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RESEARCH ARTICLE

Identification of QTLs for biomass production in maize (*Zea mays* L.) under different phosphorus levels at two sites

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Abstract **The biomass production (BP), the leaf age (LA), and the plant height (PH) as well as the quantitative trait loci (QTLs) associated with these traits were determined for $F_{2:3}$ population derived from the cross of two contrasting maize (*Zea mays* L.) genotypes: 082 and Ye107. By using composite interval mapping, a total of 12 and 12 distinct QTLs were identified at Kaixian and Southwest University under deficient phosphorus. Another 9 and 8 distinct QTLs were identified at two sites under normal phosphorus, respectively. Seven coincident QTLs for two traits (BP and LA) were detected in the interval bnlg1832-P2M8/j (bin 1.05) on Chromosome 1, and four consistent QTLs for one trait (PH) were coincident in the interval umc1102-P1M7/d (bin 3.05) on Chromosome 3. These coincident QTLs in two important genomic regions were identified under different phosphorus levels and two different environments. Therefore, the above two segments one (bnlg1832-P2M8/j) identified in Chromosome 1 and the other (umc1102-P1M7/d) identified in Chromosome 3 may be used in future for marker-assisted selection and high-resolution mapping leading to map-based cloning of QTLs for agronomically important traits under phosphorus deficiency.**

Keywords maize, QTL analysis, biomass production, leaf age, plant height

Introduction

Phosphorus is the least mobile and available to maize in most soil conditions. It is therefore frequently a major or even the prime constraint factor for maize growth. Recently, genetic mapping has been used in rice to tag quantitative trait loci (QTLs) for root traits and to dissect their relationship to abiotic stresses. Ni et al. (1998) reported that rice tolerance to low-phosphorus stress was controlled by a major QTL located on Chromosome 12 and some QTLs with relatively small effects on other chromosomes. QTLs were mapped of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean (Fredeen et al., 1989; Yan et al., 2004). The QTLs were detected of lateral rooting to phosphorus acquisition efficiency in maize seedlings (Kiniry et al., 1989; Gavito and Miller, 1998; Zhu and Lynch, 2004). QTLs of controlling root hair length were mapped in maize under phosphorus deficiency (Rogers et al., 1989; Lynch et al., 1991; Rodriguez et al., 1998a, 1998b, 1998c; Zhu et al., 2005). Using the technique of genetic map, QTLs under phosphorus stress can be detected by closely linked molecular markers that may be employed as marker-assisted selection (MAS) (Whitehead et al., 1981; Tadano et al., 1993; Steen, 1998; Silber et al., 2003; Chen et al., 2010).

The biomass production (BP) as an important index to reflect maize plant growth can be generally reduced under deficient phosphorus. Plenet et al. (2000) reported that crop growth was mainly reduced at the early stages. For several species, phosphorus deficiency was shown to severely reduce leaf growth, subsequently the amount of BP by plants and the leaf area index of a maize crop grown under temperate condition (El-Hamdi et al., 1995; Rodriguez et al., 1998a; Plénet et al., 2000). BP reflects the phosphorus utilization of

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the plant. Among the candidate traits for BP, the leaf age (LA) is believed to be very important and related to absorbing solar radiation and photosynthesis as selection parameters (Pellet et al., 1993; Colomb et al., 2000). Another trait possibly related to BP may be plant height (PH). The high PH may be favorable for plant architecture and spatial distribution of leaf (Foyer and Spencer, 1986; Plenet et al., 2000; Plenet, 2000). Consequently, the leaf can absorb solar radiation to be relatively more increasing for photosynthesis by plants (El-Hamdi et al., 1995; Plenet et al., 2000).

Genetic difference in response to deficient phosphorus has been reported in maize for BP (Plenet1, 2000; Rodriguez, 1993). A 3- to 5-fold variation in biomass accumulation has been detected among maize inbred lines after 6 weeks of growth in a low-phosphorus soil D. (Plenet et al., 2000; Rodriguez, 1993). The study of response characteristic to deficient phosphorus in maize was focused on QTL of root traits (Zhu and Lynch, 2004; Chen et al., 2008; 2009). However, there is less study on QTLs of BP. Molecular markers can be used to study the inheritance of BP, LA, and PH under phosphorus deficiency and identify specific loci associated with the expression of these traits (Roberto et al., 2003; Yan et al., 2004; Zhu and Lynch, 2004). The markers shown to be linked to specific genes may be used to facilitate the selection of desired genotypes through MAS (Yan et al., 2004; Zhu and Lynch, 2004; Zhu et al., 2005).

The objectives of our study were to detect QTLs for BP of plants, LA, and PH under different phosphorus levels at two sites, contrast the QTLs of root traits in previous studies, and analyze the common regions of QTLs for multi-traits at two sites, which may be useful for improving BP under phosphorus deficiency by means of MAS.

Materials and methods

Plant material

The mapping population included 241 $F_{2:3}$ families derived from a corresponding number of randomly chosen F_2 plants of the cross between inbreds 082 (phosphorus deficiency tolerant) and Ye107 (phosphorus deficiency susceptible), which were known to differ in phosphorus efficiency and root traits. The F_2 families were reproduced in 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 field experiment was conducted using randomized block design with three replications. $F_{2:3}$ families along with F_1 and both parent lines were sown. A set of experiments were conducted at Kaixian (KX) (31°11′N, 98°58′E; 1000 m altitude) and Southwest University (SU) (29°48′N, 106°33′E; 150 m altitude) during September to October 2007. For the Kaixian 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; the organic matter was 2.99 g/kg and the pH was 8.2. For the Southwest University soil, the contents of total N, P, and K and available N, P, and K were 0.259, 0.602, and 17.8g/kg and 14.1, 2.6, and 121 mg/kg, respectively, with 3.43 g/kg organic matter and the pH was 7.9.

Fertilizers were applied before sowing at a rate of 120 kg N and 50 kg P per hectare under normal phosphorus application but only applied at a rate of 120 kg N under deficient phosphorus application. After applying fertilizers, the available N was 21.2 mg/kg at Kaixian and 21.7 mg/kg at Southwest University. The available K remained the same as before. The normal soil available phosphorus content was 20 and 21.2 mg/kg at Kaixian and Southwest University respectively, while the deficient soil available phosphorus content was still the same as before. The area per split plot was 2.5 m^2 with 10 plants in each plot. Cultivation was carried out following the standard practices.

