

# Genetic analysis of vertical root pulling resistance (VRPR) in maize using two genetic populations

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**Abstract** Root traits are important in improving nutrient and water use efficiency. Vertical root pulling resistance (VRPR) has been shown to be closely related to root system characteristics in maize (*Zea mays* L.). In the present study, two genetic populations derived from the same parents, one containing 218 recombinant inbred lines (RILs) and the other containing 187 advanced backcross BC<sub>4</sub>F<sub>3</sub> lines, were genotyped using 184 SSR markers and evaluated for VRPR, grain yield (GY), stover yield (SY), and nitrogen uptake (N<sub>up</sub>) under field conditions over 2 years. Our aims were (1) to locate QTLs associated with VRPR, SY, GY, and N<sub>up</sub>, (2) to determine whether QTL detection is consistent between the BC<sub>4</sub>F<sub>3</sub> and RIL populations, and (3) to identify backcross lines harboring favorable VRPR QTLs for

use in future breeding programs. Using composite interval mapping (CIM), 12 and 17 QTLs were detected in BC<sub>4</sub>F<sub>3</sub> and RIL populations, respectively. An important QTL region in bin 6.02 within the interval umc1006-umc1257 was found to control VRPR, SY, and N<sub>up</sub> in both populations. These favorable alleles were contributed by the large-rooted parent Ye478. A significant positive correlation was detected between VRPR, SY, and N<sub>up</sub>, but not between VRPR and GY. Backcross lines harboring VRPR QTLs could be useful germplasm for developing near isogenic lines (NILs) and for map-based cloning of genes controlling root growth.

**Keywords** Maize (*Zea mays* L.) · Vertical root pulling resistance (VRPR) · QTL mapping · N uptake

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## Introduction

Maize is not only an important staple, but can also be used as a biofuel, starch and vegetable crop. The root system plays an essential role in maize biomass and yield formation because of its primary functions in the acquisition of water and nutrient resources and in anchorage (Bohn et al. 2006; Kenrick 2002; Tian et al. 2006; Wilson 1930). Although extensive genetic variation for root architecture has been reported (Chun et al. 2005; Hébert et al. 1992; Jenison et al. 1981; Landi et al. 1998; Tuberosa et al. 2003), root

traits have seldom been considered as selection criteria for improving yield in maize breeding, mainly due to difficulties in their measurement (Tuberosa and Salvi 2007). Vertical root pulling resistance (VRPR) has been used as a parameter to estimate root size and lodging resistance in maize (Wilson 1930; Zuber et al. 1971; Fincher et al. 1985). Compared with other root traits, VRPR can be measured more rapidly under field conditions. Many studies have shown that VRPR is closely related to root dry weight, root volume, and brace root number (Beck et al. 1988; Spencer 1940; Zuber et al. 1971). Kamara et al. (2002, 2003) found that there is a significant negative correlation between VRPR and root lodging. Landi et al. (2002) found that VRPR significantly affects maize grain yield both under well-watered and water-stressed conditions. These results suggest that VRPR can be used as a valuable trait for genetic improvement of the root system.

Quantitative trait locus (QTL) analysis is a powerful tool for explaining the genetic basis of complex traits like grain yield and root traits. In recent years, large numbers of DNA-based molecular markers have been developed for the construction of high-density genetic maps in maize, facilitating the identification of QTLs. Numerous QTLs for root traits have been identified in different mapping populations. These root QTLs are usually related to abiotic stresses such as drought (Landi et al. 2002; Lebreton et al. 1995; Tuberosa et al. 2002), nitrogen deficiency (Liu et al. 2008), phosphorus deficiency (Chen et al. 2008a; Kaeppler et al. 2000; Zhu et al. 2005, 2006), cold stress (Hund et al. 2004), root lodging (Guingo et al. 1998) and water logging (Mano et al. 2005; Qiu et al. 2007). Lebreton et al. (1995) identified 7 QTLs for VRPR and found a genetic linkage between VRPR and leaf ABA content. Landi et al. (2002) identified QTLs for VRPR, grain yield under well-watered (GY-WW) conditions, grain yield under water-stressed (GY-WS) conditions, and drought tolerance index (DTI) under different water supply conditions in the field. These results reveal that QTLs for VRPR overlap with QTLs for GW-WW, GW-WS, and DTI. VRPR has also been found to be correlated with nitrogen uptake (Kamara et al. 2002).

In the present study, vertical root pulling resistance (VRPR), whole plant nitrogen uptake ( $N_{up}$ ), grain yield (GY), and stover yield (SY) were investigated

in a recombinant inbred population and an advanced backcross  $BC_4F_3$  population derived from the same parents. Our objectives were (1) to locate QTLs associated with VRPR, SY, GY, and  $N_{up}$ , (2) to compare QTL detection in the  $BC_4F_3$  and RIL populations, and (3) to identify backcross lines harboring favorable VRPR QTLs that could be used for gene-mapped cloning of root-specific genes in the future.

