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ORIGINAL PAPER

Mapping QTLs for root system architecture of maize (*Zea mays* L.) in the field at different developmental stages

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Abstract Root system architecture (RSA) is seldom considered as a selection criterion to improve yield in maize breeding, mainly because of the practical difficulties with their evaluation under field conditions. In the present study, phenotypic profiling of 187 advanced-backcross BC_4F_3 maize lines (Ye478 × Wu312) was conducted at different developmental stages under field conditions at two locations (Dongbeiwang in 2007 and Shangzhuang in 2008) for five quantitative root traits. The aims were to (1) understand the genetic basis of root growth in the field; (2) investigate the contribution of root traits to grain yield (GY); and (3) detect QTLs controlling root traits at the seedling (I), silking (II) and maturation (III) stages. Axial root (AR)-related traits showed higher heritability than lateral root (LR)-related traits, which indicated stronger environmental effects on LR growth. Among the three developmental stages, root establishment at stage I showed the closest relationship with GY ($r = 0.33$–0.43, $P < 0.001$). Thirty QTLs for RSA were detected in the BC_4F_3 population and only 13.3 % of the QTLs were detected at stage III. Most important QTLs for root traits were located on chromosome 6 near the locus umc1257 (bin 6.02–6.04) at stage I, and chromosome 10 near the locus umc2003 (bin 10.04) for number of AR across all three developmental stages. The regions of chromosome 7 near the locus bnlg339 (bin 7.03) and chromosome 1 near the locus bnlg1556 (bin 1.07) harbored QTLs for both GY- and LR-related traits at stages I and II, respectively. These results help to understand the genetic basis of root development under field conditions and their contribution to grain yield.

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Introduction

Abiotic stresses increasingly depress crop yield as a result of global climate change and scarcity of water and nutrients (de Dorlodot et al. 2007). Given the important functions of roots for water and nutrient acquisition, the root system architecture (RSA) is fundamental for crop growth and productivity (Bohn et al. 2006; Kenrick 2002; Lynch 2007). For example, a deep root system is essential for crops to utilize nitrate and water in deeper soil layers, especially under abiotic stress conditions (Jordan et al. 1983; Wiesler and Horst 1994). However, because of the higher phosphorus availability in surface soil strata, a shallow root system with enhanced adventitious rooting is important for crops to absorb phosphorus (Lynch 2011). A cropping system simulation study in maize further suggested that the improvement of root architecture might be

sufficient to explain the historical maize yield trend in the USA Corn Belt (Hammer et al. 2009). Thus, maize breeders are turning their attention to improving root traits to increase yields with sustainable resource usage in the target environment. However, direct selection for optimal RSA in the field is not routine in maize breeding programs.

The design of ideotype root architecture to attain optimal maize productivity relies on a thorough understanding of the genetic basis of RSA. Several previous studies showed that significant genetic variation in RSA exists among maize genotypes, which may contribute to enhancement of nutrient efficiency, drought tolerance and lodging resistance (Chun et al. 2005; Hébert et al. 1992; Jenison et al. 1981; Landi et al. 1998; Tuberosa et al. 2003). Root traits are genetically controlled by a number of small-effect loci and strongly interact with the environment (de Dorlodot et al. 2007). Many QTLs that regulate RSA have been identified in several maize linkage populations, particularly in response to different environmental factors (e.g., nitrogen and phosphorus deficiency and drought stress) (Guingo et al. 1998; Hund et al. 2004; Hochholdinger and Tuberosa 2009; Kaeppler et al. 2000; Landi et al. 2002; Lebreton et al. 1995; Liu et al. 2008; Messmer et al. 2009; Ruta et al. 2010a, b; Trachsel et al. 2009; Tuberosa et al. 2002; Zhu et al. 2005, 2006). Moreover, Hund et al. (2011) performed a meta-analysis of maize root-QTLs using data from 15 QTL studies of nine mapping populations, and several putative consensus root-QTL clusters were located in bins 1.07, 2.04, 2.08, 3.06, 6.05, and 7.04.

Although maize root-QTL analysis has attracted much attention, several constraints that limit the progress of QTLs discovery remain, including the method of RSA phenotyping and the strategy of QTL mapping. Given that evaluation of maize RSA directly in the soil is difficult, RSA characterization is usually performed with seedlings grown in paper rolls, in hydroponic systems or on gel-based plates (Liu et al. 2008; Hund et al. 2009a, b; Kaeppler et al. 2000; Tuberosa et al. 2002). Root traits expressed in young seedlings, such as root growth angle, and seminal and lateral root length and number, can be evaluated easily, but more complex traits expressed at later stages of development, such as shoot-borne roots formation, are rarely evaluated (Lynch 2011; Trachsel et al. 2011). Furthermore, RSA phenotypes of seedlings grown in controlled environments may not accurately reflect root growth under field conditions (Zhu et al. 2011). To overcome these limitations, maize root-QTL analyses are required on the basis of RSA characterization in plants grown in the field at different developmental stages.

Promising mapping strategies for QTL analysis are also demanded to improve the precision of detecting small-effect loci that regulate RSA because of its genetic complexity. Compared with F_2 populations, recombinant inbred lines (RILs), or double haploid (DH) populations, QTL mapping based on an advanced-backcross population (AB) is carried out under a similar background; thus most interference from the genetic background and interactions between QTLs are minimized. In addition, QTL analysis of an AB population is an effective method for fine mapping of QTLs (Chen et al. 2008b; Salvi et al. 2011; Shimizu et al. 2008; Tian et al. 2006a, b) and subsequently for cloning of underlying candidate genes (Frary et al. 2000; Salvi et al. 2007).

In the present study, we used a BC_4F_3 population to investigate maize root growth and development under field conditions. In particular, our objectives were to (1) investigate the genetic basis of maize RSA at different stages of development, (2) study the association between RSA and grain yield, and (3) map QTLs underlying RSA at different developmental stages.