Measurement of biological traits

The plants were harvested on the 21st day after emergence of seedlings. The PH was measured and the LA was recorded before harvesting of plant. The above-ground biomass weight and the root dry weight were determined to obtain the BP after plants were deactivated at 105°C for 30 min and dried at 80°C for 72 h.

Statistical analysis of the data

Arithmetic means of three replicates were calculated for each trait of each $F_{2:3}$ families. 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. The heritability of traits for the $F_{2:3}$ families was estimated by 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 was used to identify QTLs and estimate their effects. QTL mapping was performed with the software program Windows QTL Cartographer version 2.5. Parameters for forward regression analysis consisted of a window size of 10 cM, a walking speed of 2 cM, five background control markers, and probability thresholds of 0.05 each for the partial *F*-test for both marker inclusion and exclusion. Significance threshold for QTL detection was calculated by 1000 random permutations 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, which were classified to additive (0–0.20), partial dominant (0.21–0.80), dominant (0.81–1.20), and over dominant (>1.20) according to Stuber et al. (1987) and Tuberosa et al. (1998).

Results

Segregation distortion

In $F_{2:3}$ populations, the majority of markers fitted the expected 1:2:1 to co-dominance markers and 1:3 to dominance markers ($P<0.01$). However, significant deviation from expected segregation was observed for 69 loci (Fig. 1). Distorted loci were widely distributed in all chromosomes.

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

At the two studied sites, the two parental genotypes, 082 and Ye107, differed significantly in BP, LA, and PH. 082 had higher values than Ye107 for these traits (Table 1). All traits 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 QTL mapping. Analysis of variance (ANOVA) was employed 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 different traits varied from 64.3% to 81.2% (Table 1). The heritability of the traits was generally high ($>60\%$).

Analysis of QTLs associated with traits under phosphorus deficiency

A total of 12 distinct QTLs were identified at Kaixian: five for BP, three for LA, and four for PH. A total of 12 distinct QTLs were identified for Southwest University: three for BP, six for LA, and three for PH (Table 2).

Identification of overlap regions under phosphorus deficiency

Two regions were found to influence BP at the two sites, which were located in the interval bnlg1832–P2M8/j (bins 1.05) and mmc0041–umc1013 (bins1.08) on Chromosome 1 (Table 2). Two QTLs on Chromosome 1 explained 37% to 41% of the total phenotypic variance of BP. The alleles from the QTL in bnlg1832–P2M8/j, which contribute to increase the BP, were from the lower phosphorus efficiency parental genotype Ye107 (P_2). The alleles from the QTL in mmc0041–umc1013, which contribute to increase the BP, were from the higher phosphorus efficiency parental genotype 082 (P_1). Estimates of the genetic effects both presented partial dominance.

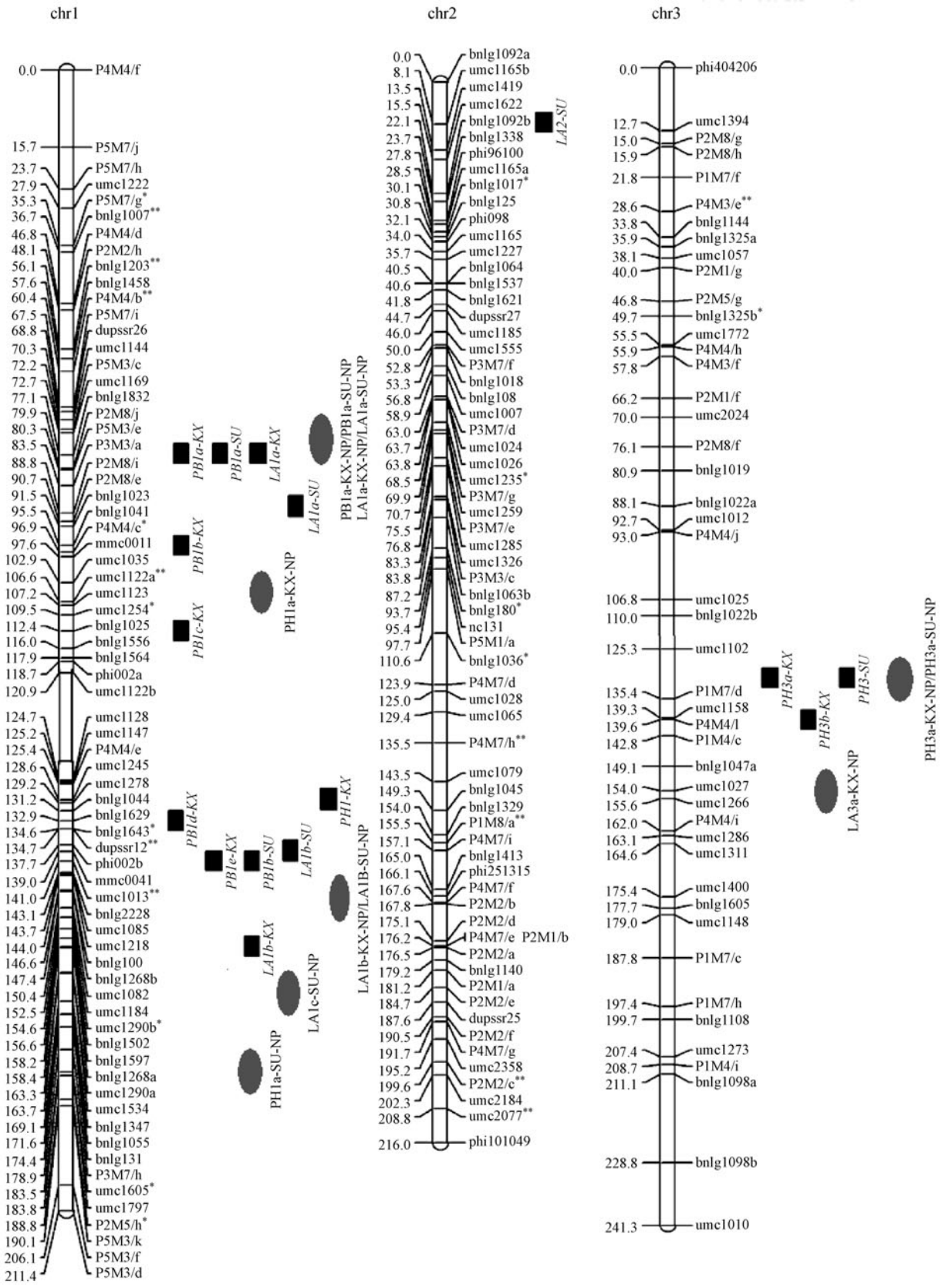
Two regions were found to influence PH at two sites located in the interval umc1102–P1M7/d (bins 3.05) on Chromosome 3 and umc1279–umc1033 (bins9.01) on Chromosome 9 (Table 2). Two QTLs on Chromosomes 3 and 9 explained 35% to 38% of the total phenotypic variance of PH. The alleles from two QTLs, which contribute to increase the PH, were from the lower phosphorus efficiency parental genotype Ye107 (P_2). Estimates of the genetic effects presented dominance and over-dominance.

