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RESEARCH ARTICLE

Comparative mapping of QTLs for H^+ secretion of root in maize (*Zea mays* L.) and cross phosphorus levels on two growth stages

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Abstract **H^+ is a root secretion that affects P acquisition and P-use efficiency (PUE) under deficient phosphorus in maize. The secretion of H^+, difference value of H^+ between deficient and normal phosphorus (DH), and relative H^+ (RH) as well as the quantitative trait loci (QTLs) associated with these traits were determined for a $F_{2:3}$ population derived from the cross of two contrasting maize (*Zea mays* L.) genotypes, 082 and Ye107. By using composite interval mapping (CIM), a total of 14, 8, and 9 distinct QTLs were identified for H^+, DH, and RH, respectively. Most loci of QTLs for traits H^+, DH, and RH had different cross environments. It showed that H^+ secretion possessed an environment-sensitive and multi-gene nature. The gene × environment interaction was actually reflected by H^+ secretion. One region for QTL of trait H^+ was detected at the interval of bnlg2228-bnlg100 (bin 1.08) on chromosome 1. Coincident QTLs in the important genomic region reflected the cross phosphorus levels, different cross growth stages, and two different cross environments. The QTL explained 10% to 14% total phenotypic variance of H^+. Therefore, the above segment (bnlg2228-bnlg100) (bin 1.08) identified on chromosome 1 may be used in the future for MAS to improve the phosphorus efficiency in maize.**

Keywords maize, QTL analysis, H^+, difference value of H^+, relative H^+

Introduction

H^+ is a root secretion that affects P acquisition and P-use efficiency (PUE) in maize and that plays a major role in P absorption as well as increases in response to P starvation in maize (Hinsinger, 1998). The increased H^+ secretion from roots in response to P starvation is thought to promote the P liberation from organic complexes in soil, which has been reported in many plant species, including maize, under P-deficient conditions. Remarkable difference in levels of H^+ secretion from roots under P-deficient conditions has been observed in many plant species. H^+ secretion from roots increases under P-deficient conditions, but the levels of secretion differ remarkably among plant species (Ae et al., 1990; Hinsinger and Gilkes, 1996; Pellet et al., 1996; Hinsinger, 1998, 2001).

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Since phosphate ions are strongly sorbed to soils (Ae et al., 1990), the charge of these P-sorbing minerals is pH dependent. Protons or H^+ are excreted into the rhizosphere by plant roots, making soil release phosphate from soil minerals and render P available for uptake. Thus, the increase in rhizosphere H^+ induced by roots can enhance P nutritional utilization in plant. However, P acquisition by plant roots is the actual flux of proton or hydroxyl equivalent excreted at the soil–root interface (Hinsinger and Gilkes, 1996; Guingo and Hebert, 1997; Hinsinger, 1998, 2001; Chen et al., 2009, 2010). H^+ exudation from roots of P-deficient seedlings has been detected in the rhizosphere in plants. However, the more important role of such H^+ exudation could be P release from Al or Fe complex and increase in solubilization of P-Al and P-Fe compounds. H^+, which can be exuded by root of plants, appears to be especially effective in enhancing P free to soil-bound P and the ability of plants to access P from soil (Jones and Darrah, 1995; Pellet et al., 1995, 1996; Jones et al., 1998; Landi et al., 1998a; Zhu and Lynch, 2004; Zhu et al., 2005).

There are close relationships between root and secretion H^+ in rhizosphere under P deficiency in plants. Molecular

markers can be used to study the inheritance of root traits and exudations and identify specific loci associated with the expression of these traits (Agrama and Moussa, 1996; Guingo et al., 1998; Agrama et al., 1999; Yan et al., 2004; Zhu et al., 2005; Chen et al., 2009, 2010). In maize hydroponics, Tuberosa et al. (2002) reported that quantitative trait locus (QTL) regions influence root diameter and root length, while Guingo et al. (1998) identified QTL for root weight. Tuberosa et al. (2002) described QTLs for root pulling force in hydroponics, root number in Polj17 × F-2, and root volume in B73 × Mo17. The QTLs of root hair length and lateral branch length in hydroponics were studied, and a QTL flanked by npi409-nc007 for root hair length was mapped on chromosome 5 (Rogers et al., 1989; Jones and Darrah, 1995; Pellet et al., 1995, 1996; Vos Hogers et al., 1995; Paterson et al., 1996; Hinsinger, 1998, 2001; Jones et al., 1998; Landi et al., 2001; Pratapbhanu, 2002; Tuberosa et al., 2003; Zhu et al., 2005; Chen et al., 2008, 2010).