Materials and methods

Plant materials

An advanced-backcross (BC_4F_3) population of maize was used as described by Liu et al. (2011). The donor parental line Ye478 was developed in China during the 1990s and was the female parent of more than 50 high-yielding hybrids. Ye478 has a larger root system than the recurrent parental line Wu312, represented as higher root biomass, higher number of axial roots, and longer root length (Liu et al. 2009; Yan et al. 2011). Both parental lines are stay-green inbreds and have similar silking and maturity stages. The F_1 plant derived from the cross between the parental lines was backcrossed with the recurrent parent to obtain ten BC_1 plants (Supplementary Fig. 1). Each BC_1 plant was backcrossed three times with the recurrent line, and a BC_4F_1 population containing 231 lines was generated. With two generations of selfing, a total of 187 BC_4F_3 lines were obtained and subsequently used for the genotypic and phenotypic analyses.

Field trials

Field trials were conducted at the China Agricultural University (CAU) experimental station in Dongbeiwang, Beijing, China (40°00′N, 116°18′E, 60 m a.s.l.) in 2007 and at the CAU experimental station in Shangzhuang, Beijing, China (40°06′N, 116°11′E, 46 m a.s.l.) in 2008. The experiments were carried out on a loamy sand soil (mixed, mesic calcareous Cambisol). The nutritional composition of the soils before the field trails is summarized in Table 1. The fields were supplied with the 750 kg/ha calcium

Table 1 Soil environment and supplementary fertilizer at the two field trial locations

Location	Soil								Fertilizer		
	Soil bulk density (g/cm^3)		Organic matter (g/kg)	Total nitrogen (mg/kg)	Mineral nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	pH	N (kg/ha)	P_2O_5 (kg/ha)	K_2O (kg/ha)
	0–20 cm	20–40 cm									
Dongbeiwang in 2007	1.34	1.51	23.0	1.0	29.3	17.4	157.5	8.0	180	120	80
Shangzhuang in 2008	1.38	1.54	15.8	0.83	36.5	26.7	103.8	7.9	180	120	80

Available phosphorus was measured as Olsen-P, and available potassium was measured as exchangeable-K

superphosphate and 135 kg/ha potassium chloride before sowing, and an additional 391 kg/ha urea was applied at the V6 growth stage. The total fertilizer amount in the pure element form of NPK is shown in Table 1.

A total of 187 BC_4F_3 families together with both parents, Ye478 and Wu312, were used in the field trials at both locations. At each location, the population was evaluated in a completely randomized block design of one-row plots with three replications. Each row was 4 m long, 50 cm wide and contained 13 plants, and the distance between plants within a row was 33 cm. Each plot was planted at a density of 60,000 plants/ha (6 plants m^{-2}). Standard cultivation management practices were used. Irrigation was applied before planting, and rainfall was sufficient during the growing season (416.5 mm in 2007 and 573.9 mm in 2008). The water content of the soil was maintained at about 75–80 % of field capacity.

Root system evaluation

The root system architecture of plants was evaluated at 50, 80, and 120 days after sowing (Supplementary Fig. 2). Two to three representative plants of similar appearance per row were selected for root excavation at each developmental stage. The harvest was performed from one side to the other side of each plot, and only fully bordered plants were selected for root excavation (Supplementary Fig. 2A). Roots of each plant were excavated with shovels by removing a cube of soil 33 cm in length, 50 cm in width, and 40 cm in depth (Supplementary Fig. 2B). The soil block was put into a plastic bag and transported to near-by place for subsequently washing the roots. Soil fractions adhering to the excavated roots were removed by light shaking and the holes were refilled by the soils. The excavated roots was soaked in water for 30–40 min with washing powder and subsequently rinsed with water to remove the remaining soil particles. A sieve (2 mm) was used to prevent the loss of fine roots severed from axial roots during the washing process. Each axial root was separated in accordance with the layers of emergence. The number of axial roots (ARN) per plant was counted and the length of axial roots (ARL) was measured. Samples of the roots were then stored at −20 °C. The WinRHIZO Pro 2004b software (Regent Instruments, Canada) was used to scan roots, and the total root surface area (TRSA) and total root length (TRL) were determined. The root dry weight (RDW) and shoot dry weight (SDW) were evaluated for the whole root system of each sampled plant after oven-drying at 70 °C until constant weight. Grain yield (GY) was evaluated separately (Supplementary Fig. 2A) and the corresponding data were obtained from Liu et al. (2011).

DNA extraction and simple sequence repeat marker analysis

Fresh leaves were collected from the 187 BC_4F_3 lines (mixed with leaves of 8–12 plants) and ground in liquid nitrogen. DNA was extracted using the cetyltrimethyl ammonium bromide (CTAB) method (Rogers and Bendich 1988). Fingerprinting was conducted using 143 polymorphic simple sequence repeat (SSR) markers as described by Senior and Heun (1993). Polymerase chain reactions were performed in a total volume of 15 μl and contained 2 ng/μl template DNA, 10 mM Tris–HCl (pH 8.0), 1.5 mmol $MgCl_2$, 0.2 μM of each primer, 2.5 mM each of dNTP, and 1 U Taq DNA polymerase. The amplification was performed with the following procedure: initial denaturing step at 94 °C for 4 min, followed by 35 cycles at 94 °C for 40 s, 56–62 °C for 40 s (depending on the T_m of each primer), and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were separated on 6 % polyacrylamide denaturing gels and stained using the silver-staining protocol described by Panaud et al. (1996). Map positions of the SSR markers were determined based on the genetic map of a RIL population derived from the cross between Ye478 and Wu312 as described by Liu et al. (2011). Genome contributions of Ye478 in a single BC_4F_3 line were determined and graphical genotypes were illustrated using the Plabsoft software package (Maurer et al. 2008).

