Genetic Dissection of Low Phosphorus Tolerance Related Traits Using Selected Introgression Lines in Rice

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Abstract: To dissect the genetic basis of low phosphorus tolerance (LPT), 114 BC_2F_4 introgression lines (ILs) were developed from Shuhui 527 and Minghui 86 (recurrent parents), and Yetuozai (donor parent). The progenies were tested for 11 quantitative traits under three treatments including normal fertilization in normal soil (as control), normal fertilization in barren soil and low phosphorus stress in barren soil in Langfang, Hebei Province, China. Moreover, the ILs were investigated at the seedling stage using nutrient solution culture method in greenhouse in Beijing, China. A total of 49 main-effect quantitative trait loci (QTLs) underlying yield related traits were identified in Langfang, and their contributions to phenotypic variations ranged from 6.7% to 16.5%. Among them, 25 (51.0%) QTLs had favorable alleles from donor parent. A total of 48 main-effect QTLs were identified for LPT-related traits in Beijing, and their contributions to phenotypic variations ranged from 7.7% to 16.6%. Among them, 21 (43.8%) QTLs had favorable alleles from donor parent. About 79.6% of the QTLs can be detected repeatedly under two or more treatments, especially QTLs associated with spikelet number per panicle, spikelet fertility and 1000-grain weight, displaying consistent phenotypic effects. Among all the detected QTLs, eight QTLs were simultaneously identified under low phosphorus stress across two environments. These results can provide useful information for the genetic dissection of LPT in rice.

Key words: rice; phosphorus tolerance; yield; introgression line; quantitative trait locus

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population (Huang et al, 2010). The developments of semi-dwarf rice varieties in the 1960s and hybrid rice varieties in the 1970s brought great increase in average yield, which have dramatically contributed to the self-sufficiency in world rice supply (Ma and Yuan, 2015). Recently, the rapid population growth and economic development have imposed a heavy pressure on rice production. To meet the global rice requirement, the total rice production needs to be increased by 0.6%–0.9% per year (Carriger and Vallee, 2007; Vinod and Heuer, 2012). It is well known that the fertilizer input plays an important role in improving yield. However, the contribution of fertilizer input to

rice yield increase has gradually decreased in the past ten years (Wu, 2013). The overuse of fertilizer not only causes the rising agricultural energy consumption, but also results in environmental degradation and pollution (Wu, 2013; Huang et al, 2014). Phosphorus (P) is a critical nutrient for rice growth and development, and it is also a basic component of many organic molecules, especially nucleic acids and proteins (Lea and Miflin, 2011). P deficiency leads to the lack of nutrition and the lag of plant growth (Guo et al, 2013). Besides, P deficiency is usually the secondary factor for low pH soils, which imposes restrictions on root growth although there are high concentrations of iron and aluminium (Ismail et al,

2007). Moreover, it causes a series of molecular and physiological responses that ultimately result in significant yield loss in rice (Dobermann and Fairhurst, 2000; Ismail et al, 2007). Currently, P deficiency occurs to about 50% of the agricultural soils in many Asian, African and South America countries (Lynch, 2011). Therefore, the balanced and sustainable use of P fertilizer is of paramount importance (Vinod and Heuer, 2012). One of the most effective ways to ensure the sustainable development of agriculture is to develop and grow rice varieties with low P tolerance (LPT) or high P-use efficiency.

In recent years, molecular-marker technology has contributed to the development of genetic map that makes it possible to identify quantitative trait loci (QTLs)/genes related to LPT in rice, including P absorption and utilization. However, very few QTLs can be directly applied in rice breeding (Gao et al, 2006; Mu et al, 2008; Vinod and Heuer, 2012; Wu, 2013), which is largely due to the complex genetic basis of LPT and the lack of QTL validation in different genetic backgrounds and environments (Loudet et al, 2003). Based on the advanced backcross QTL analysis (Tanksley and Nelson, 1996), Li (2005) proposed a molecular breeding strategy that combines QTLs/genes mining with target trait improvement using selected introgression lines (ILs) (Luo, 2005). The selected ILs were derived from elite varieties (recurrent parents) and can be directly applied in the practical breeding and QTL discovery. Research regarding QTL detection in selected ILs has been reported, especially for drought, salt and diseases (Zheng et al, 2007; Zang et al, 2008; Chen et al, 2011). However, systematic identification of the QTLs for LPT has not been conducted in a large scale.