To identify stable QTL associated with H^+ in maize, we detected the QTLs of traits of H^+, DH, and RH in multi-environments of two sites (KX and SU) and two P levels in two growth stages (SU1 and SU2).

Materials and methods

Plant materials

The mapping population included 241 $F_{2:3}$ families derived from a corresponding number of randomly chosen F_2 plants of the cross between inbred 082 tolerant to P deficiency and Ye107 susceptible to P deficiency, with different phosphorus efficiencies and root traits. The F_2 families were reproduced from October 2006 to February 2007 in Hainan, China. The $F_{2:3}$ families were reproduced in March to July 2007 in Xiema, Beibei, China.

Field experiment

The experiment was designed as split plot experiment with three replicates. P was used as main treatment and $F_{2:3}$ families along with F_1 and both parent lines were designed for vice treatment at Kaixian County (KX) and South-west University (SU). $F_{2:3}$ families along with F_1 and both parent lines were sowed. A set of experiments was conducted at KX at 31°11′N latitude, 98°58′E longitude and 1000 m altitude and SU at 29°48′N latitude, 106°33′E longitude and 150 m altitude during September to October in 2007. In KX soil, the contents of total N, P, and K and available N, P, and K were 0.236, 0.395, and 18.9 g/kg and 14.7, 2.0, and 117 mg/kg, respectively, with organic matter at 2.99 g/kg at pH 8.2. In SU soil, the contents of total N, P, and K and available N, P, and K were 0.259, 0.602, and 17.8 g/kg and 14.1, 2.6, and 121 mg/kg, respectively, with organic matter at 3.43 g/kg at pH 7.9. Fertilizers were applied before sowing at a rate of 120 kg N and 50 kg P per hectare under normal P application but only at 120 kg N under deficient P application. The area per split plot was 2.5 m^2. There were 10 plants in each plot. Cultivation was carried out according to standard practices.

Collecting of soil samples

Soils were collected on the 21st day after emergence of seedlings at KX, while soils were collected on the 21st and 35th days after emergence of seedlings at SU. The five soil samples around root system per plant were collected at random, packed in sealed plastic bags, and conserved in a –70°C refrigerator immediately.

Analysis of H^+

The 5 g sample soil at pH 6.0 was dissolved in 20 mL distilled water. The pH (later converted to H^+ concentration) was measured immediately with a pH meter (Orion 250A, Orion Research, Inc., USA) for each individual plant. The difference between measured H^+ and original H^+ was considered as total H^+ exuded (μmol/g). The difference value of H^+ (DH) was obtained when H^+ under low P was subtracted from H^+ under normal P. The relative H^+ (RH) was calculated as the rate of H^+ under low P and normal P.

Statistical analysis of data

Arithmetic means of three replicates was calculated for each trait of each $F_{2:3}$ family. The transgressive segregation was performed for genotypes that had traits with values beyond the two parents (i.e., larger than 082 or smaller than Ye107) at two sites. Heritability of traits for the $F_{2:3}$ Families was estimated by the following formula:

$$h_b^2(\%) = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_E^2} \times 100,$$

where $\hat{\sigma}_G^2$ and $\hat{\sigma}_E^2$ are the estimates of genetic variance and environment variance, respectively.

