2003) with a blocking window size of 30 cM. The cofactors were selected by forward and backward regressions with in and out thresholds set at $P = 0.01$. Data of all the traits from both the WW and WS treatments were analyzed in a combined joint analysis (Jiang and Zeng, 1995), to allow determination of the QTL × environment interaction (QEI). A QTL was considered to be significant when the logarithmic odds ratio (LOD) score was >3 for the joint analysis and 2.5 for the single-trait analysis. Both LOD thresholds represent a comparison-wise $\alpha$ significance value of 0.06 and an experiment-wise $\alpha$ significance value of 0.003, assuming that all

20 chromosome arms are segregating independently. The corresponding LOD threshold for a significant QEI was 0.8. The thresholds were determined by assuming a chi-square distribution with 3 df for the joint analysis, 2 df for the single analysis, and 1 df (comparison-wise) for the interaction. Published QTLs were available from field experiments with the same marker population; they were used to calculate collocating QTLs from those experiments and the experiments with pouches (Ribaut et al., 1996, 1997). Published QTLs for leaf and xylem ABA concentrations were available from other populations (Lebreton et al., 1995; Sanguinetti et al., 1999; Tuberosa et al., 2002a).

## RESULTS

Application of PEG reliably reduced the predawn leaf water potential to −7.4 MPa under WS compared with 0.9 MPa under WW. Under WS, the plants reached the first leaf stage after 7 d and under WW after 9 d. Under WS, P1 had significantly higher $DW_{StRt}$ and LARL (Table 1). Water treatments of the RIL population had no effect on the $No_{Se}$, whereas the $No_{Cr}$ was lower under WS. The latter was the only trait with a significant genotype × water treatment interaction. Under WS, the $DW_{St}$ was slightly lower but the $DW_{Rt}$ was higher, resulting in a lower $DW_{StRt}$ (Table 1); this translated to a 16% increase in the total $L_{Rt}$ and a 38% decrease in the total LA, resulting in a very low LARL.

### Phenotypic Correlations

Correlations between pairs of traits were always similar under WW and WS, but were usually of lower magnitude under WS (Table 2). Shoot-related traits such as $DW_{St}$ and LA showed high correlations in both water treatments ($r = 0.92$ and $0.86$ for WW and WS, respectively). Therefore, both LA and $DW_{St}$ showed similar correlation with root traits. The $DW_{St}$ was highly correlated with the $DW_{Rt}$ ($r = 0.79$ and $0.72$ for WW and WS, respectively). Furthermore, significant correlations were found between $DW_{St}$ and $L_{Rt}$ ($r = 0.67$ and $0.57$ for WW and WS, respectively).

### Overview on Identified QTLs

Fifteen QTLs were identified for eight traits, including $L_{Rt}$, $No_{Cr}$, $No_{Se}$, $DW_{Rt}$, $DW_{St}$, LA, $DW_{StRt}$, and LARL (Table 3). Two constitutive QTLs were identified with LOD scores >2.5 in both WW and WS and with no significant QEI. They included a QTL for $L_{Rt}$ (bin 2.02) and one for $No_{Se}$ (bin 6.05). Nine QTLs were significant in only one of the water treatments, three of which were specific to WS (LOD of QEI > 0.8). These QTLs were for $DW_{Rt}$, $DW_{St}$, and LARL located in bins 2.02, 9.02, and 3.04, respectively. Three QTLs were significant in the joint analysis (joint LOD > 3.0) only.

### QTL Collocations among Traits Measured at the Seedling Stage

Quantitative trait loci for LA, $DW_{St}$, $DW_{Rt}$, and $L_{Rt}$ were collocated in bin 2.02 (Table 3 and Fig. 1). All collocated

**Table 1.** Average values for maize parental lines and the recombinant inbred line (RIL) population from the cross between Ac7643 (Parent 1) × Ac7729/TZSRW (Parent 2) and heritability $(h^2)$ for the following traits: total root length ($L_{Rt}$), crown root number ($No_{Cr}$), seminal root number ($No_{Se}$), leaf area (LA), shoot dry weight ($DW_{St}$), root dry weight ($DW_{Rt}$), shoot-to-root dry weight ratio ($DW_{StRt}$), and leaf area-to- root length ratio (LARL). The experiments were performed under well-watered (WW) and water-stressed (WS) conditions.