## Materials and methods

### Plant populations

#### *RIL population*

The RIL population consisted of 218  $F_8$  lines, derived from a cross between two inbred lines, Ye478 and Wu312, using single-seed descent. Ye478, the female parent, is a popular inbred line in China and is the female parent of more than 50 high-yielding hybrids (Yu and Zhu 1996; Li et al. 2005). Wu312, the male parent, was derived from an unknown hybrid. In a previous study, we found that Ye478 has a large root system, whereas Wu312 has a small root system (Tian et al. 2006; Liu et al. 2009). On average, Ye478 reaches anthesis about 5 days earlier than Wu312 does. The average plant height of Ye478 and Wu312 is 168 and 159 cm, respectively.

#### *$BC_4F_3$ population*

Ye478 was used as the donor parent and Wu312 as the recurrent parent in a backcrossing program. The backcrossing population was developed according to the advanced backcross strategy of Tanksley and Nelson (1996). 187  $BC_4F_3$  lines were obtained for use in genotyping and phenotyping in the present study.

### Field experiments and trait evaluation

For field testing, 218 RILs and the two parents were grown at Dongbeiwang, Beijing (40°00' N latitude, 116°18' E longitude, 60 m altitude) in 2006 and 2007. The  $BC_4F_3$  population and parents were grown at Dongbeiwang in 2007 and at Shangzhuang, Beijing (40°06' N latitude, 116°11' E longitude, 46 m altitude)

in 2008. The average daytime temperature was 11.5°C. The average rainfall per year was 600 mm, with about 70% of the rainfall occurring in the summer season. The soil at Dongbeiwang was an alluvial soil, containing 23.0 g/kg organic matter, 1.0 g/kg total nitrogen, 29.3 mg/kg mineral nitrogen, 17.4 mg/kg available phosphorus (Olsen-P) and 157.5 mg/kg available potassium (NH<sub>4</sub>Ac-K). The soil at Shangzhuang contained 15.8 g/kg organic matter, 0.83 g/kg total nitrogen, 36.5 mg/kg mineral nitrogen, 26.7 mg/kg available phosphorus and 103.8 mg/kg available potassium. The experiment followed a completely random design of one-row plots with three replications. The rows were 4 m long and contained 16 plants, and the space between rows was 0.67 m. The planting density was 60,000 plants per ha. 750 kg/ha calcium superphosphate and 135 kg/ha potassium chloride were applied to the soil before sowing. An additional 391 kg/ha urea was applied at the V6 stage. Irrigation was only applied before sowing to ensure suitable soil moisture for seed germination. Rainfall was otherwise sufficient during the maize growing season. Other field management techniques followed conventional practice. Seeds were sown on May 6, 16, and 8 in 2006, 2007, and 2008, respectively. Plants were harvested on September 15, 25, and 16, in 2006, 2007, and 2008, respectively.

According to Fincher et al. (1985) and Kamara et al. (2003), maximum root-pulling resistance is reached after mid-silking. Based on this information, VRPR was determined about 2 weeks after the average date of mid-silking. There was no rain for 3 days before or during VRPR measurement. All VRPR measurements were completed within 1 week. Before VRPR was measured, one in every three plants in the row was cut to a height of 30 cm above the ground. In total six plants were cut per row, of which the middle four plants were chosen for VRPR measurements. If problems arose with the measurement, the remaining two plants were measured to make sure there were four observations. VRPR was quantified as the force (in Newton/plant) required to lift a plant vertically from the soil using a 3yc-1 maize stubble pull dynamometer (Li and Feng 1994). The remaining ten intact plants were used for determining grain yield (GY), stover (SY), and N<sub>up</sub>. Ears were separated from the stovers at harvest. Ears were air-dried and then threshed for determining GY. Stover were over-dried for determining SY and N<sub>up</sub>.

Total N concentration was measured using a Kjeldahl procedure (only in the RIL population).

#### Construction of the simple sequence repeat (SSR) molecular linkage map

Fresh leaves from plants at the V5 stage were collected from the parents, the RIL population, and the BC<sub>4</sub>F<sub>3</sub> population and were stored at –80°C. DNA was extracted as described by Saghai-Marooof et al. (1984). SSR primers were synthesized according to the sequences published by the MaizeGDB (<http://www.maizegdb.org>). A total volume of 15 µl reaction mixture, containing 2 ng/µl template DNA, 10 mM Tris–HCl (pH 8.0), 1.5 mM MgCl<sub>2</sub>, 0.2 µM of each primer, 0.25 mM of each dNTP, and 1 U of Taq DNA polymerase, was used for PCR. The amplification procedure consisted of an initial denaturing step at 94°C for 4 min, followed by 35 cycles of 40 s at 94°C, 40 s at 56–62°C (depending on the T<sub>m</sub> of different markers), 1 min at 72°C, and a final extension at 72°C for 10 min. PCR products were separated on 6% polyacrylamide denaturing gels and stained using the silver-staining protocol of Panaud et al. (1996). SSR analysis was conducted as described in Senior and Heun (1993).