Identification of distinct loci for the same QTLs at different sites under phosphorus deficiency

Three QTLs for BP in the intervals of bnlg1023–bnlg1041 (bins 1.06), bnlg1025–bnlg1556 (bins 1.07), and bnlg1044–bnlg1629 (bins 1.07) were observed on Chromosome 1 at Kaixian (Table 2). These QTLs explained 56% of the total phenotypic variance for BP. The alleles from 3 QTLs mapped on Chromosome 1, which contribute to increase the BP, were from the higher phosphorus efficiency parental genotype 082 (P_1). Estimates of the three genetic effects presented additive, additive, and partial dominance, respectively. The different loci for QTL of BP in the interval P3M8/a–bnlg1305 (bins 7.03) on Chromosome 7 were detected at Southwest University. The QTL explained 17% of total phenotypic

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	Difference	Mean	Range	$\hat{\sigma}_G^2$	$\hat{\sigma}_E^2$	h_b^2/%
at Kaixian								
BP-KX	8.54	5.52	3.02	7.64	4.68–10.96	1.42	0.33	81.2
LA-KX	4.8	3.3	1.5	3.72	2.1–5.8	1.14	0.31	78.8
PH-KX	30.4	17.2	13.2	26.21	16.3–34.4	11.92	6.62	64.3
at Southwest University								
BP-SU	8.58	6.23	2.35	7.54	5.72–10.46	0.64	0.15	80.7
LA-SU	4.7	3.3	1.4	4.39	2.3–5.8	0.71	0.22	76.7
PH-SU	37.2	24.5	12.7	33.83	23.1–41.4	11.78	6.04	66.1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15.7 P5M7/j
23.7 P5M7/h
27.9 umc1222
35.3 P5M7/g*
36.7 bnlg1007**
46.8 P4M4/d
48.1 P2M2/h
56.1 bnlg1203**
57.6 bnlg1458
60.4 P4M4/b**
67.5 P5M7/i
68.8 dupssr26
70.3 umc1144
72.2 P5M3/c
72.7 umc1169
77.1 bnlg1832
79.9 P2M8/j
80.3 P5M3/e
83.5 P3M3/a
88.8 P2M8/i
90.7 P2M8/e
91.5 bnlg1023
95.5 bnlg1041
96.9 P4M4/c*
97.6 mmc0011
102.9 umc1035
106.6 umc1122a**
107.2 umc1123
109.5 umc1254*
112.4 bnlg1025
116.0 bnlg1556
117.9 bnlg1564
118.7 phi002a
120.9 umc1122b
124.7 umc1128
125.2 umc1147
125.4 P4M4/e
128.6 umc1245
129.2 umc1278
131.2 bnlg1044
132.9 bnlg1629
134.6 bnlg1643*
134.7 dupssr12**
137.7 phi002b
139.0 mmc0041
141.0 umc1013**
143.1 bnlg2228
143.7 umc1085
144.0 umc1218
146.6 bnlg100
147.4 bnlg1268b
150.4 umc1082
152.5 umc1184
154.6 umc1290b*
156.6 bnlg1502
158.2 bnlg1597
158.4 bnlg1268a
163.3 umc1290a
163.7 umc1534
169.1 bnlg1347
171.6 bnlg1055
174.4 bnlg131
178.9 P3M7/h
183.5 umc1605*
183.8 umc1797
188.8 P2M5/h*
190.1 P5M3/k
206.1 P5M3/f
211.4 P5M3/d
PB1a-KX
PB1a-SU
LA1a-KX
LA1a-SU
PB1a-KX-NP/PB1a-SU-NP
LA1a-KX-NP/LA1a-SU-NP
PB1b-KX
PH1a-KX-NP
PB1c-KX
PB1d-KX
PB1e-KX
PB1b-SU
LA1b-SU
PH1-KX
LA1b-KX-NP/LA1B-SU-NP
LA1b-KX
LA1c-SU-NP
PH1a-SU-NP
chr2
0.0 bnlg1092a
8.1 umc1165b
13.5 umc1419
15.5 umc1622
22.1 bnlg1092b
23.7 bnlg1338
27.8 phi96100
28.5 umc1165a
30.1 bnlg1017*
30.8 bnlg125
32.1 phi098
34.0 umc1165
35.7 umc1227
40.5 bnlg1064
40.6 bnlg1537
41.8 bnlg1621
44.7 dupssr27
46.0 umc1185
50.0 umc1555
52.8 P3M7/f
53.3 bnlg1018
56.8 bnlg108
58.9 umc1007
63.0 P3M7/d
63.7 umc1024
63.8 umc1026
68.5 umc1235*
69.9 P3M7/g
70.7 umc1259
75.5 P3M7/e
76.8 umc1285
83.3 umc1326
83.8 P3M3/c
87.2 bnlg1063b
93.7 bnlg180*
95.4 nc131
97.7 P5M1/a
110.6 bnlg1036*
123.9 P4M7/d
125.0 umc1028
129.4 umc1065
135.5 P4M7/h**