| Trait | Parental lines | | Significance level[‡] | RILs | | | $h^{2}$[§] | ANOVA results[†] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Parent 1 | Parent 2 | | Mean | Range | | | G | E | G × E |
| $L_{Rt}$_WW (cm) | 83.5 | 97.6 | ** | 98.4 | 61.0 – | 170.1 | 0.74 | *** | *** | NS |
| $L_{Rt}$_WS (cm) | 100.2 | 140.4 | ** | 116.9 | 79.4 – | 158.8 | 0.65 | | | |
| $No_{Cr}$_WW (no.) | 3.65 | 2.68 | NS[¶] | 3.30 | 2.42 – | 4.24 | 0.5 | *** | *** | * |
| $No_{Cr}$_WS (no.) | 2.79 | 1.77 | NS | 2.70 | 1.77 – | 3.65 | 0.55 | | | |
| $No_{Se}$_WW (no.) | 2.65 | 3.36 | NS | 3.82 | 2.65 – | 5.11 | 0.64 | *** | NS | NS |
| $No_{Se}$_WS (no.) | 3.38 | 3.59 | NS | 3.68 | 3.02 – | 4.92 | 0.62 | | | |
| LA_WW (cm$^2$) | 18.0 | 20.6 | NS | 21.3 | 16.5 – | 35.5 | 0.63 | *** | *** | NS |
| LA_WS (cm$^2$) | 13.1 | 14.0 | NS | 13.3 | 10.8 – | 16.1 | 0.48 | | | |
| $DW_{St}$_WW (mg) | 47.1 | 48.9 | NS | 51.1 | 36.5 – | 76.8 | 0.67 | *** | ** | NS |
| $DW_{St}$_WS (mg) | 50.6 | 51.9 | NS | 48.6 | 36.7 – | 63.1 | 0.69 | | | |
| $DW_{Rt}$_WW (mg) | 34.4 | 37.6 | NS | 38.4 | 23.4 – | 67.6 | 0.76 | *** | *** | NS |
| $DW_{Rt}$_WS (mg) | 41.5 | 51.9 | NS | 43.1 | 32.8 – | 74.6 | 0.78 | | | |
| $DW_{StRt}$_WW | 1.60 | 1.30 | NS | 1.43 | 1.15 – | 2.03 | 0.63 | *** | *** | NS |
| $DW_{StRt}$_WS | 1.27 | 1.01 | ** | 1.09 | 0.82 – | 1.46 | 0.65 | | | |
| LARL_WW | 0.26 | 0.23 | NS | 0.24 | 0.19 – | 0.42 | 0.58 | *** | *** | NS |
| LARL_WS | 0.14 | 0.11 | ** | 0.12 | 0.10 – | 0.16 | 0.56 | | | |

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

[†]ANOVA results for the effect of the RILs (G), the water treatment (E), and their interaction (G × E).

[‡]Significance between the parental lines.

[§]Broad-sense heritability ($h^2$).

[¶]NS, not significant.

**Table 2.** Pearson's phenotypic correlations among traits measured in the recombinant inbred line population from the cross between Ac7643 × Ac7729/TZSR with the sample size $n$ = 208. See Table 1 for explanation of abbreviations.

| Trait | $No_{Cr}$ | $No_{Se}$ | $DW_{Rt}$ | $DW_{St}$ | LA | $DW_{StRt}$ | LARL |
|---|---|---|---|---|---|---|---|
| Well-watered | | | | | | | |
| $L_{Rt}$ | 0.30*** | 0.34*** | 0.76*** | 0.67*** | 0.64*** | −0.41*** | −0.53*** |
| $No_{Cr}$ | | 0.32*** | 0.37*** | 0.39*** | 0.37*** | −0.19** | −0.12[NS] |
| $No_{Se}$ | | | 0.43*** | 0.34*** | 0.32*** | −0.32*** | −0.13[NS] |
| $DW_{Rt}$ | | | | 0.79*** | 0.71*** | −0.62*** | −0.26*** |
| $DW_{St}$ | | | | | 0.92*** | −0.11[NS] | 0.02 [NS] |
| LA | | | | | | −0.08[NS] | 0.10 [NS] |
| $DW_{StRt}$ | | | | | | | 0.48*** |
| Water-stressed | | | | | | | |
| $L_{Rt}$ | 0.27*** | 0.36*** | 0.67*** | 0.57*** | 0.64*** | −0.44*** | −0.58*** |
| $No_{Cr}$ | | 0.20** | 0.30*** | 0.34*** | 0.30*** | −0.12[NS] | −0.03[NS] |
| $No_{Se}$ | | | 0.40*** | 0.28*** | 0.31*** | −0.34*** | −0.20** |
| $DW_{Rt}$ | | | | 0.72*** | 0.68*** | −0.67*** | −0.20** |
| $DW_{St}$ | | | | | 0.86*** | −0.08[NS] | 0.08[NS] |
| LA | | | | | | −0.12[NS] | 0.14[NS] |
| $DW_{StRt}$ | | | | | | | 0.45*** |