QTL analysis

The procedure of composite interval mapping (CIM) was used to identify QTLs and estimate their effects. QTL mapping was performed with the software program Windows QTL Cartographer version 2.5. Two-locus analysis was conducted using QTLNetwork Version 2.0 (http://ibi.zju.edu.cn/software/qtl-network/), where $P = 0.05$ was used as the threshold for detecting epistatic QTLs. Parameters for forward regression analysis were a window size of 10 cM, a walk speed of 2 cM, five background control markers, and a probability threshold of $P = 0.05$ each for the partial F test for both marker inclusion and exclusion. Significant threshold for QTL detection was calculated by 1000 random permutations

Table 1 Estimates of genetic variance $(\hat{\sigma}_G^2)$ and environment variance $(\hat{\sigma}_E^2)$ among 241 $F_{2:3}$ families from the cross of 082 × Ye107

Traits	Parents		$F_{2:3}$ families				
	082	Ye107	Mean	Range	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	$h_b^2(\%)$
H-KXDP	0.0670	0.0330	0.0525	0.0240–0.0810	0.0002	0.0001	61.3
H-KXNP	0.0223	0.0199	0.0226	0.0114–0.0455	0.0001	0.0001	67.5
H-SUDP1	0.0690	0.0380	0.0576	0.0250–0.0920	0.0002	0.0001	63.1
H-SUDP2	0.0814	0.0678	0.0675	0.0325–0.1148	0.0003	0.0002	65.7
DH-KX	0.0447	0.0131	0.0299	0.0126–0.0541	0.0001	0.0001	55.7
DH-SU1	0.0379	−0.0060	0.0233	−0.0113–0.0491	0.0001	0.0001	58.9
DH-SU2	0.0381	0.0127	0.0308	0.0108–0.0618	0.0001	0.0001	64.7
RH-KX	3.0020	1.6560	2.3780	3.0570–1.4990	0.1510	0.1400	51.9
RH-SU1	2.2200	0.8630	1.7600	2.2300–0.8640	0.0850	0.0830	50.2
RH-SU2	1.8800	1.2300	1.9147	1.2480–2.5200	0.0838	0.0731	53.4

* and ** denote significance at 0.05 and 0.01 levels, respectively. Abbreviations: H, H^+; DH, difference value of H^+ between deficient and normal phosphorus; RH, relative H^+; KX, Kaixian; SU1, 21st day at South-west University; SU2, 35th day at South-west University; KXDP, 21st day under deficient phosphorus at Kaixian; KXNP, 21st day under normal phosphorus at Kaixian; SUDP1, 21st day under deficient phosphorus at South-west University; SUDP2, 35th day under deficient phosphorus at South-west University.

of the phenotypic data at 5% level. LOD thresholds were set at 2.5 for all traits. QTL positions were assigned at the point of maximum LOD score in the regions under consideration. Gene action mode of each significant QTL (d/a) was estimated according to the additive (a) and dominant (d) effects, being classified to additive (0–0.20), partial dominant (0.21–0.80), dominant (0.81–1.20), and overdominant (>1.20) (Tuberosa et al., 1998).

Results

Variation of phenotypic traits among $F_{2:3}$ families

Phosphorus traits of 082 × Ye107 are shown in Table 2. The two parental genotypes, 082 and Ye107, differed significantly in H^+, DH, and RH in the three environments of KXDP, SUDP1, and SUDP2. 082 had higher values than Ye107 (Table 1). The $F_{2:3}$ families had a continuous segregation for all traits (H, DH, and RH), showing a quantitative pattern of inheritance for these traits, and the traits in the $F_{2:3}$ families in multi-environments were continuously segregated and approximately normally distributed with absolute values of skewness and kurtness less than 1.0, which indicated that all traits were suitable for employing QTL mapping analysis of variance (ANOVA) to estimate genetic variance $(\hat{\sigma}_G^2)$, environment variance $(\hat{\sigma}_E^2)$, and subsequently broad-sense heritability (h_b^2). The h_b^2 for H^+ in the multi-environments varied from 50.2% to 67.5% (Table 1).

The ANOVA for all traits (H^+, DH, and RH) tested on the $F_{2:3}$ families in the multi-environments was summarized in Tables 3–5. For all traits, the significant variances of genotype and environment indicated that all traits could be affected by both genotypes and environments. All genotype × environment interactions for three traits (H^+, DH, and RH) were significant.

The correlation coefficient among traits is shown in Table 6. The trait PUE was highly positive correlated with traits H^+, DH, and RH ($r > 0.70$), but three traits (H^+, DH, and RH) were not correlated with each other.