143.5 umc1079
149.3 bnlg1045
154.0 bnlg1329
155.5 P1M8/a**
157.1 P4M7/i
165.0 bnlg1413
166.1 phi251315
167.6 P4M7/f
167.8 P2M2/b
175.1 P2M2/d
176.2 P4M7/e P2M1/b
176.5 P2M2/a
179.2 bnlg1140
181.2 P2M1/a
184.7 P2M2/e
187.6 dupssr25
190.5 P2M2/f
191.7 P4M7/g
195.2 umc2358
199.6 P2M2/c**
202.3 umc2184
208.8 umc2077**
216.0 phi101049
LA2-SU
chr3
0.0 phi404206
12.7 umc1394
15.0 P2M8/g
15.9 P2M8/h
21.8 P1M7/f
28.6 P4M3/e**
33.8 bnlg1144
35.9 bnlg1325a
38.1 umc1057
40.0 P2M1/g
46.8 P2M5/g
49.7 bnlg1325b*
55.5 umc1772
55.9 P4M4/h
57.8 P4M3/f
66.2 P2M1/f
70.0 umc2024
76.1 P2M8/f
80.9 bnlg1019
88.1 bnlg1022a
92.7 umc1012
93.0 P4M4/j
106.8 umc1025
110.0 bnlg1022b
125.3 umc1102
135.4 P1M7/d
139.3 umc1158
139.6 P4M4/l
142.8 P1M4/c
149.1 bnlg1047a
154.0 umc1027
155.6 umc1266
162.0 P4M4/i
163.1 umc1286
164.6 umc1311
175.4 umc1400
177.7 bnlg1605
179.0 umc1148
187.8 P1M7/c
197.4 P1M7/h
199.7 bnlg1108
207.4 umc1273
208.7 P1M4/i
211.1 bnlg1098a
228.8 bnlg1098b
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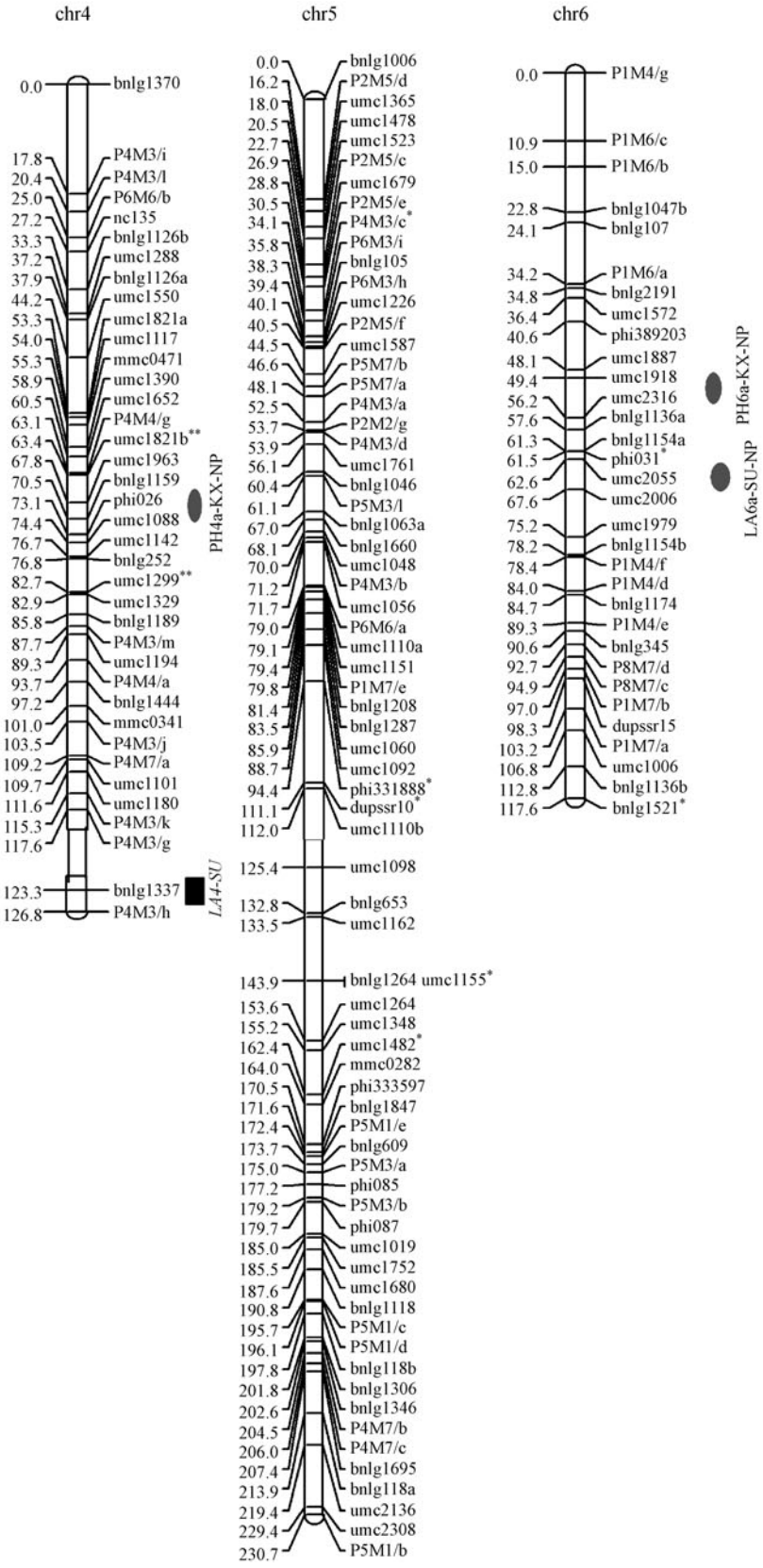
PH3b-KX
PH3-SU
PH3a-KX-NP/PH3a-SU-NP
LA3a-KX-NP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37.2 umc1288
37.9 bnlg1126a
44.2 umc1550
53.3 umc1821a
54.0 umc1117
55.3 mmc0471
58.9 umc1390
60.5 umc1652
63.1 P4M4/g
63.4 umc1821b**
67.8 umc1963
70.5 bnlg1159
73.1 phi026
74.4 umc1088
76.7 umc1142
76.8 bnlg252
82.7 umc1299**
82.9 umc1329
85.8 bnlg1189
87.7 P4M3/m
89.3 umc1194
93.7 P4M4/a
97.2 bnlg1444
101.0 mmc0341
103.5 P4M3/j
109.2 P4M7/a
109.7 umc1101
111.6 umc1180
115.3 P4M3/k
117.6 P4M3/g
123.3 bnlg1337
126.8 P4M3/h
PH4a-KX-NP
LA4-SU
chr5
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16.2 P2M5/d
18.0 umc1365
20.5 umc1478
22.7 umc1523
26.9 P2M5/c
28.8 umc1679
30.5 P2M5/e*
34.1 P4M3/c*
35.8 P6M3/i
38.3 bnlg105
39.4 P6M3/h
40.1 umc1226
40.5 P2M5/f
44.5 umc1587
46.6 P5M7/b
48.1 P5M7/a
52.5 P4M3/a
53.7 P2M2/g
53.9 P4M3/d
56.1 umc1761
60.4 bnlg1046
61.1 P5M3/l
67.0 bnlg1063a
68.1 bnlg1660
70.0 umc1048
71.2 P4M3/b
71.7 umc1056
79.0 P6M6/a
79.1 umc1110a
79.4 umc1151
79.8 P1M7/e
81.4 bnlg1208
83.5 bnlg1287
85.9 umc1060
88.7 umc1092
94.4 phi331888*
111.1 dupssr10*
112.0 umc1110b
125.4 umc1098
132.8 bnlg653
133.5 umc1162
143.9 bnlg1264 umc1155*
153.6 umc1264
155.2 umc1348
162.4 umc1482*
164.0 mmc0282
170.5 phi333597
171.6 bnlg1847
172.4 P5M1/e
173.7 bnlg609
175.0 P5M3/a
177.2 phi085
179.2 P5M3/b
179.7 phi087
185.0 umc1019
185.5 umc1752
187.6 umc1680
190.8 bnlg1118
195.7 P5M1/c
196.1 P5M1/d
197.8 bnlg118b
201.8 bnlg1306
202.6 bnlg1346
204.5 P4M7/b
206.0 P4M7/c
207.4 bnlg1695
213.9 bnlg118a
219.4 umc2136
229.4 umc2308
230.7 P5M1/b
chr6
0.0 P1M4/g
10.9 P1M6/c
15.0 P1M6/b
22.8 bnlg1047b
24.1 bnlg107
34.2 P1M6/a
34.8 bnlg2191
36.4 umc1572
40.6 phi389203
48.1 umc1887
49.4 umc1918
56.2 umc2316
57.6 bnlg1136a
61.3 bnlg1154a
61.5 phi031*
62.6 umc2055
67.6 umc2006
75.2 umc1979
78.2 bnlg1154b
78.4 P1M4/f
84.0 P1M4/d
84.7 bnlg1174
89.3 P1M4/e
90.6 bnlg345
92.7 P8M7/d
94.9 P8M7/c
97.0 P1M7/b
98.3 dupssr15
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PH6a-KX-NP
LA6a-SU-NP