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

QTLs, except that for $L_{Rt}$, were significant only in the WS treatment with positive additive effects of alleles contributed by P1. Collocating QTLs for $DW_{StRt}$ and LARL were identified in bins 1.03 and 3.04. In bin 3.04 the QTL for LARL was significant only under WS, while the QTL for $DW_{StRt}$ was significant only under WW. A QTL for $No_{Cr}$ was collocated with a QTL for $DW_{St}$ in bin 9.02. The QTL for $No_{Cr}$ was significant only under WW, while that for $DW_{St}$ was specific to WS. The two QTLs showed positive additive effects of the allele contributed by P1.

## QTL Collocations with Traits Measured under Field Conditions

Collocations between QTLs for $No_{Cr}$ and for ASI (Ribaut et al., 1996) were found in bins 1.08 and 2.08. The QTLs for both traits showed the same signs of corresponding additive effects in bin 1.08, while the loci in bin 2.08 showed the opposite signs. The QTL for $No_{Se}$ in bin 6.05 overlapped with QTLs for ASI in both treatments, and with ear number (ENO) under WS. At this locus the P2 allele increased the ASI and decreased $No_{Se}$ in both treatments.

Two linked QTLs for $DW_{StRt}$ and LARL in bins 1.03 and 3.04 overlapped with QTLs for ENO under inter-mediate and severe water stress (Ribaut et al., 1997). In

Table 3. Detected quantitative trait loci (QTLs) (joint logarithmic odds ratio [LOD] score ≥ 3) for maize traits evaluated under well-watered (WW) and water-stressed (WS) conditions. Evaluated traits were total root length ($L_{Rt}$), crown root number ($No_{Cr}$), seminal root number ($No_{Se}$), leaf area (LA), shoot dry weight ($DW_{St}$), root dry weight ($DW_{Rt}$), shoot-to-root dry weight ratio ($DW_{StRt}$), and leaf area-to-root length ratio (LARL). The QTL characteristics include the chromosome (Chr), the position of the QTL peak in centimorgans, location on the Bin map, the LOD score for the joint analysis, the individual environment (significant above 2.5) and the QTL × environment interaction (QEI), the closest markers and the QTL interval as well as the additive (A) effect at the QTL peak, and the percentage of phenotypic variance ($R^2$) explained by the individual QTLs, considering all significant QTLs together.

| Trait | Chr | cM | Bin | Closest marker | LOD score | | | | Interval[†] | A[‡] | $R^2$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Joint | WW | WS | QEI | | | WW | WS |
| $L_{Rt}$ | 2 | 18.5 | 2.02 | umc53a | 5.18 | 2.82 | 5.10 | 0.00 | 4 – 41 | 4.984 | 5.0 | 8.0 |
| | 3 | 131.1 | 3.05 | csu134d(thf) | 3.16 | 2.33 | 2.94 | 0.10 | 107 – 156 | −4.730 | 6.5 | 8.2 |
| $No_{Cr}$ | 1 | 228.8 | 1.08 | umc83a | 3.79 | 2.44 | 2.02 | 0.00 | 213 – 244 | 0.084 | 5.5 | 4.2 |
| | 2 | 178.2 | 2.08 | umc137a | 3.77 | 1.49 | 2.87 | 0.38 | 165 – 199 | 0.089 | 2.4 | 5.7 |
| | 9 | 57.6 | 9.02 | umc105a | 3.62 | 3.03 | 1.62 | 0.06 | 44 – 108 | 0.091 | 7.9 | 4.2 |
| $No_{Se}$ | 6 | 129.4 | 6.05 | csu116a(elf1) | 4.68 | 4.38 | 2.86 | 0.58 | 88 – 171 | 0.142 | 11.0 | 7.4 |
| LA | 2 | 32.5 | 2.02 | umc53a | 3.17 | 0.98 | 3.14 | 0.06 | 17 – 85 | 0.336 | 3.0 | 10.0 |
| | 8 | 58.0 | 8.02 | umc91b | 3.23 | 1.44 | 0.12 | 2.71§ | 31 – 86 | 0.129 | 4.0 | 0.3 |
| $DW_{St}$ | 2 | 36.5 | 2.02 | umc53a | 3.47 | 1.31 | 3.44 | 0.25 | 17 – 80 | 1.614 | 3.1 | 9.4 |
| | 9 | 55.6 | 9.02 | umc105a | 3.10 | 0.37 | 2.57 | 0.95§ | 46 – 73 | 1.174 | 1.7 | 6.9 |
| $DW_{Rt}$ | 2 | 30.5 | 2.02 | umc53a | 3.74 | 0.89 | 3.46 | 2.08§ | 6 – 53 | 1.788 | 2.8 | 8.3 |
| $DW_{StRt}$ | 1 | 89.1 | 1.03 | umc185(p1) | 3.05 | 2.30 | 2.33 | 0.21 | 78 – 115 | −0.027 | 5.4 | 5.4 |
| | 3 | 67.0 | 3.04 | umc50a | 3.10 | 3.06 | 1.23 | 1.25§ | 55 – 129 | 0.024 | 6.7 | 4.5 |
| LARL | 1 | 82.4 | 1.03 | UMC11a | 6.05 | 4.34 | 3.85 | 2.70§ | 54 – 109 | −0.003 | 6.3 | 7.1 |
| | 3 | 67.9 | 3.04 | npi114b | 3.42 | 1.91 | 2.87 | 0.90§ | 57 – 103 | 0.003 | 3.5 | 6.5 |