Data analysis

Analysis of variance (ANOVA) was performed for all investigated traits under each environment (Cochran and

Cox 1957). Adjusted means and effective error mean squares from the calculations were used in the combined analyses across the two locations. Variance components were estimated as described by Snedecor and Cochran (1980) and their standard errors as described by Searle (1971). Broad-sense heritability (h^2) on an entry mean basis was estimated for each trait as described by Hallauer and Miranda (1981). Genotypic correlations were computed in accordance with the procedures developed by Mode and Robinson (1959). We assumed fixed genotypic effects to determine the best linear unbiased estimate for QTL analysis. All statistical computations were performed with the PLABSTAT software (Utz 1993). The associations among traits were further visualized with a principal coordinate analysis (PCA) (Gower 1966) based on Pearson correlation coefficients. The PCA was performed using the R package (R Development Core Team 2010).

Composite interval mapping for QTL analysis was performed by stepwise multiple regression in combination with the use of selected markers as cofactors. Cofactors were selected by stepwise regression with an "F-to-enter" and an "F-to-delete" value of 3.5. Testing for the presence of a putative QTL in an interval by a likelihood ratio test was performed using a LOD threshold of 2.5. The detected QTLs and their estimated positions were used for a simultaneous multiple regression to obtain final estimates of the additive effects. The proportion of the phenotypic variance explained by a QTL was determined by the estimator R^2_{adj} as described by Utz et al. (2000). The proportion of the genotypic variance explained by all detected QTLs was estimated from the ratio $p_G = R^2_{adj}/h^2$. All computations were performed with the PLABQTL software package (Utz and Melchinger 1996).

Results

Evaluation of genotypes

On the basis of the 143 SSR markers, the 187 BC_4F_3 lines with the background of the recurrent parent Wu312 covered a total of 99.3 % of the genome of the donor parent Ye478 (Supplementary Fig. 3). Single BC_4F_3 lines contained an average of 6.8 introgression segments (range 1–29). The average size of introgression segments was 25.6 cM (minimum 5.4 cM, maximum 135.0 cM). The average portion of the Ye478 genome present in each single BC_4F_3 line was 6.6 % (range 0.2–27.7 %).

Evaluation of RSA

In the field trials at the two locations, the RSA traits and shoot growth of plants were evaluated at 50 days (stage I), 80 days (stage II), and 120 days (stage III) after planting, which represented the seedling (V6), silking (R1) and maturation (R6) developmental stages. Compared with the recurrent parent Wu312, the donor parent Ye478 had a larger root system and higher shoot biomass at all developmental stages (Table 2). Within the BC_4F_3 population, considerable phenotypic variation existed for all investigated traits, and the median value for each trait was significantly higher than that of the recurrent parent Wu312. The axial root (AR)-related traits were mainly determined by the number and length of axial roots (ARN and ARL), and the lateral root (LR)-related traits were mainly determined by total root length (TRL) and total root surface area (TRSA) because ARL only accounted for a small proportion of TRL (10–30 %). Whereas shoot growth, as indicated by SDW, increased continuously from stage I to III, all root traits peaked at stage II and then declined at stage III (Table 2). This trend was more pronounced for LR-related traits (TRL and TRSA), probably because of significant senescence of lateral roots during the post-silking period.

ANOVA showed that genotypic variances were significantly ($P < 0.01$) for all root traits at the three growth stages (Supplementary Table 1). Each root trait within the population was significantly correlated across the two locations ($r = 0.25$–0.66, $P < 0.05$). However, the effects of location and genotype × location were also significant for most root traits, which suggested the presence of strong environmental effects on root growth across the two environments. The LR-related traits (TRL and TRSA) had higher CV values compared with those of AR-related traits (ARL and ARN) at all stages, which indicated the greater genetic variation of LR within the population. Moreover, heritability (h^2) of LR-related traits (TRL and TRSA) was rather low (<30 %) at stage I and II, and further decreased significantly at stage III. By contrast, heritability of AR-related traits (ARL and ARN) was at moderate levels with a maximum of 55 % for ARN at stage II (Table 2).

Relationship between RSA, SDW and GY

Within the BC_4F_3 population, the percentage of Ye478 alleles present in each line was significantly correlated with GY ($r = 0.25$), SDW ($r = 0.19$–0.34), and RSA ($r = 0.14$–0.37) (Table 3). The phenotypic correlation between root traits (RDW, TRSA, TRL, ARL, and ARN) and GY was significantly different from zero ($P < 0.01$) at stage I ($r = 0.33$–0.43). At stage II, TRSA, TRL, ARL, and ARN were also significantly correlated with GY, but with lower coefficients ($r = 0.15$–0.23, $P < 0.05$). By contrast, no significant correlation was observed between RDW and GY. Furthermore, the correlations were not

Table 2 First- and second-degree statistics for the parents and BC_4F_3 population for grain yield (GY), shoot dry weight (SDW), root dry weight (RDW), total root surface area (TRSA), total root length (TRL), axial root length (ARL), and axial root number (ARN) of plants measured at 50 days (I), 80 days (II), and 120 days (III) after planting

Trait	GY (g/m^2)	SDW (g)			RDW (g)			TRSA (cm^2)		
		I	II	III	I	II	III	I	II	III
Parents										
Ye478	475.2	16.4	125.3	242.4	2.6	25.4	21.8	444.5	3,207.0	2,003.7
Wu312	358.8	12.3	103.6	174.0	1.2	17.9	13.9	222.2	1,949.6	1,048.5
BC_4F_3 population										
Median	328.1	14.1	111.6	221.5	1.3	20.1	16.0	266.3	2,333.3	1,526.7
Minimum	129.6	7.4	73.1	131.0	0.5	13.1	9.2	117.0	1,479.4	792.9
Maximum	513.5	28.7	180.2	311.9	2.7	33.4	24.0	512.3	5,740.1	2,364.6
$LSD_{0.05}$	79.9	4.0	13.8	46.3	0.6	4.9	4.0	115.2	620.3	364.7
h^2 (%)	54.2	19.9	41.4	43.2	21.5	37.0	0.0	19.3	21.8	0.0
CV (%)	20.5	24.8	13.0	13.5	32.8	17.6	19.6	27.5	19.7	19.2