Therefore, 114 BC_2F_4 ILs in two populations deriving from three parents, Minghui 86 (recurrent parent), Shuhui 527 (recurrent parent) and Yetuozai (donor parent), were selected to investigate the genetic basis for LPT both at the seedling and maturity stages of rice. The phenotypic evaluation and QTL identification of LPT-related traits in ILs were also conducted. This study will probably provide better understanding of LPT and useful information for marker-assisted selection in rice breeding.

MATERIALS AND METHODS

Population development

Two widely used elite indica restorer lines (Shuhui 527 and Minghui 86) were used as recurrent parents to develop two backcross populations with Yetuozai (donor parent), an indica rice variety. The F_1 combinations were backcrossed with the corresponding recurrent parents to generate BC_1F_1 seeds, and 25 random BC_1F_1 plants were backcrossed with the recurrent parents to obtain 25 BC_2F_1 lines. All BC_2F_1 lines were planted in the fields and allowed to self-produce BC_2F_2 seeds which were bulk-harvested as a random BC_2F_2 population. A total of 1000 plants from each BC_2F_2 population were screened randomly in a low P red soil field without P fertilizer input at the experimental station of Xuancheng Institute of Agricultural Science, Anhui Province, China, in the summer season (May to September) of 2010, and 60 and 54 ILs were selected from Shuhui 527/Yetuozai (SY) and Minghui 86/Yetuozai (MY) populations, respectively. Then, 114 BC_2F_4 lines were developed from self-crossed generations at the experimental station of the Institute of Crop Science, Chinese Academy of Agricultural Sciences in Sanya the winter season (November of 2010 to April of 2011).

Phenotypic evaluation

The phenotypic evaluation was conducted under field conditions in Langfang, Hebei Province and nutrient solution culture conditions in Beijing, China.

Field conditions

The phenotypic evaluation under field conditions was conducted in the summer of 2013 at the experimental field of the International Agricultural High-Tech Industrial Center of the Chinese Academy of Agricultural Sciences (Langfang, China). Three treatments included normal fertilization in normal soil (treatment A, as control group), normal fertilization in barren soil (treatment B) and low P stress in barren soil (treatment C). The 50 cm topsoil was replaced with uniform barren soil except the plot of the control group. The organic matter content, total contents of nitrogen, phosphorus and potassium in the soil are listed in Table 1. All 114 BC_2F_4 lines and 3 parental lines with three treatments were planted under a randomized block design. Each treatment consisted of three replications (plots), and each IL was in one row with 12 plants. The seeds of each IL were sown in the seedbeds in late April. Then the seedlings were transplanted to experimental fields on 6 June with a spacing of 25.0 cm between rows and 17.0 cm within each row. Fertilization was conducted in two phases, the stem elongation stage (11 July) and booting stage

Table 1. Soil organic matter and nitrogen (N), phosphorus (P) and potassium (K) contents of trial area for different treatments in Langfang, Hebei Province, China.

Sampling stage	Treatment	Organic matter (g/kg)	Total N (g/kg)	Alkali-hydrolyzable N (g/kg)	Olsen-P (g/kg)	Available K (g/kg)	pH
Before sowing	A	13.71	1.11	0.06	0.04	0.10	8.4
	B	7.85	0.57	0.04	0.01	0.07	8.6
	C	8.20	0.62	0.04	0.01	0.07	8.6
Elongation stage (7 d after the first fertilizer application)	A	-	1.12	0.08	0.04	-	-
	B	-	0.55	0.04	0.01	-	-
	C	-	0.56	0.04	0.01	-	-
Before booting stage (before the second fertilizer application)	A	-	0.96	0.07	0.03	-	-
	B	-	0.51	0.04	0.01	-	-
	C	-	0.53	0.04	0.01	-	-
Booting stage (7 d after the second fertilizer application)	A	-	0.95	0.06	0.03	-	-
	B	-	0.55	0.05	0.01	-	-
	C	-	0.48	0.04	0.01	-	-
After harvest	A	13.35	1.02	0.06	0.03	0.08	8.2
	B	7.42	0.53	0.03	0.01	0.06	8.2
	C	7.05	0.54	0.03	0.01	0.06	8.2

A, Normal fertilization treatment in normal soil; B, Normal fertilization in barren soil; C, Low phosphorus stress in barren soil.