The genetic map was constructed using the MAPMAKER/EXP 3.0 software program (LOD > 3.0) (Lincoln et al. 1993). The Kosambi mapping function was selected for calculating map distances. The ‘Sequence’ command was used to construct linkage groups for all the markers. The ‘Compare’ command determined the order of markers within the linkage group and the ‘Ripple’ command was used to validate it. The  $\chi^2$  test was applied to identify any distorted segregation of markers ( $P < 0.01$ ) from the expected ratios.

#### Statistical analysis and QTL detection

Data from the parental lines in the field experiment was analyzed with SAS (SAS Institute, 1999) using the GLM procedure. Combinations of year-location were treated as environments (E). Genotype (G) was treated as fixed, with E and interaction of genotype-by-environment (G × E) as random. Then LSMEANS was used to estimate BLUE values for genotypes. Bartlett test was applied to check the homogeneity of random error variances within each environment.

Procedure VARCOMP was used to estimate genetic variance ( $\sigma_G^2$ ), interaction variance ( $\sigma_{GE}^2$ ), and environment variance ( $\sigma_E^2$ ). Following equation was applied to estimate heritability ( $h^2$ ).  $h^2(\%) = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/e + \sigma_E^2/re} \times 100\%$  (Hallauer and Miranda 1981), where  $e$  is the number of environments, and  $r$  is the number of replications. Exact 95% confidence intervals (CI) of  $h^2$  were calculated according to Knapp et al. (1985).

QTL detection was performed by composite interval mapping (Zeng 1994) using Windows QTL Cartographer version 2.5 (Model 6, Wang et al. 2005). Forward regression was analyzed using a window size of 10 cM, a walk speed of 2 cM and five control markers. The threshold LOD values were determined using 1,000-times permutations at  $P < 0.05$  level (Churchill and Doerge 1994). The resulting LOD values were 2.8–3.2 for different traits in two populations. As a result, the LOD thresholds were set at 2.8 for all traits to retain more QTLs information. Putative QTLs which were detected in the two populations within the same or adjacent marker intervals (within 10 cM), and were in the same chromosome bin, were considered to be common QTLs.

The VRPR phenotype of BC<sub>4</sub>F<sub>3</sub> lines that showed a significant difference ( $P < 0.01$ ) from the recurrent parent Wu312 was selected. The percentage difference in VRPR, SY, and GY phenotypes between the mean of each of these lines and the mean of the recurrent parent Wu312 was defined as phenotypic improvement and was calculated as  $(\text{Line}-\text{Wu312})/\text{Wu312} \times 100$ .

## Results

### Genetic linkage map

A total of 662 SSR markers were selected on the basis of their bin location in the IBM2 2008 maize neighbor linkage map (<http://www.maizegdb.org>) and screened for polymorphisms between Ye478 and Wu312. 213 markers (32.2%) were found to show polymorphism, of which 29 were eliminated because of significant segregation distortion or unstable polymorphism in the RIL population. The remaining 184 markers were used to construct a genetic linkage map. The map has 21, 20, 24, 20, 20, 17, 17, 19, 12, and 14 markers on chromosomes 1, 2, 3, 4, 5, 6, 7, 8,

9 and 10, respectively. The map covers 2084.1 cM with an average interval of 11.3 cM. Most SSR markers were at the same locations as that in the IBM2 2008 neighbor linkage map for maize. Based on this genetic linkage map constructed using the 218 RIL lines, 143 markers were selected for the analysis of phenotypic data in the BC<sub>4</sub>F<sub>3</sub> population.

### Phenotypes of the RILs and BC<sub>4</sub>F<sub>3</sub> lines

VRPR, GY, SY, and  $N_{up}$  differed greatly between the two parents and among the lines in the two populations. The frequency of all the traits showed a pattern of continuous distribution around the mean (Table 1; Fig. 1). VRPR was lower in the BC<sub>4</sub>F<sub>3</sub> population than that in the RIL population. There were no obvious differences between the populations in GY and SY. The broad sense heritability of the four traits was calculated according to the formula of Hallauer and Mirada (1981) and ranged from 38.2 to 67.4% in the RIL population and from 41.6 to 54.2% in the BC<sub>4</sub>F<sub>3</sub> population (Table 1). For VRPR, GY, and SY, the heritability was higher in the RIL population than in the BC<sub>4</sub>F<sub>3</sub> population. However, the coefficient of variation was lower in the BC<sub>4</sub>F<sub>3</sub> population compared with the RIL population, because the alleles of Ye478 in the backcross lines decreased with continuous backcrossing against the recurrent parent Wu312 and the performance of most BC<sub>4</sub>F<sub>3</sub> lines was close to the recurrent parent Wu312.

There were significant correlations between VRPR and SY and  $N_{up}$  in the RIL population (Table 2). A correlation between VRPR and SY was found in the BC<sub>4</sub>F<sub>3</sub> population though the correlation coefficients were lower than in the RIL population. This data suggested that VRPR, SY, and  $N_{up}$  were at least partly controlled by a common set of genes. However, the correlation between VRPR and GY was not consistently positive in both the RIL and the BC<sub>4</sub>F<sub>3</sub> populations.