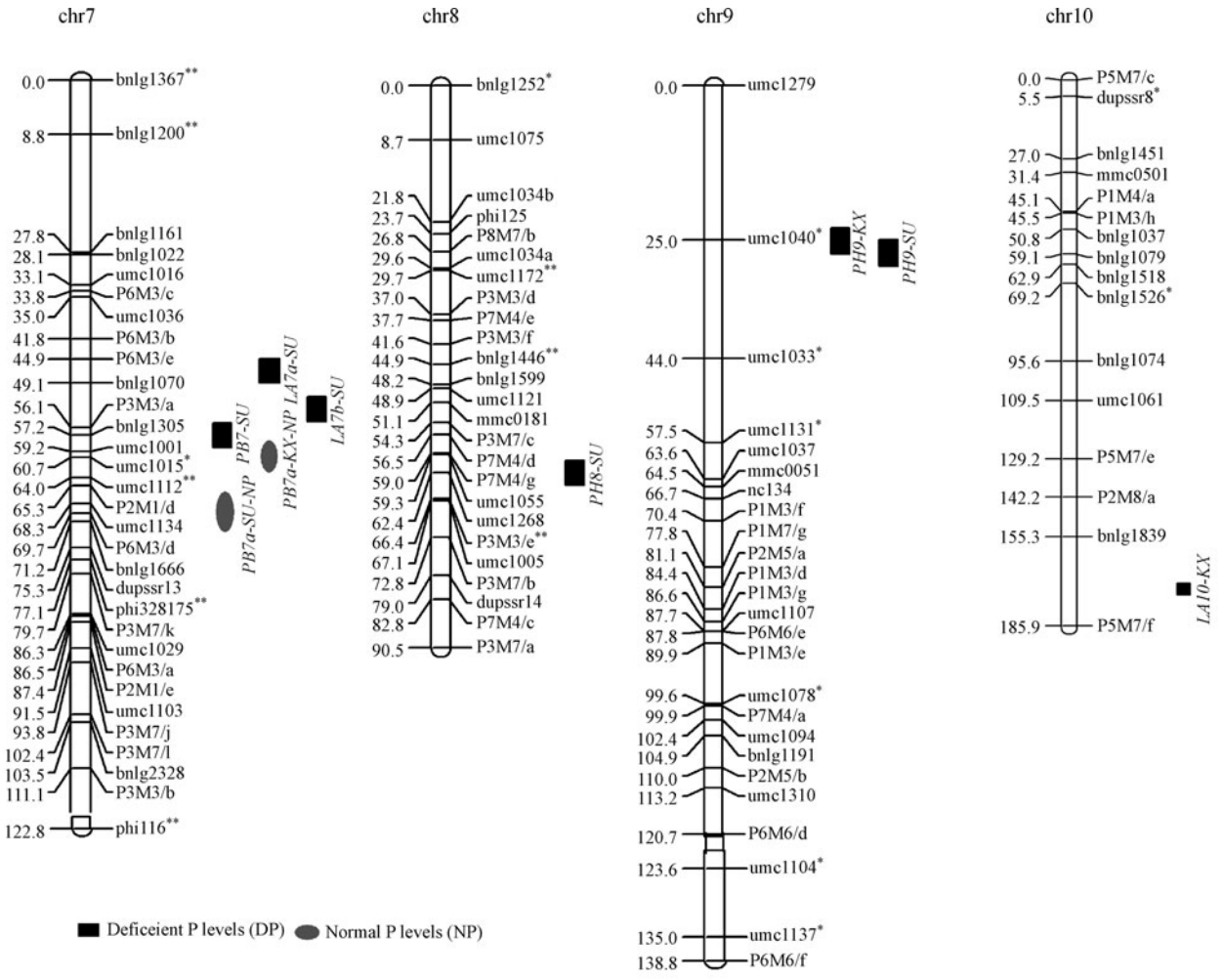

Figure 1 Linkage map of QTLs conferring BP, LA, and PH. Note: * and ** denote significant deviation at 5% and 1% level, respectively.

variance for BP. The alleles from the QTL mapped on Chromosome 7, which contribute to increase the BP, were from the higher phosphorus efficiency parental genotype 082 (P_1). Estimates of the genetic effects presented partial dominance.