(11 August). The specific amount of fertilizer for treatments A and B were: 195 kg/hm^2 urea, 750 kg/hm^2 calcium superphosphate and 150 kg/hm^2 potassium sulphate at the stem elongation stage; 112 kg/hm^2 urea, 279 kg/hm^2 calcium superphosphate and 89 kg/hm^2 potassium sulphate at the booting stage. For treatment C, they were 195 kg/hm^2 urea, 250 kg/hm^2 calcium superphosphate and 150 kg/hm^2 potassium sulphate at the stem elongation stage; 112 kg/hm^2 urea, 93 kg/hm^2 calcium superphosphate and 89 kg/hm^2 potassium sulphate at the booting stage. The amount of phosphate fertilizer for treatment C is one third of that for treatment A. The recommended agronomical practices for rice were applied in the experimental plots. The middle five plants in the central row of each line were used for data collection. The eleven quantitative traits investigated were heading date (HD, d), plant height (PH, cm), panicle number per plant (PN), panicle length (PL, cm), spikelet number per panicle (SNP), filled-grain number per panicle (FGP), spikelet fertility (SF, %), 1000-grain weight (TGW, g), grain yield per plant (GY, g), biomass (BI, g) and harvest index (HI). HD was the days from sowing to the heading of 50% panicles.

Nutrient solution culture conditions

Firstly, all 114 BC$_2$F$_4$ lines and 3 parents were soaked. After germination, they were transferred to the nutrient solutions including normal nutrient solution (treatment D), 1/3 P nutrient solution (treatment E) and P deficient nutrient solution (treatment F). Each treatment had three replications. For each nutrient solution, pH was set to 5.5. Other nutrient contents were 1.4 mmol/L NH$_4$NO$_3$, 0.3 mmol/L NaH$_2$PO$_4$, 0.5 mmol/L K$_2$SO$_4$, 1.0 mmol/L CaCl$_2$, 1.6 mmol/L

MgSO$_4$, 0.2 mmol/L Na$_2$SiO$_3$, 50 µmol/L Fe-EDTA, 0.06 µmol/L (NH$_4$)$_6$Mo$_7$O$_{24}$, 15 µmol/L H$_3$BO$_3$, 8 µmol/L MnCl$_2$, 0.12 µmol/L CuSO$_4$, 0.12 µmol/L ZnSO$_4$, 29 µmol/L FeCl$_3$ and 40.5 µmol/L citric acid. The nutrient solution was replaced every six days. The low P stress was conducted at the two-leaf stage for 30 d. Then, three uniform plants of each group were used for data collection. The five investigated quantitative traits included relative root length (RRL, cm), relative root dry weight (RRW, g), relative shoot dry weight (RSW, g), relative total dry weight (RTW, g) and relative root to shoot ratio of dry weight (RWRSR, %). The trait value was calculated as the value difference between treatments E or F and D, i.e., RRL$_{(E-D)}$= Root length under treatment E − Root length under treatment D.

Genotyping and QTL mapping

Based on the genetic linkage map of SSR markers from Cornell University and the reference physical map of SSR markers of GRAMENE (http://www. gramene.org/), 98 bins with an average length of 0.8 ± 1.0 Mb were distributed on 12 chromosomes with the distance unit of about 3 Mb (Zheng et al, 2007). About 650 pairs of SSR primers were screened for polymorphisms among the parents Shuhui 527, Minghui 86 and Yetuozai. Totally, 60 and 65 pairs of SSR primers evenly distributing across 12 chromosomes were finally selected for genotyping Shuhui 527/ Yetuozai (SY) and Minghui 86/Yetuozai (MY) populations, respectively. The putative QTL associated with the target trait was judged by SAS PROC GLM (SAS Institute Inc., 2008). The significance threshold $P = 0.01$ was used in Langfang. If a QTL at $P < 0.01$

level was detected, and it was simultaneously detected in other population or other environment at $P < 0.05$ level, it is considered as a QTL (Moncada et al, 2001). In Beijing, the significance threshold $P = 0.05$ was used, and the trait value, calculated as the value difference between treatments E or F and D, was used for QTL mapping.