### QTL detection

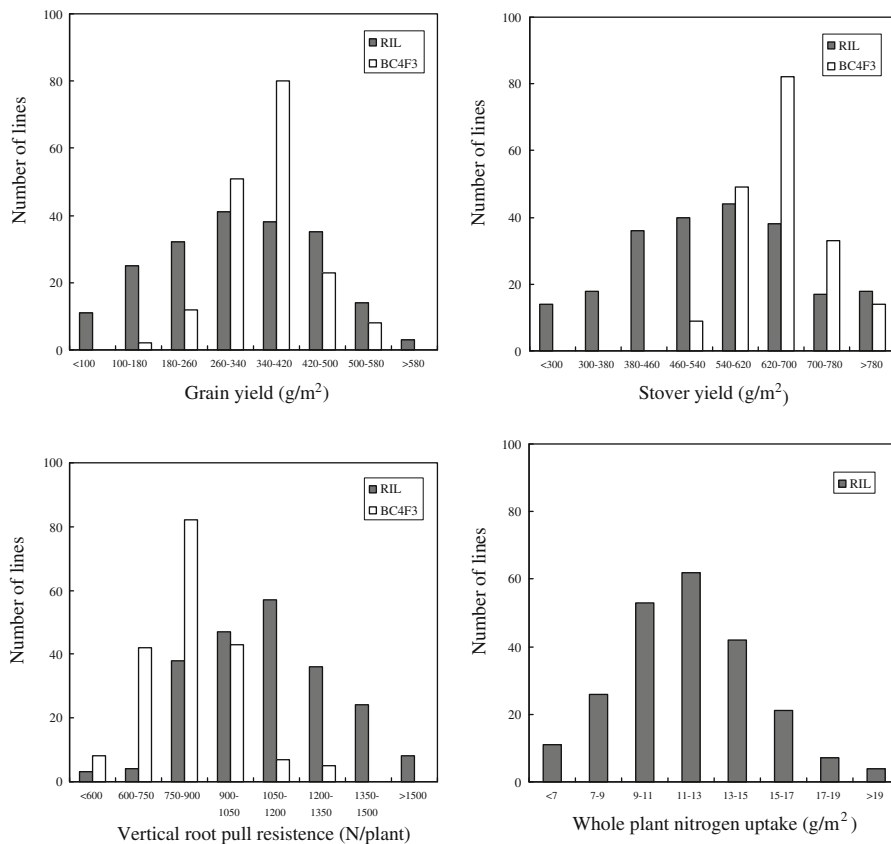
A total of 12 and 17 QTLs which were significantly associated with the four traits were detected in the BC<sub>4</sub>F<sub>3</sub> and RIL populations, respectively. The phenotypic variation explained by each QTL ranged from 6.0 to 25.4%. QTLs were located on all chromosomes except for chromosome 9 (Table 3; Fig. 2).

**Table 1** Means and heritability ( $h^2$ ) of VRPR, GY, SY and  $N_{up}$  for the parents, RIL population, and  $BC_4F_3$  population

Traits	Ye478	Wu312	RIL population				$BC_4F_3$ population			
			Mean	Range	$h^2$ (%)	95% CI on $h^2$	Mean	Range	$h^2$ (%)	95% CI on $h^2$
VRPR (Newton/plant)	1,031.7 a	825.0 b	1,100 ± 229.8	482.1–1,932.0	61.3	30.8–91.8	833.9 ± 113.1	553.3–1,290.1	44.1	27.9–60.3
GY ( $g/m^2$ )	475.2 a	358.8 b	319.9 ± 131.0	62.4–644.3	67.4	50.0–84.8	328.1 ± 72.9	129.6–513.5	54.2	43.8–64.6
SY ( $g/m^2$ )	689.0 a	619.5 b	549.5 ± 156.8	224.3–993.1	62.0	41.2–82.8	673.8 ± 74.9	466.2–913.8	41.6	30.9–52.3
$N_{up}$ ( $g/m^2$ )	14.6 a	11.8 b	12.8 ± 3.0	5.2–20.3	38.2	37.8–38.6	–	–	–	–

Data with the same letter within a row are not significantly different ( $P < 0.05$ )

CI confidence interval



**Fig. 1** Frequency histograms for VRPR, GY, SY and  $N_{up}$  of the RIL and  $BC_4F_3$  population

**VRPR**

Single QTLs for VRPR were detected on chromosomes 1, 2, 3, and 6 in the RIL population, and on chromosomes 1, 2, and 10 in the  $BC_4F_3$  population. The contribution to phenotypic variation of each QTL

ranged from 6.1 to 14.9%. Of the detected QTLs, only one QTL on chromosome 2 (umc1518-bnlg2248) in the  $BC_4F_3$  population was near its equivalent QTL (bnlg2248-umc2248) in the RIL population. Their contributions were 8.9 and 6.1% for the RIL and  $BC_4F_3$  populations, respectively.