Trait	TRL (cm)			ARL (cm)			ARN		
	I	II	III	I	II	III	I	II	III
Parents									
Ye478	1,740.0	13,172.0	6,458.7	426.6	1,534.1	1,357.1	32.5	97.4	83.8
Wu312	910.3	7,667.7	3,615.6	253.7	1,037.5	903.6	21.8	67.4	61.1
BC_4F_3 population									
Median	1,074.5	9,394.7	4,374.8	305.8	1,183.0	988.5	25.2	71.9	65.0
Minimum	522.2	5,183.2	2,064.4	190.5	853.0	670.6	17.8	51.1	49.1
Maximum	1,928.1	21,084.4	7,667.9	437.5	1,904.5	1,476.2	34.9	105.3	90.8
$LSD_{0.05}$	403.6	2,443.6	1,189.3	74.9	200.0	175.6	4.6	8.9	9.8
h^2 (%)	30.0	22.1	7.9	0.0	41.9	29.3	26.0	54.7	49.2
CV (%)	25.2	23.4	23.2	15.0	13.5	14.3	13.7	10.7	10.3

The data for grain yield (GY) were obtained from Liu et al. (2011)

significantly different from zero at stage III except for ARN ($r = 0.16$, $P < 0.05$). We performed a PCA to visualize the correlation between GY and root traits at different developmental stages (Fig. 1). The first three principal coordinates explained in total 66.8 % of the total variance. All root traits at stage I were closely associated with GY, which indicated that GY was more closely related with RSA traits in the early developmental stages of maize plants.

In contrast to root traits, the phenotypic correlations between SDW and GY were significantly different from zero ($P < 0.01$) at all developmental stages, and the associations increased from stage I ($r = 0.33$) to III ($r = 0.63$) (Table 3). This indicated that shoot biomass contributed substantially to grain yield with the developmental stages. Although correlations significantly different from zero were observed between SDW and RSA traits at all stages, the coefficients decreased from stage I ($r = 0.61$–0.75) to III ($r = 0.24$–0.31) (Table 3).

We also observed that the five root traits (RDW, TRSA, TRL, ARL, and ARN) were significantly correlated at all developmental stages (Table 3). The mean correlation coefficients among root traits in stage I was highest ($r_{mean} = 0.71$), compared with that at stage II ($r_{mean} = 0.60$) and III ($r_{mean} = 0.59$). Of all the root traits, the highest correlations were found between the LR-related traits TRL and TRSA ($r = 0.81$–0.89), and between the AR-related traits ARL and ARN ($r = 0.77$–0.83). By contrast, the correlations between LR- and AR-related traits were relatively low ($r = 0.41$–0.71).

QTL mapping

Thirty-six putative QTLs were detected in the BC_4F_3 population on all chromosomes except for chromosome 3, including six QTLs for SDW and 30 QTLs for RSA traits at the three developmental stages (Table 4; Fig. 2). From the 36 identified QTLs, 80.6 % carried a favorable allele

Table 3 Pearson's correlation coefficients[a] (*r*) between grain yield (GY), shoot dry weight (SDW), root dry weight (RDW), total root surface area (TRSA), total root length (TRL), axial root length (ARL), and axial root number (ARN) of plants measured at 50 days (I), 80 days (II), and 120 days (III) after planting

Stage	Trait	RDW	TRSA	TRL	ARL	ARN	GY[b]	*P* (478)[c]
I	SDW	0.746***	0.732***	0.618***	0.641***	0.646***	0.329***	0.282***
	RDW		0.800***	0.638***	0.670***	0.648***	0.372***	0.201**
	TRSA			0.871***	0.707***	0.682***	0.412***	0.283***
	TRL				0.639***	0.590***	0.433***	0.349***
	ARL					0.825***	0.391***	0.282***
	ARN						0.331***	0.310***
II	SDW	0.556***	0.528***	0.483***	0.349***	0.381***	0.445***	0.339***
	RDW		0.684***	0.597***	0.536***	0.580***	0.075 ns	0.367***
	TRSA			0.887***	0.512***	0.504***	0.226**	0.311***
	TRL				0.425***	0.412***	0.225**	0.277***
	ARL					0.817***	0.150*	0.334***
	ARN						0.158*	0.303***
III	SDW	0.302***	0.312***	0.256***	0.240***	0.291***	0.633***	0.185**
	RDW		0.741***	0.566***	0.463***	0.486***	0.107 ns	0.200**
	TRSA			0.814***	0.598***	0.540***	0.097 ns	0.220**
	TRL				0.506***	0.429***	0.058 ns	0.137*
	ARL					0.768***	0.114 ns	0.314***
	ARN						0.162*	0.255***
	GY							0.252***

[a] The trait performances were calculated using the least-squares means of the BC_4F_3 lines averaged across all environments. The significance thresholds for *r* values: *ns* not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[b] The data for grain yield (GY) were obtained from Liu et al. (2011)

[c] The trait *P* (478) is defined as the percentage of 478 alleles present in a BC_4F_3 line

that originated from the donor parent Ye478. Few QTLs for SDW were detected at each growth stage and explained 11.9–26.1 % of the phenotypic variation, but none of the common QTLs were detected across all three growth stages (Table 4).