Three QTLs for LA in the intervals of bnlg1832–P2M8/j (bins 1.05), bnlg1597–bnlg1268a (bins 1.09), and bnlg1839–P5M7/f (bins 10.07) were observed on Chromosome 1, Chromosome 1, and Chromosome 10, respectively, at Kaixian (Table 2). These QTLs explained 25% of the total phenotypic variance for LA. The alleles from QTL in the interval bnlg1832–P2M8/j (bins 1.05), which contribute to increase the LA, were from the lower phosphorus efficiency parental genotype Ye107 (P_2). The favorable alleles from the other two QTLs (bins 1.09 and 10.07) were from the higher phosphorus efficiency parental genotype 082 (P_1). Estimates of the genetic effects presented additive, partial dominance, and over dominance, respectively.

Six QTLs for LA were observed at Southwest University, which were located in the intervals of P3M3/a-P2M8/i (bins 1.05) on Chromosome 1, mmc0041-umc1013 (bins 1.08) on Chromosome 1, umc1165b-umc1419 (bins 2.00) on Chromosome 2, P4M3/g–bnlg1337 (bins 4.10) on Chromosome 4, P6M3/e–bnlg1070 (bins 7.03) on Chromosome 7, and bnlg1070–P3M8/a (bins 7.03) on Chromosome 7, respectively (Table 2). These QTLs explained 94% of the total phenotypic variance for LA. The alleles increasing LA from QTLs (bins 1.05 and 2.00) were from the lower phosphorus efficiency parental genotype Ye107 (P_2). The favorable alleles from other four QTLs (bins 1.08, 4.10, 7.03, and 7.03) were from the higher phosphorus efficiency parental genotype 082 (P_1). The estimates of the genetic effects of the six QTLs presented additive, partial dominance, dominance, over dominance, over dominance, and over dominance effects, respectively.

Two QTLs for PH were observed at Kaixian in the intervals of umc1245–umc1278 (bins 1.07) on Chromosome 1 and umc1158–P4M4/l (bins 3.05) on Chromosome 3 (Table 2). These QTLs explained 43% of the total phenotypic variance for PH. The alleles from the QTL mapped on Chromosome 1,

Table 2 QTLs detected for BP, LA, and PH with the $F_{2:3}$ families from the cross of 082 × Ye107 under deficient phosphorus

Names	C	QP	Interval markers	Closest markers	Bins	LOD	R^2 (%)	TR^2 (%)	A	D	GA	Dir
BP1a-KX	1	77.1	bnlg1832–P2M8/j	bnlg1832	1.05	3.35	9	20	−0.5051	0.3348	PD	Ye107
BP1b-KX	1	95.5	bnlg1023–bnlg1041	bnlg1041	1.06	2.85	5	20	0.4407	−0.0291	A	082
BP1c-KX	1	112.5	bnlg1025–bnlg1556	bnlg1025	1.07	2.57	4	18	0.3777	0.0298	A	082
BP1d-KX	1	132.9	bnlg1044–bnlg1629	bnlg1629	1.07	3.78	9	18	0.5502	−0.3035	PD	082
BP1e-KX	1	141.0	mmc0041–umc1013	umc1013	1.08	4.67	12	21	0.6536	−0.3925	PD	082
BP1a-SU	1	77.1	bnlg1832–P2M8/j	bnlg1832	1.05	3	8	19	−0.3269	0.197	PD	Ye107
BP1b-SU	1	141.0	mmc0041–umc1013	umc1013	1.08	3.68	9	18	0.3906	−0.23	PD	082
BP7-SU	7	57.2	P3M8/a–bnlg1305	P3M8/a	7.03	2.61	7	17	0.3106	−0.1421	PD	082
LA1a-KX	1	77.1	bnlg1832–P2M8/j	bnlg1832	1.05	2.62	4	6	−0.315	−0.0517	A	Ye107
LA1b-KX	1	158.2	bnlg1597–bnlg1268a	bnlg1597	1.09	3.71	6	12	0.3636	−0.5732	OD	082
LA10-KX	10	173.3	bnlg1839–P5M7/f	P5M7/f	10.07	10.94	0	7	0.0239	1.9504	OD	082
LA1a-SU	1	87.5	P3M3/a–P2M8/i	P2M8/i	1.05	3.07	7	17	−0.3093	0.0325	A	Ye107
LA1b-SU	1	139.0	mmc0041–umc1013	mmc0041	1.08	2.54	2	12	0.1883	0.107	PD	082
LA2-SU	2	8.1	umc1165b–umc1419	umc1165b	2.00	3.53	8	19	−0.3472	0.3016	D	Ye107
LA4-SU	4	123.3	P4M3/g–bnlg1337	bnlg1337	4.10	2.81	3	16	0.2104	−0.3562	OD	082
LA7a-SU	7	47.0	P6M3/e–bnlg1070	bnlg1070	7.03	2.58	3	16	0.1982	0.2384	OD	082
LA7b-SU	7	53.1	bnlg1070–P3M8/a	P3M8/a	7.03	3.31	1	14	0.1429	0.2287	OD	082
PH1-KX	1	128.6	umc1245–umc1278	umc1245	1.07	2.57	5	19	1.1318	−0.3201	PD	082
PH3a-KX	3	131.3	umc1102–P1M7/d	P1M7/d	3.05	3.08	9	23	−1.4165	1.4513	D	Ye107
PH3b-KX	3	139.6	umc1158–P4M4/l	P4M4/l	3.05	3.88	9	24	−1.4758	1.3721	D	Ye107
PH9-KX	9	25.0	umc1279–umc1033	umc1040	9.01	3.74	2	15	−0.7027	−0.8912	OD	Ye107
PH3-SU	3	131.3	umc1102–P1M7/d	P1M7/d	3.05	3.09	8	22	−1.3495	1.6192	D	Ye107
PH8-SU	8	62.4	umc1268–P3M3/e	umc1268	8.07	3.84	7	18	−1.2678	0.2641	PD	Ye107
PH9-SU	9	27.0	umc1279–umc1033	umc1040	9.01	4.24	2	13	−0.8089	−1.0771	OD	Ye107

which contribute to increasing the PH, were from the higher phosphorus efficiency parental genotype 082 (P_1). The favorable alleles from the QTL mapped on Chromosome 3 were from the lower phosphorus efficiency parental genotype Ye107 (P_2). The estimates of the genetic effects presented partial dominance and dominance. One QTL for PH was observed at Southwest University in the interval umc1268–P3M3/e (bins8.07) on Chromosome 8. The QTL explained 18% of total phenotypic variance for PH. The alleles from the QTL, which contribute to increasing the PH, were from the lower phosphorus efficiency parental genotype Ye107 (P_2) with partial dominance effect.