**Table 2** Pearson's correlation coefficients for VRPR, GY, SY, and  $N_{up}$  in the RIL and  $BC_4F_3$  populations

Population	Trait	GY	SY	$N_{up}$
RIL	VRPR	-0.12	0.43**	0.39**
	GY		0.38**	0.43**
	SY			0.85**
$BC_4F_3$	VRPR	-0.13	0.28**	-
	GY		0.41**	-
	SY			-

\*\*  $P < 0.01$ **GY**

Single QTLs for GY were detected on chromosomes 1, 2, 3, 5, and 8 in the RIL population, and on chromosomes 1, 2, 5, 7, and 8 in the  $BC_4F_3$  population, and contributed between 6.9 and 16.2% to phenotypic variation. The QTL *umc1335-bnlg1556* on chromosome 1 was found in both populations, and its contribution to phenotypic variation was 14.6 and 10.3% in the RIL and  $BC_4F_3$  populations, respectively. Another QTL found in the  $BC_4F_3$  population

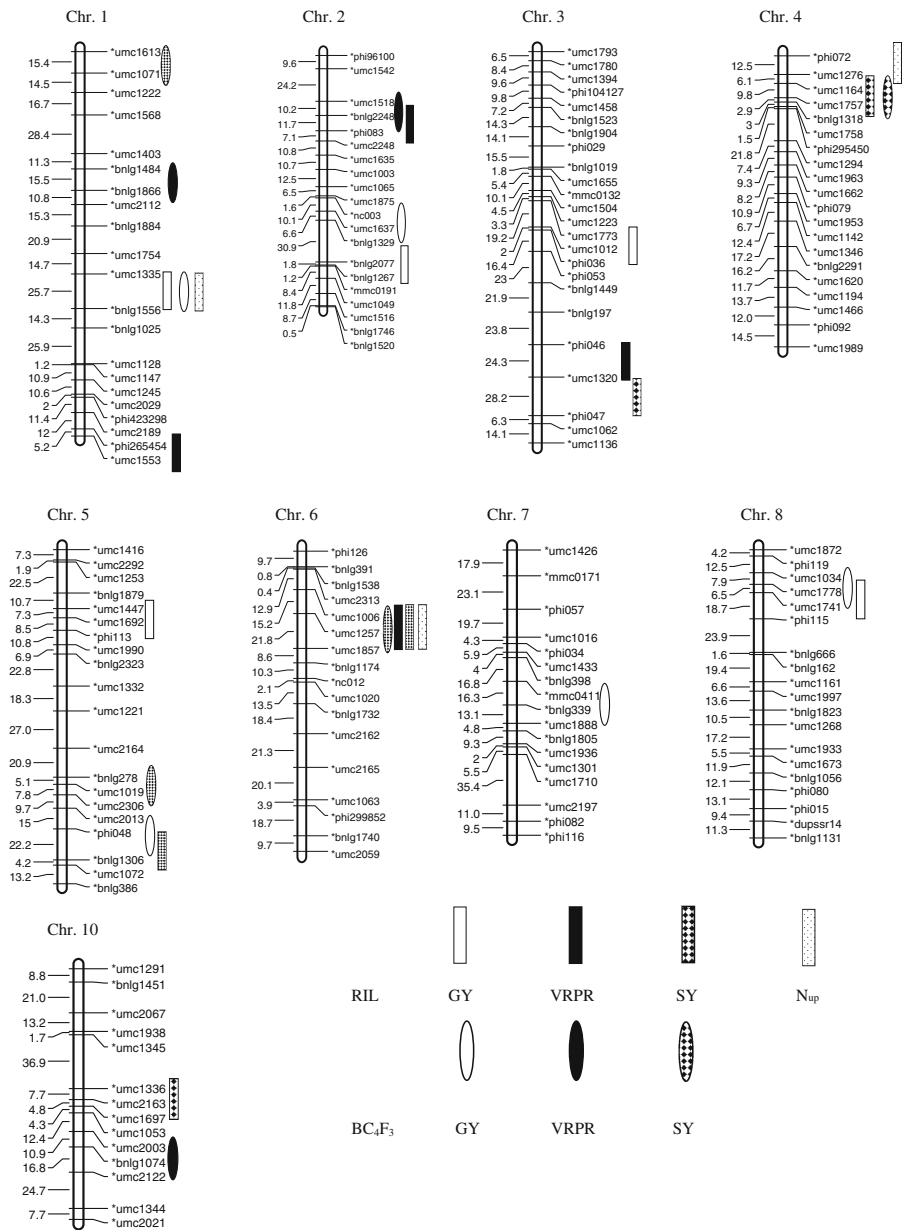
**Table 3** VRPR, GY, SY, and  $N_{up}$  QTLs identified in the RIL and  $BC_4F_3$  populations