Table 2 Phosphorus traits of 082 × Ye107

Traits	Parents	
	082	Ye107
PE[a]	0.803	0.548
PAE[a]	3.230	2.237
WPUE-NP[a]	5.24	6.01
WPUE-NP[a]	5.11	5.87
H-DP[a]	0.0670	0.0330
H-NP[a]	0.0223	0.0199

PE, phosphorus efficiency; PAE, phosphorus absorption efficiency; WPUE, phosphorus utilization efficiency; H, H^+; DP, deficient phosphorus; NP, normal phosphorus.

Analysis of QTLs for H^+

One region located at the interval bnlg2228-bnlg100 (bin 1.08) on chromosome 1 was found to influence H^+ in all environments of KXDP, KXNP, SUDP1, and SUDP2 in across phosphorus levels, sites, and growth stages. The QTL explained 10% to 14% of total phenotypic variance of H^+. The alleles from the QTL in bnlg2228-bnlg100, which contribute to increasing H^+, were from the lower phosphorus efficiency parental genotype Ye107 (P2). Estimates of the genetic effects presented partial dominance.

Three different regions were found to influence H^+ on the 21st day under deficient phosphorus at Kaixian (KXDP), which were located at the intervals of phi098-umc1165 (bins2.02) on chromosome 2, umc1185-umc1555 (bins 2.03) on chromosome 2, and P3M7/b-dupssr14 (bins 8.09) on chromosome 8 (Table 7), respectively. These QTLs (H2a-KXDP, H2b-KXDP, and H8-KXDP) explained 65% of the total variation for H^+. The alleles from these QTLs mapped, which contribute to increasing the phosphorus efficiency,

Table 3 *F* value of ANOVA for effects on H^+

Treatments	Source of variation	SS	Degree of freedom	*F* value
Sites	Genotypes	0.0888	240	2.14**
	Environments	0.0031	1	4.56**
	G × E	—	—	3.54**
P levels	Genotypes	0.0391	240	1.59**
	Environments	0.1076	1	2.17*
	G × E	—	—	4.55**
Stages	Genotypes	0.1116	240	0.673*
	Environments	0.0119	1	4.23**
	G × E	—	—	0.412**

* and ** denote significance at 0.05 and 0.01 levels, respectively.

Table 4 *F* value of ANOVA for effects on DH

Treatments	Source of variation	SS	Degree of freedom	*F* value
Sites	Genotypes	0.0339	240	3.05**
	Environments	0.0052	1	112.84**
	G × E	—	—	24.67*
Stages	Genotypes	0.0350	240	6.72**
	Environments	0.0067	1	310.60**
	G × E	—	—	43.26*

* and ** denote significance at 0.05 and 0.01 levels, respectively.

Table 5 *F* value of ANOVA for effects on RH

Treatments	Sources of variation	SS	Degree of freedom	*F* value
Sites	Genotypes	36.53	240	1.80**
	Environments	45.91	1	543.99**
	G × E	—	—	36.65*
Stages	Genotypes	33.78	240	4.96**
	Environments	2.87	1	101.34**
	G × E	—	—	37.85*

* and ** denote significance at 0.05 and 0.01 levels, respectively.

Table 6 Analyze of correlation among PUE, H^+, DH, and RH

Item	PUE	H^+	DH
H^+	0.7374**	—	—
DH	0.7905**	0.4028	—
RH	0.7033**	0.4129	0.4046

** denote significance at 0.01 level.

were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects ranged from partial dominance to overdominance.

The region located at the interval bnlg1006-P2M5/d (bins 5.00) on chromosome 5 (Table 7) was found to influence H^+ on the 35th day under deficient phosphorus at South-west University (SUDP2). Two QTLs (H5a-SUDP2) explained 15% of the total variation for H^+. The alleles from two QTLs mapped, which contribute to increasing the phosphorus efficiency, were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects presented dominance.