[†]Interval around the peak where the LOD score of the joint analysis dropped by one-half from its peak value.

[‡]Additive (A) effects of the joint analysis are defined as the contribution of the allele of Ac7643 (P1).

§Significant >0.8 probability level.

bin 1.03, the P2 allele increased $DW_{StRt}$ and LARL, but decreased ENO, while in bin 3.04 the P1 allele increased $DW_{StRt}$ and LARL and decreased ENO. Therefore, both parental lines contributed favorable alleles to these traits, as expected for polygenic traits presenting transgressive segregation (Table 1). Independent of the nature of the allelic contribution, from P1 or P2, an increase in the development of the upper part of the plant as opposed to root system corresponded in both cases to a reduction of ENO. In bin 9.02, QTLs for $No_{Cr}$ and $DW_{St}$ collocated with QTLs for ENO in both WW and WS and with a QTL for kernel number (KNO) in WS only. In this genomic region the allele increased $No_{Cr}$ and $DW_{St}$, while it decreased ENO and KNO.

## DISCUSSION

The PEG treatment successfully induced water stress, which resulted in leaf water potentials of about −7.0 MPa, similar to results reported for maize, barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) seedlings (Lu and Neumann, 1998). Water stress retarded shoot development and promoted root development, consistent with results for stressed plants in solid substrates (Sharp and Davies, 1989; Weerathaworn et al., 1992a, 1992b). These observations indicate that the stress was mild, allowing an adaptation in the allocation of available biomass.

In rice, water deficit favors the development of seminal and lateral roots, but not of adventitious roots (Ling et al., 2003). The number of crown, that is, adventitious, roots decreased under WS, while the number of seminal roots did not change significantly. The absence of common QTLs for the number of seminal and crown roots may suggest control by different genetic loci.

Collocations of QTLs for different traits could indicate that the genes underlying the QTLs are related by linkage and/or pleiotropy (Lebreton et al., 1995; Agrama and Moussa, 1996; Tuberosa et al., 2002b). The major QTLs for $L_{Rt}$, LA, $DW_{St}$, and $DW_{Rt}$ in bin 2.02 may imply a common dependence of their expression on the availability of readily transportable biomass at the heterotrophic stage (Cooper and McDonald, 1970; Deleens et al., 1984). Interestingly, with the exception of root length, most of the QTLs in this region were only significant under WS; all the positive alleles at the collocated QTLs were contributed by the drought-tolerant P1. According to Moussa and Abdel-Aziz (2008), mechanisms of tolerance to drought stress are already active in maize seedlings, as corroborated here.