Trait	Population	Chr/Bin	Position (cM)	Marker interval	LOD	Add. <sup>a</sup>	$R^2$ (%) <sup>b</sup>		
VRPR	RIL	1.11	282	<i>phi265454-umc1553</i>	5.38	-94.2	9.8		
		2.03/2.04	49	<i>bnlg2248-phi083</i>	4.36	62.7	8.9		
		3.08	225	<i>phi046-umc1320</i>	3.36	-100.5	7.1		
		6.02	27	<i>umc1006-umc1257</i>	4.29	85.8	10.6		
	$BC_4F_3$	1.03	97	<i>bnlg1484-bnlg1866</i>	4.35	-79.7	8.8		
		2.03	41	<i>umc1518-bnlg2248</i>	2.97	66.7	6.1		
		10.04	116	<i>umc2003-bnlg1074</i>	7.65	122.5	14.9		
		GY	RIL	1.06/1.07	172	<i>umc1335-bnlg1556</i>	5.53	47.0	14.6
				2.07	154	<i>bnlg2077-bnlg1267</i>	6.96	-43.9	16.2
				3.04	125	<i>umc1773-umc1012</i>	3.20	39.7	9.6
5.03	43			<i>umc1447-umc1692</i>	4.05	48.9	13.2		
8.03	25			<i>umc1778-umc1741</i>	3.99	-33.4	10.3		
$BC_4F_3$	1.06/1.07			176	<i>umc1335-bnlg1556</i>	4.91	45.1	10.3	
	2.06	110	<i>nc003-umc1637</i>	4.23	37.6	9.0			
	5.07	202	<i>umc2013-phi048</i>	7.48	-60.9	15.2			
	7.02/7.03	99	<i>mmc0411-bnlg339</i>	3.22	27.3	6.9			
	8.02/8.03	20	<i>umc1034-umc1778</i>	5.37	49.1	11.1			
	SY	RIL	3.08	250	<i>umc1320-phi047</i>	3.24	55.1	9.3	
4.01			19	<i>umc1164-umc1757</i>	2.92	-36.2	8.0		
5.07			209	<i>phi048-bnlg1306</i>	5.11	-55.9	15.0		
6.02			25	<i>umc1006-umc1257</i>	13.09	104.6	25.4		
10.03/10.04			89	<i>umc1336-umc2163</i>	5.55	-66.6	14.8		
$BC_4F_3$			1.01	1	<i>umc1613-umc1071</i>	4.23	50.3	8.7	
		4.01	21	<i>umc1164-umc1757</i>	2.93	-45.3	6.0		
		5.05/5.06	165	<i>bnlg278-umc1019</i>	3.67	-46.8	7.5		
		6.02	26	<i>umc1006-umc1257</i>	4.10	51.2	7.8		
$N_{up}$		RIL	1.06/1.07	168	<i>umc1335-bnlg1556</i>	3.38	0.72	8.7	
	4.00/4.01		4	<i>phi072-umc1276</i>	3.02	-0.66	7.3		
	6.02		25	<i>umc1006-umc1257</i>	4.58	1.53	13.7		

<sup>a</sup> A positive value means that Ye478 carried the allele for an increase in the trait, and a negative value means that Wu312 carried the allele for an increase in the trait

<sup>b</sup> The proportion of phenotypic variation explained by each QTL

**Fig. 2** SSR linkage map for Ye478 × Wu312 RIL population and QTLs for all traits detected from RIL and BC<sub>4</sub>F<sub>3</sub> populations



on chromosome 8 (umc1034-umc1778) was near the QTL detected in the RIL population (umc1778-umc1774). However, their additive effects were in the opposite direction.

**SY**

Single QTLs for SY were detected on chromosomes 3, 4, 5, 6, and 10 in the RIL population, and on chromosomes 1, 4, 5, and 6 in the BC<sub>4</sub>F<sub>3</sub> population.

The phenotypic variation explained by a single QTL varied between 6.0 and 25.4%. The QTLs on chromosome 4 (umc1164-umc1757) and on chromosome 6 (umc1006-umc1257) were found in both of the populations.

**N<sub>up</sub>**

Single QTLs for N<sub>up</sub> were detected on chromosomes 1, 4, and 6 in the RIL population. The contribution to

phenotypic variation of a single QTL ranged from 7.3 to 13.7%. The QTL for SY was identified in the chromosome interval umc1006-umc1257 in both populations, while the QTL for GY was found in the chromosome interval umc1335-bnlgl1556 in both populations.