The number of identified QTLs for RSA traits decreased at the late stage of root development, because 14 QTLs for RSA traits were detected at stage I, 12 QTLs at stage II, and four QTLs at stage III (Table 4). Total phenotypic variation explained by QTLs for each RSA trait ranged from 6.6 % (ANR at stage III) to 61.5 % (TRL at stage I), and the contribution of each single QTL to the phenotypic variation varied from 5.4 to 13.5 %. For LR-related traits (TRSA and TRL), many QTLs were detected at stage I, whereas none of these QTLs was detected at stage III (Table 4). However, despite the lower number, QTLs for AR traits (ARN and ARL) were detected across all three developmental stages.

The QTLs for RSA traits were distributed throughout the maize genome with putative clusters on chromosome 1 (bin 1.07), chromosome 6 (bin 6.02–6.04), chromosome 7 (bin 7.03–7.04), and chromosome 10 (bin 10.04–10.06) (Fig. 2). At stage I, a QTL for RDW (*qRDW16-1*) was closely linked with *qTRSA16-1*, *qTRL16-1* and *qARN16-1* on chromosome 6 near the locus umc1257, and another QTL for RDW (*qRDW110-1*) was linked with *qTRSA110-1*, and *qTRL110-1* in the middle of chromosome 10 near the locus umc2003. In the latter chromosomal region, three QTLs for ARN covering all developmental stages (*qARN110-1*, *qARN210-1* and *qARN310-1*) were also present together with marker umc2003. Two QTLs for ARL at stage II (*qARL210-1*) and stage III (*qARL310-1*) were closely linked with marker umc2067 on chromosome 10. Furthermore, there were chromosomal regions where QTLs for RSA traits were colocalized with those for GY. For example, on chromosome 1 near the locus bnlg1556 and chromosome 7 near the locus bnlg339, the QTLs for LR-related traits (TRSA and TRL) at stage I and II were closely linked with two QTLs for GY (Fig. 2).

Discussion

Genotypic evaluation of advanced-backcross BC_4F_3 population

The introgressed segments in the BC_4F_3 lines almost covered the whole genome of the donor parent (99.3 % as

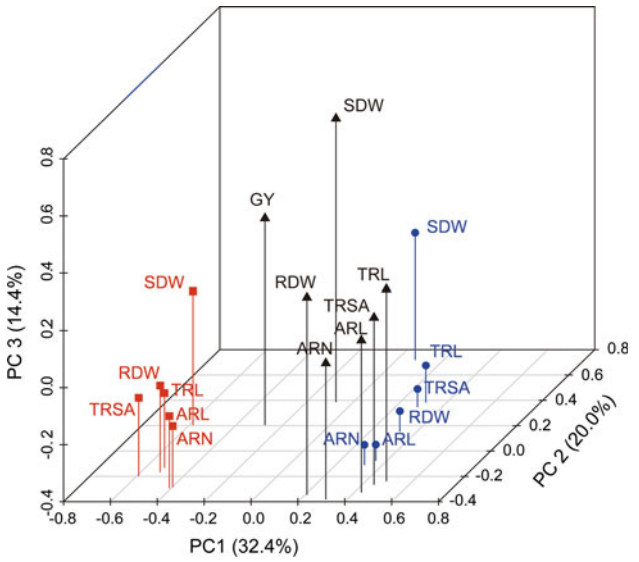


Fig. 1 Principal component analysis (PCA) of grain yield (GY), root dry weight (RDW), total root surface area (TRSA), total root length (TRL), axial root length (ARL), axial root number (ARN), and shoot dry weight (SDW) of plants measured at 50 days (I, in *red*), 80 days (II, in *blue*), and 120 days (III, in *black*) after planting. Nineteen traits were projected onto the first three principal components. The data of different traits were standardized with the formula: $(P_i - P_{mean})/SD$ (standard deviation) (color figure online)

shown in Supplementary Fig. 3). This proportion is slightly higher than that reported previously in maize using 89 near-isogenic lines (NILs) derived from a $BC_3F_{2:3}$ population with a marker-assisted selection (MAS) approach by Szalma et al. (2007). The average size of introgressed segments in the BC_4F_3 population (25.6 cM) was less than that in the $BC_3F_{2:3}$ population (60 cM) because of the additional backcross performed. Consequently, the established BC_4F_3 population represents an excellent resource for genetic studies in maize.

The expected segregation ratio of the three possible marker genotypes is equal to 123:2:3 (AA:AB:BB) in the BC_4F_3 population. Therefore, the expected average portion of the Ye478 genome is 3.1 % (Supplementary Fig. 1). Using the 143 informative SSRs we revealed that the proportion of the donor genome averaged 6.6 % and showed a skewness from homozygote or heterozygote alleles of the donor parent Ye478 (data not shown). In addition, the percentage of Ye478 alleles in the population showed a significant positive correlation with grain yield (GY) and RSA traits (Table 3). Consequently, the higher proportion of the Ye478 genome can be explained by slight selection pressure during the establishment of the population.

Phenotypic evaluation of RSA under field conditions

Although it is difficult to evaluate RSA directly in the field, in the present study the root systems were excavated and subsequently scanned for measurement of root traits. This method enabled direct estimation of root growth in the intact soil environment, but it was labor-intensive and precluded high-throughput analysis. By contrast, Trachsel et al. (2011) developed an advanced method called "shovelomics", which visually scored ten root architectural traits (the number, angles and branching density of brace and crown roots) of an adult maize plant in the field in a few minutes. Thus, such method can be used for high-throughput phenotyping of RSA in maize, especially for quantitative genetic studies on root traits. Nevertheless, our study allowed estimation of root length traits (ARL and TRL), which are also an important component of RSA in the field conditions.