In a different segregating population for drought studies, a QTL for KNO was also identified in this bin 2.02 (Messmer et al., 2009); it was significant only under drought conditions (at flowering time) and explained a large percentage of the phenotypic variance for a yield
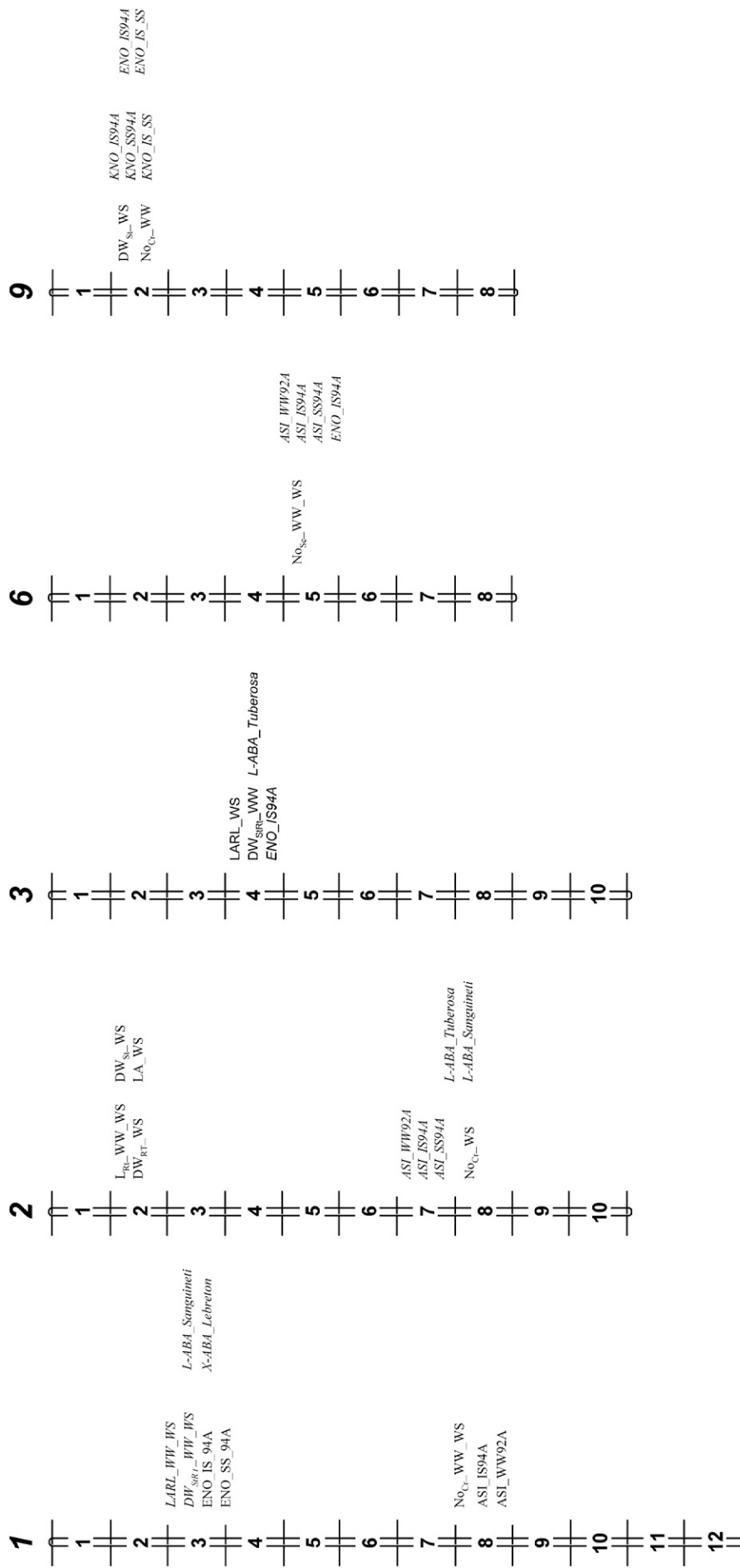
component (8%). Within the same marker population (Ribaut et al., 1996), collocating QTLs for $DW_{StRt}$ and LARL overlapped with published QTLs for ENO under WS in the field in bins 1.03 and 3.04. The directions of these additive effects of QTLs for $DW_{StRt}$ and LARL were opposite to QTLs for ENO in both bins 1.03 and 3.04. This may reflect the importance of a continuous coordination of the size of the root system and of the shoot ($DW_{StRt}$ and LARL) for the number of ears; under WS an increase in $DW_{StRt}$ and LARL may be at the expense of number of ears, a mechanism likely to minimize grain yield. The maintenance of functional equilibrium between resource-capturing organs, above and below ground, could stabilize yield components under drought, favoring the exploitation of water to guarantee survival and reproduction. The higher LOD scores and significance levels of the QTLs for LARL, as compared with QTLs for $DW_{StRt}$, may indicate that the ratio between the surface areas was a better indicator of the maintenance of this equilibrium than the ratio of the dry weights. However, they could also indicate the presence of stronger and perhaps fewer QTLs for LARL. The QTLs for axile root number ($No_{Cr}$ and $No_{Se}$) were collocated with published QTLs for the ASI under WW and WS (Ribaut et al., 1996) in bins 1.08, 2.08, and 6.05. Interestingly, the significant QTLs