RESULTS

Phenotypic performances of two IL populations and their parents under three treatments in Langfang

The means and ranges of 11 quantitative traits measured in the two populations and their parents in Langfang are shown in Table 2. There were significant differences in HD and TGW between their recurrent parents (Shuhui 527 and Minghui 86) and donor parent (Yetuozai) under three treatments. For Shuhui 527, treatment B caused significant decreases in PH, GY and SNP compared with treatment A, and no significant changes were observed in the other traits, which ultimately led to the yield loss of Shuhui 527. Treatment C had no significant influences on PN, PL, SNP, BI and SF, but had significant influences on HD, PH, FGP, TGW, GY and HI. Therefore, the yield loss of Shuhui 527 under treatment C was mainly due to the reduction of yield components FGP and TGW. For Minghui 86, treatment B caused significant decreases in PH and SNP compared with the control condition, but no significant changes in the other traits, which ultimately led to the yield loss of Minghui 86. The means of PH, FGP, TGW, GY and HI of Minghui 86 under treatment C were significantly lower than the corresponding values under treatment A. Thus, the main reason for the reduction of GY in Minghui 86 is the decrease of FGP and TGW under treatment C.

Transgressive variations for each trait were observed in SY and MY populations under three treatments, indicating the presence of a wide range of separations among the progenies (Table 2). On average, for SY population, ILs showed significantly higher SF, lower PH and HI under treatment B than under treatment A. ILs showed significantly delayed HD, reduced PH, decreased TGW and HI under treatment C compared with treatment A. For MY population, ILs showed significantly lower PH under treatment B than treatment A. ILs showed significantly delayed HD, reduced PH, decreased PN, GY and BI under treatment C compared with treatment A. These results demonstrated that treatment B caused a significant

Table 2. Phenotypic performances of yield and its related traits for introgression lines and their parents under field conditions in Langfang, Hebei Province, China.