Analysis of QTLs associated with traits under normal phosphorus

A total of nine distinct QTLs were identified at Kaixian: two for BP, three for LA, and four for PH. A total of eight distinct QTLs were identified for Southwest University: one for BP, five for LA, and two for PH (Table 2).

Identification of overlap regions under normal phosphorus

One region was found to influence BP at two sites located in the interval bnlg1832–P2M8/j (bins 1.05) on Chromosome 1 (Table 3). The QTLs on Chromosome 1 explained 20% to 21% of the total phenotypic variance of BP. The alleles from the QTL, which contribute to increasing the BP, were from the lower phosphorus efficiency parental genotype Ye107 (P_2), with partial dominance effect.

One region was found to influence LA at the two sites located in the interval bnlg1832–P2M8/j (bins 1.05) on Chromosome 1 (Table 3). The QTLs on Chromosome 1 explained 9% to 14% of the total phenotypic variance of LA. The alleles from the QTL, which contribute to increasing the LA, were from the lower phosphorus efficiency parental genotype Ye107 (P_2) with additive. Another region was found to influence LA at two sites located in the interval bnlg2228–umc1085 (bins 1.09) on Chromosome 1. The QTLs on Chromosome 1 explained 10% to 15% of the total phenotypic variance of LA. The alleles from the QTL, which contribute to increasing the LA, were from the higher phosphorus efficiency parental genotype 082 (P_1), with partial dominance and over dominance effects.

One region was found to influence PH at the two sites located in the interval umc1102–P1M7/d (bins 3.03) on Chromosome 3 (Table 3). The QTL explained 20% to 21% of

Table 3 QTLs detected for BP, LA, and PH with the $F_{2:3}$ families from the cross of 082 × Ye107 under normal phosphorus

Names	C	QP	Interval markers	Closest markers	Bins	LOD	R^2 (%)	TR^2 (%)	A	D	GA	Dir
BP1a-KX-NP	1	77.1	bnlg1832-P2M8/j	bnlg1832	1.05	3.00	15	21	−0.4321	0.3067	PD	Ye107
BP1a-SU-NP	1	77.1	bnlg1832-P2M8/j	bnlg1832	1.05	3.10	9	20	−0.4208	0.234	PD	Ye107
BP7a-KX-NP	7	60.3	umc1001-umc1015	umc1001	7.03	2.75	8	18	0.3106	−0.1421	PD	082
LA1a-KX-NP	1	77.1	bnlg1832-P2M8/j	bnlg1832	1.05	2.66	8	9	−0.435	−0.1217	A	Ye107
LA1b-KX-NP	1	143.5	bnlg2228-umc1085	umc1085	1.09	3.55	8	15	0.3336	−0.3453	OD	082
LA3a-KX-NP	3	155.4	umc1027-umc1266	umc1027	3.06	3.55	8	12	0.0198	1.9664	OD	082
LA1a-SU-NP	1	77.1	bnlg1832-P2M8/j	bnlg1832	1.05	3.15	9	14	−0.2985	0.1221	A	Ye107
LA1b-SU-NP	1	143.5	bnlg2228-umc1085	umc1085	1.09	2.88	6	10	0.4883	0.117	PD	082
LA1c-SU-NP	1	163.4	umc1290a-umc1534	umc1534	1.09	3.45	9	6	−0.3435	0.3005	D	Ye107
LA6a-SU-NP	6	63.3	umc2055-umc2006	umc2055	6.05	2.81	3	16	0.2135	−0.3542	OD	082
LA7a-SU-NP	7	68.8	umc1134-P6M3/d	umc1134	7.05	2.68	3	16	0.1982	0.2384	OD	082
PH1a-KX-NP	1	103.8	umc1035-umc1122a	umc1035	1.07	2.85	7	18	1.0225	−0.2201	PD	082
PH1a-SU-NP	1	172.5	bnlg1055-bnlg131	bnlg131	1.10	3.25	9	20	−1.4008	1.4335	D	Ye107
PH3a-KX-NP	3	131.3	umc1102-P1M7/d	P1M7/d	3.05	3.50	9	21	−1.4855	1.3656	D	Ye107
PH4a-KX-NP	4	68.5	umc1963-bnlg1159	umc1963	4.05	3.54	2	15	−0.6232	−0.8768	OD	Ye107
PH3a-SU-NP	3	131.3	umc1102-P1M7/d	P1M7/d	3.05	3.09	10	20	−1.4436	1.5563	D	Ye107
PH6a-KX-NP	6	52.4	umc1918-umc2316	umc2316	6.04	3.25	7	18	−1.1856	0. 2547	PD	Ye107

the total phenotypic variance of PH. The alleles from the QTL, which contribute to increasing the PH, were from the lower phosphorus efficiency parental genotype Ye107 (P_2), with dominance effect.

Identification of distinct loci for same QTLs at different sites under normal phosphorus

One QTL for BP was observed on Chromosome 7 at Kaixian in the interval umc1001–umc1015 (bins 7.03) (Table 3). The QTLs explained 18% of the total phenotypic variance for BP. The alleles from the QTL mapped on Chromosome 7, which contribute to increasing the BP, were from the higher phosphorus efficiency parental genotype 082 (P_1) with partial dominance effect.