Root complexity and root development depend on genetic and environmental factors and their interactions (Lynch 1995, 2007). In the present study the lower heritability of RSA traits ($h^2 < 0.5$, Table 1) was detected in maize plants grown under field conditions in comparison with those of plants grown in nutrient solutions ($h^2 = 0.6$–0.8; Trachsel et al. 2009; Ruta et al. 2010a). This difference indicated that environmental factors in the field had a major influence on maize root growth, whereas these factors were generally well controlled in the artificial environment. The heritability of LR-related traits (TRL and TRSA), in particular at a late stage of root development (stage III), was rather low (Table 2), which suggested that these traits are strongly influenced by environment, genotype × environment interactions, and the residual error. The evaluation of lateral roots at stage III would be problematic because the root systems become variable with age in response to microenvironments. It is even more relevant that, because carbon allocation from the shoots to the root system was limited at the late stage of root development, the senescence processes in roots would increase which strongly affect root plasticity. In addition, at the two later growth stages, the competition of roots of neighborhood genotypes grown in each row plot might also produce more environmental effects. By contrast, ARN showed the highest heritability ($h^2 = 0.55$), which was similar to that of brace root number evaluated in the field ($h^2 = 0.67$–0.8; Ku et al. 2011). The presence of consistent QTLs for ARN across all three growth stages also indicated that the genetic control of ARN was development-independent (Table 4). Although QTLs for both AR- and LR-related traits were colocalized on the chromosome bins 6.02–6.04 and 10.4, other QTLs were distributed differently across the chromosomal regions (Fig. 2). Thus, the development of distinct classes of roots is probably under differential genetic control and also responds differentially to environmental factors (Lynch and van Beem 1993; Zobel 1996; Zhu et al. 2006).

Table 4 List of 36 putative QTLs detected from the BC_4F_3 population for shoot dry weight (SDW), root dry weight (RDW), total root surface area (TRSA), total root length (TRL), axial root length (ARL), and axial root number (ARN) of plants measured at 50 days (I), 80 days (II), and 120 days (III) after planting

Trait	Stage	QTL	Chromosome bin[a]	Marker interval	LOD (%)	R^{2b}	Add.[c]
SDW11	I	*qSDW16-1*	6.02	umc1006–umc1257	3.2	8.1	2.6
		qSDW18-1	8.02	phi119–umc1034	4.9	9.3	−3.1
		qSDW110-1	10.03	umc1336–umc2163	4.4	8.7	−3.6
Total						26.1	
SDW12	II	*qSDW29-1*	9.03	bnlg430–umc1191	3.2	11.9	7.4
Total						11.9	
SDW13	III	*qSDW35-1*	5.05	bnlg278–umc1019	3.2	7.1	−12.5
		qSDW35-1	5.07	bnlg1306–umc1072	2.7	5.9	−21.5
Total						13.0	
RDW11	I	*qRDW16-1*	6.02–6.04	umc1257–umc1857	6.1	12.0	0.5
		qRDW110-1	10.04–10.06	umc2003–umc2122	6.9	13.5	0.5
Total						25.5	
RDW12		*qRDW26-1*	6.02–6.04	umc1257–umc1857	2.6	5.4	2.0
		qRDW29-1	9.03	bnlg430–umc1191	2.9	5.9	2.6
		qRDW210-1	10.06–10.07	umc2122–umc1344	3.9	7.9	3.0
Total						19.2	
TRSA11	I	*qTRSA14-1*	4.07–4.08	umc1620–phi092	6.7	13.1	−121.4
		qTRSA16-1	6.02–6.04	umc1257–umc1857	5.2	10.4	103.4
		qTRSA17-1	7.03–7.04	bnlg339–umc1888	3.4	7.0	69.5
		qTRSA110-1	10.04–10.06	umc2003–umc2122	5.1	10.3	105.8
Total						40.8	
TRSA12	II	*qTRSA21-1*	1.07	bnlg1556–bnlg1025	5.1	10.1	372.2
Total						10.1	
TRL11	I	*qTRL11-1*	1.10–1.11	umc2189–umc1553	4.0	8.1	−275.6
		qTRL14-1	4.06–4.07	bnlg2291–umc1620	6.9	13.5	−513.2
		qTRL16-1	6.02–6.04	umc1257–umc1857	6.9	13.5	542.3
		qTRL17-1	7.02–7.03	mmc0411–bnlg339	4.2	8.5	276.7
		qTRL19-1	9.02–9.03	umc1033–umc1191	4.9	10.6	486.8
		qTRL110-1	10.04	umc1697–umc2003	3.6	7.3	208.7
Total						61.5	
TRL12	II	*qTRL21-1*	1.07	bnlg1556–bnlg1025	3.5	7.0	1,480.7
Total						7.0	
ARL12	II	*qARL21-1*	1.03	umc1403–bnlg1484	3.8	9.0	81.8
		qARL22-1	2.07–2.08	bnlg2077–umc1049	3.9	9.3	104.6
		qARL26-1	6.05	bnlg1174–nc012	3.3	7.9	84.9
		qARL28-1	8.07–8.08	umc1268–umc1933	4.5	9.0	103.1
		qARL210-1	10.01–10.03	bnlg1451–umc2067	5.4	10.8	120.2
Total						46.0	
ARL13	III	*qARL35-1*	5.06	umc1019–umc2306	3.4	6.9	94.8
		qARL36-1	6.00–6.01	phi126–umc2313	3.9	7.9	118.1
		qARL310-1	10.03	umc2067–umc2016	4.4	8.9	120.6
Total						23.7	
ARN11	I	*qARN16-1*	6.02–6.04	umc1257–umc1857	5.5	11.0	3.8
		qARN110-1	10.04–10.06	umc2003–umc2122	6.5	12.8	4.6
Total						23.8	
ARN12	II	*qARN26-1*	6.05	bnlg1174–nc012	5.2	12.1	6.7
		qARN210-1	10.04	umc1053–umc2003	2.9	6.0	3.2

Table 4 continued

Trait	Stage	QTL	Chromosome bin[a]	Marker interval	LOD (%)	R^{2b}	Add.[c]
Total						18.1	
ARN13	III	*qARN310-1*	10.04–10.06	umc2003–umc2122	3.3	6.6	5.1
Total						6.6	