Material	Treatment	HD (d)	PH (cm)	PN	PL (cm)	FGP	SNP	SF (%)	TGW (g)	GY (g)	BI (g)	HI
Shuhui 527												
Mean ± SD	A	112.3 ± 2.5 b	105.0 ± 1.9 a	5.7 ± 0.7 a	26.2 ± 1.1 a	168.2 ± 23.6 a	195.9 ± 27.3 a	85.8 ± 0.8 a	29.8 ± 0.3 a	19.9 ± 2.4 a	35.5 ± 4.2 a	0.56 ± 0.00 a
	B	112.3 ± 1.5 b	98.9 ± 3.7 b	5.3 ± 0.8 a	25.0 ± 0.9 a	132.9 ± 16.4 ab	153.1 ± 17.9 b	86.8 ± 3.0 a	29.1 ± 0.5 a	16.1 ± 2.0 b	32.0 ± 4.0 a	0.50 ± 0.00 ab
	C	116.3 ± 0.6 a	98.1 ± 2.5 b	4.6 ± 0.3 a	25.6 ± 1.2 a	130.3 ± 12.4 b	162.1 ± 16.9 ab	80.6 ± 5.9 a	27.6 ± 1.0 b	16.2 ± 0.8 b	34.1 ± 2.0 a	0.48 ± 0.10 b
Minghui 86												
Mean ± SD	A	112.0 ± 1.7 a	107.1 ± 0.8 a	5.0 ± 0.8 a	25.7 ± 2.0 a	157.1 ± 12.5 a	191.0 ± 14.7 a	82.4 ± 5.8 a	25.8 ± 0.1 a	14.3 ± 0.8 a	30.2 ± 3.8 a	0.48 ± 0.00 a
	B	110.7 ± 1.2 a	95.6 ± 2.8 b	4.3 ± 0.6 a	22.9 ± 0.2 a	123.8 ± 20.3 ab	145.2 ± 16.9 b	85.0 ± 4.2 a	25.4 ± 1.2 a	11.8 ± 1.8 ab	25.7 ± 3.4 a	0.46 ± 0.00 ab
	C	113.7 ± 2.1 a	92.7 ± 2.1 b	4.3 ± 1.2 a	23.4 ± 2.6 a	120.8 ± 17.3 b	159.5 ± 21.3 ab	75.8 ± 5.6 a	23.8 ± 0.7 b	9.1 ± 2.4 b	24.3 ± 4.0 a	0.37 ± 0.00 b
Yetuozai												
Mean ± SD	A	122.3 ± 1.2 a	100.5 ± 4.1 a	4.9 ± 1.4 a	25.0 ± 0.2 ab	160.9 ± 14.0 a	184.6 ± 9.9 a	87.2 ± 5.4 a	19.5 ± 1.1 ab	11.8 ± 2.0 a	26.4 ± 3.6 a	0.45 ± 0.10 a
	B	120.3 ± 1.2 b	100.5 ± 3.0 a	5.4 ± 0.5 a	25.9 ± 0.6 a	177.3 ± 16.3 a	195.5 ± 12.3 a	90.6 ± 3.2 a	20.4 ± 0.2 a	13.0 ± 0.6 a	32.4 ± 2.9 a	0.40 ± 0.00 a
	C	122.7 ± 0.6 a	95.9 ± 2.1 a	4.8 ± 1.1 a	24.3 ± 0.8 b	155.6 ± 12.5 a	179.5 ± 10.7 a	86.6 ± 2.8 a	18.4 ± 0.5 b	10.2 ± 1.9 a	28.1 ± 4.2 a	0.41 ± 0.00 a
Shuhui 527/Yetuozai population (SY)												
Mean ± SD	A	114.2 ± 2.5 b	106.0 ± 4.6 a	5.4 ± 0.7 a	25.5 ± 1.7 a	137.7 ± 21.9 a	171.0 ± 25.1 a	80.5 ± 4.5 b	28.5 ± 2.2 a	16.4 ± 3.0 a	32.7 ± 5.5 a	0.50 ± 0.00 a
	B	114.5 ± 2.2 b	102.8 ± 4.4 b	5.4 ± 0.7 a	25.0 ± 1.7 a	137.3 ± 20.8 a	164.7 ± 22.2 a	83.7 ± 4.3 a	28.8 ± 2.3 a	17.2 ± 2.6 a	35.7 ± 6.1 a	0.48 ± 0.00 b
	C	116.7 ± 2.3 a	100.6 ± 4.4 c	5.3 ± 0.8 a	25.1 ± 1.5 a	142.8 ± 22.4 a	173.0 ± 24.5 a	82.5 ± 5.0 ab	27.7 ± 1.9 b	16.2 ± 3.5 a	34.7 ± 7.2 a	0.47 ± 0.00 b
Range		107.7–120.0	97.7–120.7	3.9–7.9	21.4–29.4	92.7–186.1	118.6–226.2	69.8–88.9	21.3–32.4	8.7–23.9	20.0–46.2	0.39–0.62
		108.0–121.0	92.3–115.5	3.9–7.0	19.8–28.6	100.9–200.0	113.9–225.4	71.3–92.7	21.2–32.9	11.4–22.8	23.7–55.4	0.40–0.56
		111.7–123.3	88.5–112.9	3.7–7.4	21.0–28.1	93.5–191.8	118.7–221.2	67.8–91.7	22.0–31.4	8.9–29.2	22.5–60.0	0.30–0.56
Minghui 86/Yetuozai population (MY)												
Mean ± SD	A	115.4 ± 1.7 b	109.4 ± 6.1 a	5.4 ± 0.7 a	27.1 ± 1.5 a	156.6 ± 27.5 a	208.0 ± 34.0 a	75.3 ± 6.4 a	27.4 ± 1.9 a	17.9 ± 3.4 a	36.7 ± 6.1 a	0.49 ± 0.00 a
	B	115.3 ± 1.2 b	106.1 ± 6.0 b	5.0 ± 0.7 ab	26.7 ± 1.3 a	164.1 ± 21.2 a	206.4 ± 25.0 a	79.8 ± 4.6 a	27.5 ± 1.7 a	17.7 ± 2.6 ab	35.5 ± 5.5 ab	0.50 ± 0.00 a
	C	116.9 ± 1.4 a	101.7 ± 5.2 c	4.8 ± 0.8 b	26.4 ± 1.2 a	159.3 ± 26.3 a	202.9 ± 29.8 a	78.4 ± 6.3 a	26.9 ± 1.9 a	16.1 ± 3.6 b	33.2 ± 7.5 b	0.49 ± 0.00 a
Range		112.0–121.7	96.7–123.7	4.1–7.0	22.8–30.9	104.7–223.3	130.6–283.9	57.3–84.9	21.8–31.5	10.9–24.9	23.1–51.6	0.41–0.60
		113.3–118.0	95.5–119.4	4.0–6.3	24.1–29.3	119.9–213.9	159.3–266.1	70.9–90.2	22.3–30.7	12.2–25.3	23.9–47.7	0.34–0.68
		114.0–120.3	89.8–111.0	2.9–6.7	23.7–29.0	114.3–239.0	158.0–278.2	65.0–89.5	20.6–30.8	10.4–26.3	21.9–51.6	0.39–0.60