Two regions located at the intervals of bnlg1325b-umc1772 (bins3.02) on chromosome 3 and P2M8/a-bnlg1839 (bins 10.07) on chromosome 10 (Table 7) were found to influence H^+ on the 21st day under normal phosphorus at Kaixian (KXNP). These QTLs (H3-KXNP and H10-KXNP) explained 19% of the total variation for H^+. The alleles from QTL mapped on chromosome 3, which contributed to increasing the phosphorus efficiency, were

Table 7 QTLs detected for H^+, DH, and RH with the $F_{2:3}$ families from the cross of 082 × Ye107

Name	C	QP	Interval markers	Closest markers	Bins	LOD	TR2 (%)	A	D	GA	Dir
H1-KXDP	1	146.7	bnlg2228-bnlg100	bnlg100	1.08	2.83	13	−0.0027	−0.002	PD	Ye107
H2a-KXDP	2	32.1	phi098-umc1165	phi098	2.02	3.45	14	0.0009	0.0056	OD	Ye107
H2b-KXDP	2	46.0	umc1185-umc1555	umc1185	2.03	2.84	22	−0.0065	0.0052	PD	Ye107
H8-KXDP	8	79.0	P3M7/b-dupssr14	dupssr14	8.09	2.95	16	−0.0021	−0.0024	D	Ye107
H1-SUDP1	1	146.7	bnlg2228-bnlg100	bnlg100	1.08	2.86	12	−0.0026	−0.0032	OD	Ye107
H1-SUDP2	1	146.7	bnlg2228-bnlg100	bnlg100	1.08	2.98	10	−0.0019	0.0074	OD	Ye107
H1-KXNP	1	146.7	bnlg2228-bnlg100	bnlg100	1.08	3.16	11	−0.0217	0.0276	OD	Ye107
H5-SUDP2	5	88.7	umc1092-phi331888	umc1092	5.04	2.86	12	0.0522	−0.0530	OD	082
H5a-SUDP2	5	16.0	bnlg1006-P2M5/d	P2M5/d	5.00	2.69	15	−0.0057	0.0051	D	Ye107
H3-KXNP	3	49.7	bnlg1325b-umc1772	bnlg1325b	3.02	2.93	12	0.0027	−0.0023	D	082
H5b-KXNP	5	88.7	umc1092-phi331888	umc1092	5.04	2.50	10	0.0020	−0.0027	OD	082
H10-KXNP	10	154.2	P2M8/a-bnlg1839	bnlg1839	10.07	3.39	7	−0.0010	0.0106	OD	Ye107
DH4-KX	4	121.6	P4M3/g-bnlg1337	bnlg1337	4.10	2.60	15	0.0013	−0.0047	OD	082
DH5-KX	5	16.0	bnlg1006-P2M5/d	P2M5/d	5.00	3.34	16	−0.0034	0.0022	PD	Ye107
DH8-KX	8	79.0	P3M7/b-dupssr14	dupssr14	8.09	3.59	11	−0.0015	−0.0022	OD	Ye107
DH1-SUDP1	1	116.0	bnlg1556-bnlg1564	bnlg1556	1.07	2.72	10	−0.0015	0.0044	OD	Ye107
DH9a-SUDP1	9	68.7	nc134-P1M3/f	P1M3/f	9.03	2.54	17	−0.0046	0.0055	D	Ye107
DH9b-SUDP1	9	86.6	P1M3/d-P1M3/g	P1M3/g	9.04	3.38	7	0.0017	0.0047	OD	082
DH9c-SUDP1	9	104.4	umc1094-bnlg1191	bnlg1191	9.06	2.68	11	−0.0024	0.0062	OD	Ye107
DH6-SUDP2	6	51.4	umc1918-umc2316	umc1918	6.03	3.23	19	0.0039	−0.0023	PD	082
RH6-KX	6	36.4	umc1572-phi389203	umc1572	6.02	3.51	9	−0.1687	0.1135	PD	Ye107
RH2-SUDP1	2	95.4	nc131-P5M1/a	nc131	2.05	2.90	6	−0.1000	0.1068	D	Ye107
RH3-SUDP1	3	207.4	umc1273-P1M4/i	umc1273	3.08	4.75	1	0.0096	−0.1437	OD	082
RH8-SUDP1	8	0.0	bnlg1252-umc1075	bnlg1252	8.00	3.08	3	−0.0691	0.1339	OD	Ye107
RH1-SUDP2	1	93.5	bnlg1023-bnlg1041	bnlg1023	1.06	3.04	23	0.1215	−0.0487	PD	082
RH3a-SUDP2	3	49.7	bnlg1325b-umc1772	bnlg1325b	3.02	2.64	19	−0.1093	0.0566	PD	Ye107
RH3b-SUDP2	3	207.4	umc1273-P1M4/i	umc1273	3.08	2.67	14	0.0093	−0.1037	OD	082
RH5a-SUDP2	5	111.1	dupssr10-umc1110b	dupssr10	5.04	3.25	19	−0.1306	0.0463	PD	Ye107
RH5b-SUDP2	5	145.9	umc1155-umc1264	umc1155	5.05	3.00	14	0.0795	0.0315	PD	082