Figure 1. Collocations of quantitative trait loci (QTLs) for root and shoot traits of maize seedlings in the Ac7643 × Ac7729/TZSRW population (see Table 1 for explanation of abbreviations) with QTLs for anthesis-silking interval (ASI) (Ribaut et al., 1996) and for ear number (ENO) and kernel number (KNO) (Ribaut et al., 1997) in the same population. Labels in italics indicate traits increasing alleles derived from Ac7729/TZSRW (P2). Quantitative trait loci for leaf and xylem abscisic acid (ABA) concentrations (L-ABA and X-ABA, respectively) (Sanguineti et al., 1999; Tuberosa et al., 2002a; Lebreton et al., 1995) from other populations. Test conditions were well watered (WW), water stressed (WS), both well watered and water stressed (WW_WS), intermediate stressed (IS), and severely stressed (SS). For field traits and ABA, only matching QTLs were presented.

for axile root number in bins 2.08 and 6.05 were reversed compared with the QTLs for ASI. The allele from the drought-tolerant P1 increased $No_{Cr}$ and $No_{Se}$, while the allele from P2 increased ASI in these two regions. Therefore, an increase in the number of seedling axile roots was related to a short ASI under field conditions, reflecting drought tolerance at flowering. As the rooting strategy cannot be assessed within a large segregating population at anthesis, a better understanding of the genetic control of root traits at the seedling stage can provide valuable information for determining drought tolerance mechanisms at later stages of plant development.

Quantitative trait loci for $No_{Cr}$ (under WW) and $DW_{St}$ (under WS) in bin 9.02 collocated with QTLs for ENO and KNO under WS (Ribaut et al., 1997). The signs of additive effects of the QTLs for $No_{Cr}$ and $DW_{St}$ were opposite to those for yield components. Early $No_{Cr}$ was correlated more closely to $DW_{St}$ than to $No_{Se}$; thus, the former might indicate accelerated seedling development, important at this collocation for a more stable yield under WS.

A seedling QTL for $No_{Cr}$ collocated with published QTLs for leaf ABA in another marker population (Sanguinetti et al., 1999; Tuberosa et al., 2002a) in bin 2.08; this supports the hypothesis of Lebreton et al. (1995) that ABA regulates root pulling resistance. The ABA concentration was not measured here, but it was shown elsewhere that the endogenous ABA concentration and root growth of seedlings are highly correlated under conditions of simulated drought stress (Sharp et al., 1994; McDonald and Davies, 1996).

## CONCLUSIONS

It was shown that relevant root data can be generated at an early stage with easy-to-handle pouches, allowing for the phenotyping of large sets of plants as is typically required for genetic analysis. Some collocations of seedling root and shoot traits in bin 2.02 may be due to their common dependency on readily transportable assimilates at the heterotrophic stage, although the average seed size as reported by the seed producer was not related to these traits. Collocating QTLs for the relationship between the shoot and the root system ($DW_{StRt}$ and LARL) and the ENO under water stress were reversed at these loci, indicating that the strategic preference of seminal roots over shoot development under drought translates to more stable yield components under drought in the field. At a couple of loci the drought-tolerant parental line, P1, contributed to better growth of axile roots at the seedling stage while in the same genomic regions P1 allele contributed to a shorter ASI for drought at flowering. Again, an increase in the root development at seedling stage is related to drought tolerance under field conditions, as reflected by a short ASI. A collocation between a QTL for $No_{Cr}$ with a QTL for ABA concentration (Tuberosa et al., 2002a) may be linked to the general role of leaf ABA as a signaling hormone at the start of drought in sections of the root system; the beginning of sensed drought may differ depending on the size of the root system.

Little is known about the correlation between root structure and drought tolerance under field conditions. This study presents promising perspectives for the application of QTL data on traits of roots at the seedling stage to predict the yield of maize under water-limited conditions at flowering.

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