[a] Chromosome bins of the marker and position taken from IBM 2008

[b] The squared partial correlation coefficient, which is the coefficient of determination between the respective QTL and the phenotypic observation, with all other QTL effects fixed

[c] Estimated additive QTL effects at the location of scanning

Association of RSA and GY

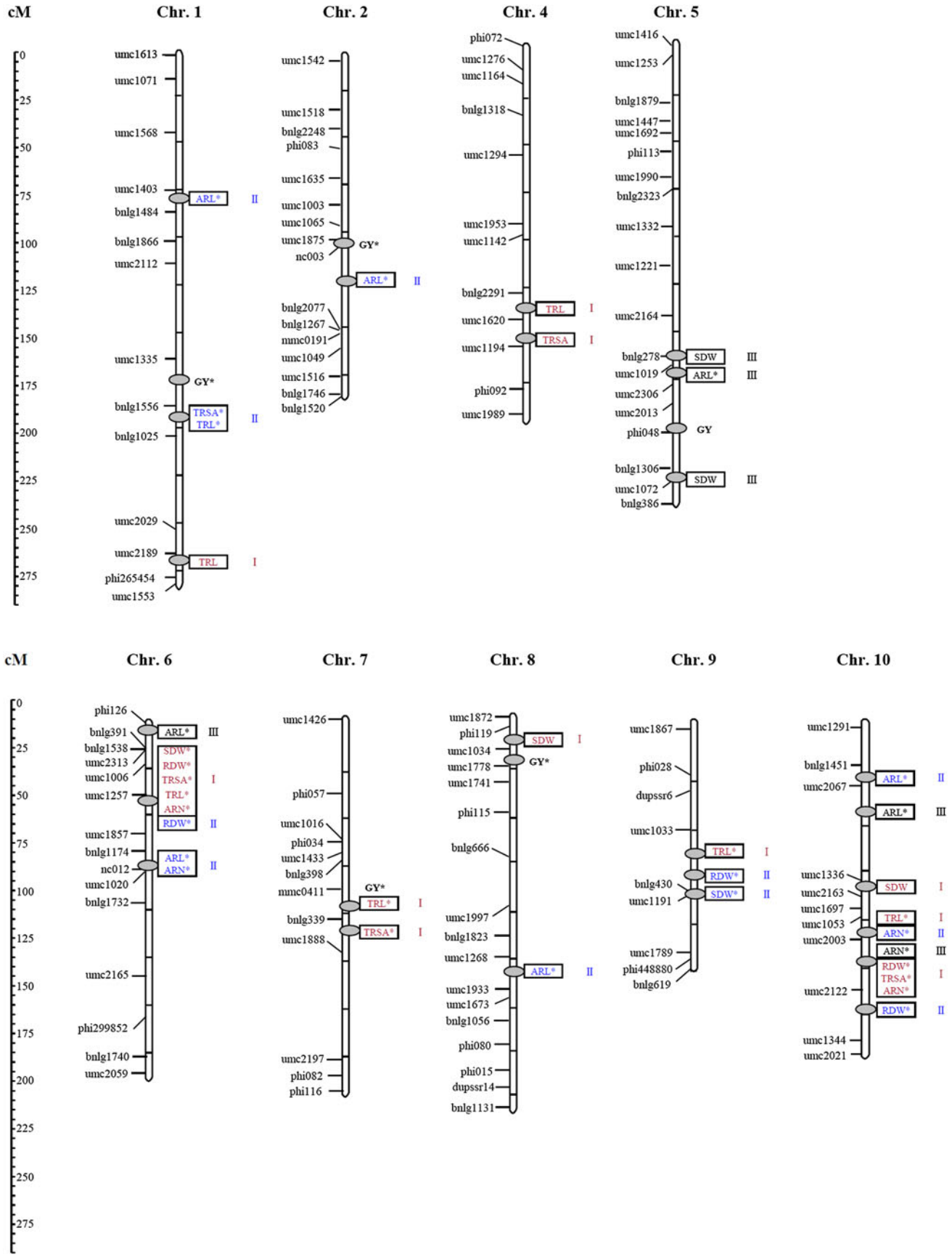
The importance of the root system on grain yield in maize was recognized many years ago (Wilson 1930). Tuberosa et al. (2002) reported significant, albeit low, positive correlations ($r = 0.2$–0.3, $P < 0.05$) between root traits and GY under water stress conditions. Similarly, in the present study a significant association was observed between GY and RSA evaluated under field conditions (Table 3). Importantly, we further revealed that the establishment of roots at an early developmental stage, rather than that at the late stage of root development has a close relationship with grain yield (Table 3; Fig. 1). Although vertical root pulling resistance (VRPR) is related to root system characteristics and yield in maize (Landi et al. 2002), Liu et al. (2011) observed no significant correlation between GY and VRPR at the mid-silking stage in the same BC_4F_3 population used in the present study. This finding further supported the conclusion that RSA traits at the late stage of root development are unlikely to be related to GY in maize. In addition, the QTLs for GY reported by Liu et al. (2011) were colocalized with those for root traits, particularly at stages I and II (Fig. 2). The QTLs *qTRL21-1*, *qTRSA21-1,* and *qGY1-1* were associated with marker bnlg1556 on chromosome region 1.06–1.07 where a major QTL (*root-yield-1.06*) was also identified that regulates both root traits and GY (Tuberosa et al. 2003; Landi et al. 2010). The QTLs *qTRL17-1*, *qTRSA17-1,* and *qGY7-1* were associated with marker bnlg339 on chromosome region 7.03 in which the QTLs for NoAx (elongation rate of axile roots), ERAx (the number of axile roots), GY, kernel number, and hundred kernel fresh weight were detected in other maize populations (Messmer et al. 2009; Trachsel et al. 2009). Although the flowering time plays fundamental role in determining crop yield, it did not identify any overlapped QTLs for root traits in this study (data not shown). However, it is worth noting that these QTLs associated with RSA and GY could be also involved in the control of plant's vigor and, hence, further contributing to both root and above-ground traits (mainly GY) (Landi et al. 2010).