#### Performance of backcross lines containing VRPR QTLs

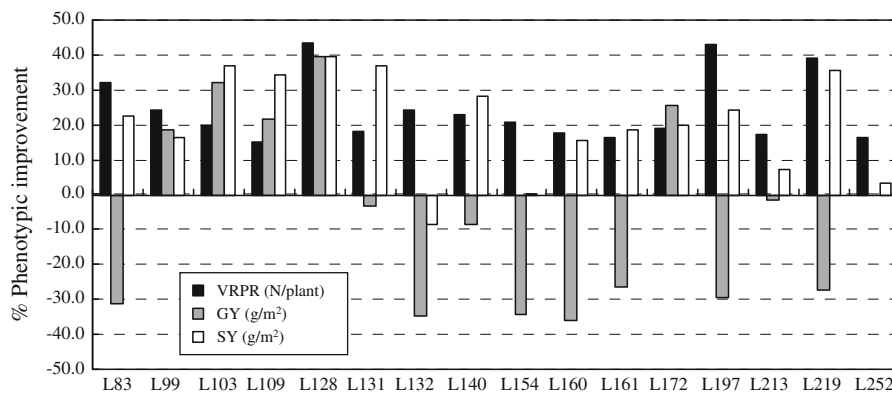
Sixteen lines which contained VRPR QTLs from the donor parent Ye478 and also had higher VRPR phenotypes were selected from the BC<sub>4</sub>F<sub>3</sub> population. The mean percentage phenotypic improvement in VRPR, GY, and SY in the high-VRPR lines is illustrated in Fig. 3. Compared with Wu312, these lines showed an apparent improvement in VRPR of 15.0–43.2% and in SY of 3.2–39.5%. However, grain yield in these selected lines did not show a consistent improvement compared with Wu312 (Fig. 3). This result is consistent with our results from correlation analysis (Table 2) and QTL mapping (Table 3).

#### Discussion

The overlap between QTLs for VRPR and GY, SY, and N<sub>up</sub>

Analysis of correlation coefficients between VRPR and GY, SY, and N<sub>up</sub> in the two populations indicated that a significant positive correlation exists between

VRPR, SY and N<sub>up</sub>, but not between VRPR and GY (Table 2). QTL analysis also indicated that two of the QTLs controlling VRPR were linked to the QTL controlling SY in bins 3.08 and 10.04 (Table 3; Fig. 2). In bin 6.02, four QTLs controlling VRPR, SY, and N<sub>up</sub> were detected within the same marker interval umc1006-umc1257, all favorable alleles being contributed by Ye478. These results suggest that improving VRPR can enhance N acquisition and above-ground growth. Previously it was found that the large-rooted parent Ye478 has greater N acquisition ability, and this involves the coordination of leaf and root growth (Tian et al. 2006). Vigorous leaf growth causes a large demand for N which can be met by this genotype's large root system. Besides providing a strong sink for N uptake, the larger leaf area of Ye478 might also guarantee the carbohydrate supply necessary for its greater root growth. In addition, the other seven VRPR QTLs detected did not overlap with QTLs for GY in the two populations, indicating that VRPR and GY are probably unrelated. This may be because yield is determined not only by dry matter production, but also by the efficient allocation of dry matter into grains. Thus, a large root system as indicated by high VRPR may not necessarily result in a higher grain yield (Arihara and Crosbie 1982; Rahman et al. 1994). Under low N or drought stress when water and nutrient acquisition has become the most limiting factor in biomass production and yield formation, a large root system is generally positively correlated to grain yield (King et al. 2003). For example, Kamara et al. (2002; 2003)



**Fig. 3** Analysis of sixteen lines with strong root (VRPR) which were inferior to the recurrent parent Wu312 over 10% in BC<sub>4</sub>F<sub>3</sub> population at 2007 and 2008. The percent phenotypic

difference between the mean of each lines and the mean of the recurrent parent Wu312 was estimated as: % phenotypic improvement = 100(L - Wu312)/Wu312



who showed that VRPR is positively correlated with maize grain yield ( $r = 0.71$ ) and N-uptake ( $r = 0.68$ ) in 18 selected breeding lines from a tropical low nitrogen population. Landi et al. (2002) also found that 11 of the 19 QTLs identified for VRPR overlapped at least once with QTLs for GY, especially under water-stressed conditions.

#### Comparison of QTLs in the RIL and BC<sub>4</sub>F<sub>3</sub> populations

QTL detection can be influenced by factors such as environment, genetic background, selection during backcrossing, and generations used (Austin and Lee 1996; Beavis et al. 1994; Li et al. 2007; Moreno-Gonzalez 1993; Xu and Crouch 2008). In our study, 12 and 14 QTLs were detected in the BC<sub>4</sub>F<sub>3</sub> and RIL populations, respectively, for the same three traits investigated. Three pairs of QTLs were located within the same chromosome intervals in the two populations, while two pairs were located near the same chromosome intervals. The frequency of identifying common QTLs in our RIL and BC<sub>4</sub>F<sub>3</sub> populations was similar to that of Li et al. (2007) in which common QTLs for grain yield components were detected using a BC<sub>2</sub>F<sub>2</sub> and an F<sub>2:3</sub> populations. Compared with the RIL population, the number and contribution of QTLs detected were lower in the BC<sub>4</sub>F<sub>3</sub> population (Table 3). In the RIL population, we detected an important QTL region associated with SY and VRPR on chromosome 6 within the interval umc1006-umc1257, with LOD values of 13.09 and 4.29, respectively. At the same locus in the BC<sub>4</sub>F<sub>3</sub> population, however, the LOD value of the QTL for SY decreased to 4.10 and the QTL for VRPR was not significant (LOD = 2.3). Based on Mendelian expectations, the theoretical frequency of the three possible marker genotypes in the BC<sub>4</sub>F<sub>3</sub> population is 123:2:3 (AA: AB: BB). So a “dilution effect” could decrease the effects of the donor parent QTL alleles, and even mask small effects at low frequencies (Ho et al. 2002; Li et al. 2007). In three QTL regions common to both populations (interval umc1335-bnlg1556 of chromosome bin 1.06/1.07, interval umc1164-umc1757 of chromosome bin 4.01, and interval umc1006-umc1257 of chromosome bin 6.02), the low  $R^2$  values in the BC<sub>4</sub>F<sub>3</sub> population could be attributed to the low allele frequencies of the donor parent. Since RILs represent a permanent mapping population in which the expected