C, chromosome; QP, QTL position; LR, likelihood ratio test statistic; R2, proportion of variance explained by QTL; A, additive; D, dominance; GA, gene action; Dir, direction.

from the higher phosphorus efficiency parental genotype 082 (P1), and the favorable alleles from the QTL mapped on chromosome 10 were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects presented dominance and overdominance.

Analysis of QTLs for DH

Three regions were found to influence DH on the 21st day at KX, which were located at the intervals of P4M3/g-bnlg1337 (bins4.10) on chromosome 4, bnlg1006-P2M5/d (bins5.00) on chromosome 5, and P3M7/b-dupssr14 (bins 8.09) on chromosome 8, respectively (Table 7). These QTLs (DH4-KX, DH5-KX, and DH8-KX) explained 42% of the total variation for DH. The alleles from one of three QTLs mapped (33%), which contributed to increasing the phosphorus efficiency, were from the higher phosphorus efficiency parental genotype 082(P1) and the favorable alleles from the two remaining QTLs mapped (67%) were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects ranged from partial dominance to overdominance; two and one QTLs presented overdominance and partial dominance, respectively. The average level of the genetic effects was overdominance.

Four regions that were found to influence DH at SU1 were located at the intervals bnlg1556-bnlg1564 (bins1.07) on chromosome 1, nc134-P1M3/f (bins9.03) on chromosome 9 (Table 7), P1M3/d-P1M3/g (bins 9.04) on chromosome 9, and umc1094-bnlg1191 (bins 9.06) on chromosome 9, respectively. These QTLs (DH1-SUDP1, DH9a-SUDP1, DH9b-SUDP1, and DH9c-SUDP1) explained 45% of the total variation for DH. The alleles from one of four QTLs mapped (25%), which contributed to increasing the phosphorus efficiency, were from the higher phosphorus efficiency parental genotype 082(P1) and the favorable alleles from the three remaining QTLs mapped (75%) were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects ranged from dominance to

overdominance. Three and one QTLs presented overdominance and dominance, respectively. The average level of the genetic effects was overdominance.

One region that influenced DH at SUDP2 was located at the interval of umc1918-umc2316 (bins 6.03) on chromosome 6 (Table 7). The QTL (DH6-SUDP2) explained 19% of the total variation for DH. The alleles from the QTL mapped, which contributed to increasing the phosphorus efficiency, were from the higher phosphorus efficiency parental genotype 082(P1). Estimates of the genetic effects presented partial dominance.

Analysis of QTLs for RH

One region that influenced RH at KX was located at the interval of umc1572-phi389203 (bins 6.02) on chromosome 6 (Table 7). The QTL (RH6-KX) explained 9% of the total variation for RH. The alleles from the QTL mapped, which contributed to increasing the phosphorus efficiency, were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects presented partial dominance.

Three regions that influenced RH at SUDP1 were located at the intervals of nc131-P5M1/a (bins 2.05) on chromosome 2 (Table 7), umc1273-P1M4/i (bins 3.08) on chromosome 3, and bnlg1252-umc1075 (bins 8.00) on chromosome 8. These QTLs (RH2-SUDP1, RH3-SUDP1, and RH8-SUDP1) explained 10% of the total variation for RH. The alleles from one of three QTLs mapped (33%), which contributed to increasing the phosphorus efficiency, were from the higher phosphorus efficiency parental genotype 082(P1) and the favorable alleles from the two remaining QTLs mapped (67%) were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects ranged from dominance to overdominance. Two and one QTLs presented overdominance and dominance, respectively. The average level of the genetic effects was partial dominance.