Collectively, these results indicate that GY is likely to be genetically associated with root traits, and selection of optimal root traits at an early developmental stage is essential to optimize maize productivity. These findings provide valuable information to the maize-breeding community for future selection of high-yielding cultivars. Additionally, fine-tuning the phenotyping platform for maize RSA at an early growth stage should be thereby focused on in the further studies.

Identification of QTLs controlling RSA

Identification of important QTLs for RSA traits is essential for the improvement of root traits via a MAS approach and for cloning key genes that regulate RSA development in maize. In the present study several important root-QTL clusters were localized on chromosome region bins 1.06–1.07, 6.02–6.04, 7.02–7.04, and 10.04–10.06 (Fig. 2). Using the same BC_4F_3 population, the QTLs for the root traits that were evaluated around flowering stages were also clustered in the chromosome region bins 6.02 and 10.04 (Cai et al. 2011). The importance of these regions is further substantiated by meta-QTL analysis in maize, which suggests the presence of key genes that regulate RSA development occur in a wide range of genetic backgrounds (Hund et al. 2011). Thus, the chromosome regions that contain important QTLs for root traits could be targets for root improvement by MAS, which would be more productive than direct phenotypic evaluation of roots in the field.

Many previous studies also revealed that the putative QTLs of root traits on chromosome region bins 1.06/1.07, 6.02, and 10.04 were associated with adaptation to abiotic stress, such as low nitrogen stress (Liu et al. 2008, 2011), low phosphorus stress (Zhu et al. 2006; Chen et al. 2008a), and water stress (Ribaut et al. 1996; Tuberosa et al. 2002; Landi et al. 2010). Although it is still not possible to distinguish between pleiotropy and close linkage of RSA and abiotic stress tolerance, this could point to a possible genetic association between root growth and adaptation of plants to abiotic stress.


cM
Chr. 1
Chr. 2
Chr. 4
Chr. 5
0
25
50
75
100
125
150
175
200
225
250
275
umc1613
umc1071
umc1568
umc1403
bnlg1484
bnlg1866
umc2112
umc1335
bnlg1556
bnlg1025
umc2029
umc2189
phi265454
umc1553
ARL*
GY*
TRSA*
TRL*
TRL
II
I
umc1542
umc1518
bnlg2248
phi083
umc1635
umc1003
umc1065
umc1875
nc003
bnlg2077
bnlg1267
mmc0191
umc1049
umc1516
bnlg1746
bnlg1520
GY*
ARL*
II
phi072
umc1276
umc1164
bnlg1318
umc1294
umc1953
umc1142
bnlg2291
umc1620
umc1194
phi092
umc1989
TRL
TRSA
I
umc1416
umc1253
bnlg1879
umc1447
umc1692
phi113
umc1990
bnlg2323
umc1332
umc1221
umc2164
bnlg278
umc1019
umc2306
umc2013
phi048
bnlg1306
umc1072
bnlg386
SDW
ARL*
GY
III
cM
Chr. 6
Chr. 7
Chr. 8
Chr. 9
Chr. 10
0
25
50
75
100
125
150
175
200
225
250
275
phi126
bnlg391
bnlg1538
umc2313
umc1006
umc1257
umc1857
bnlg1174
nc012
umc1020
bnlg1732
umc2165
phi299852
bnlg1740
umc2059
ARL*
SDW*
RDW*
TRSA*
TRL*
ARN*
RDW*
ARL*
ARN*
III
I
II
umc1426
phi057
umc1016
phi034
umc1433
bnlg398
mmc0411
bnlg339
umc1888
umc2197
phi082
phi116
GY*
TRL*
TRSA*
I
umc1872
phi119
umc1034
umc1778
umc1741
phi115
bnlg666
umc1997
bnlg1823
umc1268
umc1933
umc1673
bnlg1056
phi080
phi015
dupssr14
bnlg1131
SDW
GY*
ARL*
I
II
umc1867
phi028
dupssr6
umc1033
bnlg430
umc1191
umc1789
phi448880
bnlg619
TRL*
RDW*
SDW*
I
II
umc1291
bnlg1451
umc2067
umc1336
umc2163
umc1697
umc1053
umc2003
umc2122
umc1344
umc2021
ARL*
SDW
TRL*
ARN*
RDW*
TRSA*
ARN*
II
III
I

◀ **Fig. 2** Maize SSR map containing QTLs for root dry weight (RDW), total root surface area (TRSA), total root length (TRL), axial root length (ARL), axial root number (ARN), and shoot dry weight (SDW) of plants measured at 50 days (I, in *red*), 80 days (II, in *blue*), and 120 days (III, in *black*) after planting. QTLs for grain yield (GY) were plotted according to the data from Liu et al. (2011). A *vertical line* represents the marker interval where the QTL was located. An *asterisk* indicates donor QTL alleles with a favorable effect (color figure online)

Tuberosa et al. (2003) suggested that root traits are good candidates for the application of advanced-backcross QTL analysis, which is able to identify quickly and exploit beneficial QTL alleles by integrating QTL discovery and crop improvement simultaneously. In the present study, because of the genetic nature of the population, an average number of 6.8 rather than one introgression segment within each BC_4F_3 line might affect the accuracy of QTL mapping. To overcome this limitation, Salvi et al. (2011) recently produced an introgression library by means of marker-assisted backcrossing, which could serve as a permanent source of near-isogenic materials for multiple studies of QTL analysis and cloning. Nevertheless, in the present study we also obtained 23 introgression lines with 1–2 introgression segments and 43 lines with 3–4 segments, which will be used further to construct near-isogenic lines for fine mapping and eventually cloning of the important root QTLs.

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