ratio of homozygous parental genotypes is 1:1, only additive effects can be detected. For that reason we have only listed the additive effects for the RIL and BC<sub>4</sub>F<sub>3</sub> populations here (Table 3). The direction of the additive effects of the three traits investigated in the RIL population was the same as that in the BC<sub>4</sub>F<sub>3</sub> population.

#### Comparison between QTLs detected in this study and other studies

It is important to emphasize the relevance of a comparative analysis of QTL studies investigating similar traits in different genetic backgrounds and environmental situations (Tuberosa et al. 2003). Due to differences in the mapping populations, direct comparisons of QTL mapping results across studies are difficult. One way to make comparisons is to use information provided by markers that are common to the different genetic maps. Alternatively, one can compare each mapping population indirectly to a reference map, such as the IBM map (<http://www.maizemap.org/>). In the present study, we found major QTLs for VRPR, SY, and N<sub>up</sub> between umc1006 and umc1257 in chromosome bin 6.02. In the same location, Landi et al. (2002) found a QTL for VRPR, Bertin and Gallais (2001) found a QTL for nitrogen utilization efficiency, Hund et al. (2004) found a QTL for lateral root length, and Mechin et al. (2001) found a QTL for dry matter yield. These results suggest that this chromosome region between umc1006 and umc1257 is a key location for genes associated with root growth, N uptake and biomass accumulation in maize.

Tuberosa et al. (2003) summarized the mapping results of four populations which had been used to detect QTLs for root traits and grain yield under controlled conditions or in the field. Some important QTL regions were detected in chromosome bins 1.03, 1.06, 1.08, 2.03, 2.04, 7.02, 8.06, and 10.04. In the present study, three QTLs for VRPR were found in the same chromosome bins as reported in previous studies, namely bin 1.03 (bnlg1484-bnlg1866) (Tuberosa et al. 2002; Lebreton et al. 1995), bin 1.11 (phi265454-umc1553) (Tuberosa et al. 2002), and bin 3.08 (phi046-umc1320) (Lebreton et al. 1995). Furthermore, QTLs controlling grain yield have been detected in bins 1.03 (Ribaut et al. 1996; Tuberosa et al. 2002), 1.11 (Tuberosa et al. 2002), and 3.08

(Agrama and Moussa 1996). Of the ten QTLs detected for grain yield in the present study, a QTL in chromosome bin 1.06–1.07 (umc1335–bnlg1556) falls in a chromosome region rich in QTLs for grain yield and root related traits (Liu et al. 2008). These loci may be targets for root improvement.

#### The significance of VRPR QTLs and utilization of the BC<sub>4</sub>F<sub>3</sub> population

The breeder's objective is to identify favorable QTL alleles with stable effects across genetic populations and environments (Ho et al. 2002; Li et al. 2007). A deeper root system is essential for combating nitrogen and water shortage in the field (Jordan et al. 1983; Wiesler and Horst 1994). Practice in maize breeding has revealed that improvement in root traits has played a very important role in increasing the size of the root system for hybrids (Duvick 1992). Since VRPR is easily measured and is closely related to root characteristics such as axial root length and number, lateral root length and density (Sanguineti et al. 1998), QTLs or genes controlling VRPR should have potential value in marker assisted selection (MAS)-based improvement of the root system, and hence, nutrient and water use efficiency. In the RIL population, we detected a major QTL region on chromosome 6 between umc1006 and umc1257. In the same fragment, four QTLs for VRPR overlapped with QTLs for SY in both populations. This QTL may be used as a potential marker in breeding for maize cultivars with bigger and deeper root system through an MAS approach. Furthermore, Beneficial QTL alleles identified in the RIL population will continue to exert their positive effects in near isogenic backgrounds of the same recipient parent (Ho et al. 2002). In advanced backcross populations some superior lines which contain the target QTLs and lower genotypic ratios of the donor parent can be selected for studying the stability of the effects of the target QTLs (Li et al. 2007), their fine mapping (Chen et al. 2008b), and even their functions (Tan et al. 2008). The sixteen high-VRPR lines from the BC<sub>4</sub>F<sub>3</sub> population used here which contained at least one VRPR QTL from the donor parent Ye478 showed improvements in VRPR and SY by an average of 24.3 and 20.6%, respectively (Fig. 3). After a further 1–2 backcrosses with Wu312, these lines could be useful germplasm in drought and low nitrogen stress

for conducting MAS for target QTLs and for developing QTL-NILs which could then be used for identifying superior root genes using map-based cloning strategies.

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