Five regions that influenced RH at SUDP2 were located at the intervals of bnlg1023-bnlg1041 (bin 1.06) on chromosome 1, bnlg1325b-umc1772 (bin 3.02) on chromosome 3, umc1273-P1M4/i (bin 3.08) on chromosome 3, dupssr10-umc1110b (bin 5.04) on chromosome 5, and umc1155-umc1264 (bin 5.05) on chromosome 5 (Table 7). These QTLs (RH1-SUDP2, RH3a-SUDP2, RH3b-SUDP2, RH5a-SUDP2, and RH5b-SUDP2) explained 89% of the total variation for RH. The alleles from three of five QTLs mapped (60%), which contributed to increasing the phosphorus efficiency, were from the higher phosphorus efficiency parental genotype 082(P1) and the favorable alleles from the two remaining QTLs mapped (40%) were from the lower phosphorus efficiency parental genotype Ye107(P2). Estimates of the genetic effects ranged from partial dominance to overdominance. Four and one QTLs presented partial dominance and overdominance, respectively. The average level of the genetic effects was partial dominance.

Discussion

Most loci for QTLs of all traits had a difference in the cross environments, which showed that the H^+ secretion possessed an environment-sensitive and multi-gene nature. The loci of two QTLs (RH3-SU1 and RH3b-SU2) were the same in different growth stages at South-west University; however, the QTL for RH was not detected at the same locus at KX. The QTL × environment interaction actually reflected the H^+ secretion. QTL effects for phenotypic traits were largely dependent on the environments and the presence of significant genotype × environment interaction. In the present study, the epistatic interaction also existed; different QTLs could mask the effects of each other due to the epistatic interaction (data not shown). Since traits H^+, DH, and RH were not correlated with each other, the QTLs of the three traits had no co-locations or clusters, while QTL for PUE and H^+ had a co-location (Tuberosa et al., 2003; Chen et al., 2009).

A total of 31 QTLs were identified as being associated with traits (H^+, DH, and RH); however, two of them, located within the intervals of bnlg2228-bnlg100 (bin 1.08) on chromosome 1 and umc1092-phi331888 (bins5.04) on chromosome 5, were commonly detected in all experimental conditions, while others were only detected in one P level, one site, or one growth stage.

The interactions of QTLs for H^+ at experimental sites and growth stages, especially in the treatment in different phosphorus levels, were observed in our study. Of the several QTL × environment interactions, only two regions were free of interactions from the detection of a significant QTL in all environments (cross phosphorus levels, sites, and growth stages), while the remaining interactions resulted from differential effects within the two phosphorus levels, two sites, or growth stages. Maize is unusual among major crops grown in that large acreages under both deficient phosphorus and normal phosphorus conditions, making QTL × environment interactions even more important than usual in crop improvement.

Understanding QTL × environment interactions is important not only for understanding the genetic traits of phosphorus absorption but also for practically improving the phosphorus efficiency. QTL × environment interactions can affect root H^+ secretion challenges in the improvement of maize, such as phosphorus efficiency. It is accepted that some genotypes are better suited to the deficient phosphorus and others to the normal phosphorus; it is important to study and manipulate the specific genes that confer adaptation to these very different environments.

Through comparing the QTLs between normal and deficient conditions or using the ratios (data under P-deficient/normal conditions), the putative QTL only related to P utilization efficiency can be detected and used for genetic improvement. One region was found to influence H^+ at the interval bnlg2228-bnlg100 (bin 1.08) on chromosome 1,

which was detected in all environments, i.e., KXDP, KXNP, SUDP1, and SUDP2. It was across phosphorus levels, sites, and growth stages. The region was relative stable QTL and might be the candidate QTL for improvement phosphorus efficiency. It showed that two parents contributed to increasing the H^+, indicating that lower phosphorus efficiency parental genotype Ye107 (P2) can also be utilized to improve the phosphorus efficiency in maize.

In our present study, one important genomic region was detected at the interval of bnlg2228-bnlg100 (bin 1.08) on chromosome 1. There exist largely coincident QTLs in all cross environments. The QTLs detected in more than one phosphorus levels or detected in more than one environment prove useful for marker-assisted selection. Therefore, the interval of bnlg2228-bnlg100 (bin 1.08) on chromosome 1 is greatly important to MAS for improving the phosphorus efficiency in maize.

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