One QTL for LA was observed at Kaixian in the interval umc1027–umc1266 (bins 3.06) on Chromosome 3 (Table 3). The QTLs explained 12% of the total phenotypic variance for LA. The alleles from the QTL, which contribute to increasing the LA, were from the higher phosphorus efficiency parental genotype 082 (P_1) with over dominance. Three QTLs for LA were observed at Southwest University in the intervals of umc1290a–umc1534 (bins 1.09) on Chromosome 1, umc2055–umc2006 (bins 6.05) on Chromosome 6, and umc1134–P6M3/d (bins 7.05) on Chromosome 7. These QTLs explained 38% of the total phenotypic variance for LA. The alleles from QTL mapped on Chromosome 1, which contribute to increasing the LA, were from the lower phosphorus efficiency parental genotype Ye107 (P_2) and the alleles from QTLs mapped on Chromosomes 6 and 7, which contribute to increasing the LA, were from the higher phosphorus efficiency parental genotype 082 (P_1) with dominance, over dominance, and over dominance effects, respectively.

Three QTLs for PH were observed on Chromosomes 1, 4, and 6 at Kaixian in the intervals of umc1035–umc1122a (bins 1.07), umc1963–bnlg1159 (bins 4.05), and umc1918–umc2316 (bins 6.04), respectively. These QTLs explained 51% of the total phenotypic variance for PH. The alleles from QTL mapped on Chromosome 1, which contribute to increasing the PH, were from the higher phosphorus efficiency parental genotype 082 (P_1) with partial dominance effect. The alleles from QTLs mapped on Chromosomes 4 and 6, which contribute to increasing the PH, were from the lower phosphorus efficiency parental genotype Ye107 (P_2) with over dominance and partial dominance effects, respectively. Different QTL for PH was observed at Southwest University in the interval bnlg1055–bnlg131 (bins 1.10) on Chromosome 1 (Table 3). The QTL explained 20% of the total phenotypic variance for PH. The alleles from the QTL, which contribute to increasing the PH, were from the lower phosphorus efficiency parental genotype Ye107 (P_2) with dominance effect.

Discussion

Two important regions

In the present study, there are two important genomic regions: seven coincident QTLs for two traits (PB and LA) were detected in the interval bnlg1832–P2M8/j (bin 1.05) on

chromosome 1 and four consistent QTL for one trait (PH) were coincident in the interval umc1102–P1M7/d (bin 3.05) on Chromosome 3. These coincident QTLs were largely pleiotropic and consistent under phosphorus levels and different two environments. The QTL detected in more than one phosphorus levels or detected in more than one environments was proven useful for MAS. Therefore, the interval 'bnlg1832–P2M8/j' on Chromosome 1 associated with QTLs for two traits (PB and LA) was important. Another important genomic region associated with QTLs for trait PH was detected on Chromosome 3 covering the segment 'umc1102–P1M7/d'. In our earlier studies conducted by 082 × Ye107, these two regions were also found associated with phosphorus efficiency and root traits (Chen et al., 2008). Tuberosa et al. (2002) also identified QTLs for brace root number trait in the same region (bin 1.05) using Lo964 × Lo1016, thus confirming the significance of this region of Chromosome 1. Therefore, the above two segments, bnlg1832–P2M8/j identified in Chromosome 1 and umc1102-P1M7/d identified in Chromosome 3, may be used in the future for MAS and high-resolution mapping leading to map-based cloning of QTLs for agronomically important traits.

Plant biomass affected by LA and PH can be reflected by linkage of the QTLs. Significant positive correlation between plant biomass, PH, and LA (data not shown) was supported by their linked QTLs. This suggests that estimating plant biomass, PH, or LA will have the same meaning for simultaneous improvement under deficient/normal phosphorus in maize.

Gene action

Most QTLs (controlling BP, LA, and PH) showed the levels of partial to over dominance effects, suggesting the complexity of these traits. Table 2 indicates that both 082 and Ye107 contributed favorable alleles for BP, LA, and PH. We also detected significant transgressive segregation, confirming that both parents contributed alleles for these traits. It is likely that there are multiple QTLs controlling the traits, with favorable alleles coming from both parents. From a breeding perspective, it implies that favorable alleles are to be found in a poorly performing genotype under deficient phosphorus condition.

Comparison with QTL of root traits in previous study

Compared with QTL of root traits in previous study, two overlaps of QTLs of BP occurred on bins 1.05 and 1.08. On bin 1.05, the region for QTLs of BP was found for QTLs of root traits (brace root number) in Lo964 × Lo1016 (Tuberosa et al., 2002). On bin 1.08, the region for QTLs of BP was found for QTLs of root traits (root pulling force) in Lo964 × Lo1016 (Tuberosa et al., 2002). The root itself is a significant source of BP.

QTL analysis under different environments

The loci for QTLs of all traits had difference under environments except a few QTLs. It showed that all traits were environment sensitive and multi-gene in nature. The gene × environment interaction was actually reflected these traits. Several studies reported that QTL effects for phenotypic traits are largely dependent on the environment and the presence of significant genotype × environment interaction. The practical implication of this is that MAS may be more highly heritable genetic markers as selection criteria.

In this study, the loci of some QTLs were the same for BP and PH under environments, two for BP (bins 1.05 and 1.08) and one for PH (bin 3.05). These were major QTLs that were extremely stable under environments. In maize, the QTLs identified on the same chromosomal region under environments reduced environmental effects, increasing the precision and the power of mapping QTLs. Therefore, it is an efficient approach to QTL detection and is considered only the most stable one.

The BP was found in the same region as LA in bnlg1832–P2M8/j (bins 1.05) on the chromosome at Kaixian; it verified the role of linkage between two traits. It is interesting to note that three common regions at two sites are in bnlg1832–P2M8/j (bins 1.05) (for QTL of BP), mmc0041–umc1013 (bins 1.08) (for QTL of BP), and umc1102–P1M7/d (bins 3.05) (for QTLs of PH). The work described here was in the first comprehensive analysis of the location of QTL for BP, LA, and PH under phosphorus deficiency at two different sites. The QTLs mapped here can represent regions that are extremely important to improve genetically BP under phosphorus deficiency. It is important to stress here that in this case these QTLs are extremely stable, justifying even more importance in assisted selection programs. If true, the intervals of bnlg1832–P2M8/j (bins 1.05), mmc0041–umc1013 (bins 1.08), and umc1102–P1M7/d (bins 3.05) may be useful for improving BP by means of MAS.

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