A, Normal fertilization treatment under normal soil; B, Normal fertilization treatment under barren soil; C, Low phosphorus stress under barren soil; HD, Heading date; PH, Plant height; PN, Panicle number per plant; PL, Panicle length; FGP, Filled grain number per panicle; SNP, Spikelet number per panicle; SF, Spikelet fertility; TGW, 1000-grain weight; GY, Grain yield per plant; BI, Biomass; HI, Harvest index.
The same lowercase letter followed the values means no significant difference among the different treatments in the same materials.

reduction of PH, whereas significantly delayed HD and reduced PH were caused by treatment C.

Phenotypic performances of two IL populations and their parents under three treatments in Beijing

The performances of each trait in the two populations and their parents in Beijing are shown in Table 3. There were significant differences in RRL, RSW, RWRSR and RTW between the recurrent (Shuhui 527) and donor (Yetuozai) parents under treatment F. For Shuhui 527, significant differences were observed in RRW and RWRSR between stress conditions (treatments E and F) and control condition (treatment D). For Minghui 86, significant differences were observed in RWRSR between stress conditions and control condition.

On average, for SY population, ILs showed higher RRL, RRW and RWRSR under treatments E and F than under treatment D. ILs showed increased RSW and RTW under treatment E while decreased RSW and RTW under treatment F compared with treatment D. The means of RRW, RSW, RWRSR and RTW of ILs were significantly lower than the corresponding values of their recurrent parent Shuhui 527 under treatment E. The means of RRL, RRW, RSW and RTW of ILs were significantly lower than the

corresponding values of Shuhui 527 under treatment F. For MY population, the means of RRL and RWRSR of ILs were higher while the means of RRW, RSW and RTW were lower than the corresponding values of their recurrent parent Minghui 86 under treatment E. The means of RRL of ILs were higher while the means of RRW, RSW and RTW were lower than the corresponding values of their recurrent parent Minghui 86 under treatment F.

QTL detection and analysis

QTL detection in Langfang

The QTLs detected in the two IL populations for yield and yield-related traits (PN, FGP, SNP, SF and GY) are shown in Table 4 and Fig. 1. A total of 49 main QTLs affecting yield and yield-related traits were identified. These QTLs can independently explain 6.7%–16.5% of the phenotypic variations, and 25 (51%) of them had favorable alleles from the donor. For SY population, 38 QTLs affecting yield and yield-related traits were identified. These QTLs were distributed on chromosomes 1, 4, 5, 6, 7, 9, 11 and 12. For MY population, 11 QTLs affecting yield and yield related traits were identified. These QTLs were distributed on chromosomes 1, 3, 4, 5 and 12. No

Table 3. Phenotypic performances of measured traits for introgression lines and their parents under nutrient solution culture conditions in Beijing, China.

Material	Treatment	RRL (cm)	RRW (g)	RSW (g)	RWRSR	RTW (g)
Shuhui 527						
Mean ± *SD*	E-D	1.8 ± 2.6 a	0.015 ± 0.003 b	0.048 ± 0.018 a	0.039 ± 0.027 b	0.062 ± 0.021 a
	F-D	4.0 ± 0.3 a	0.026 ± 0.000 a	0.030 ± 0.009 a	0.207 ± 0.035 a	0.056 ± 0.009 a
Minghui 86						
Mean ± *SD*	E-D	0.1 ± 1.0 a	0.006 ± 0.008 a	0.011 ± 0.015 a	0.011 ± 0.067 b	-0.005 ± 0.062 a
	F-D	0.6 ± 0.9 a	0.008 ± 0.009 a	-0.005 ± 0.023 a	0.160 ± 0.086 a	0.003 ± 0.027 a
Yetuozai (1)						
Mean ± *SD*	E-D	0.5 ± 1.8 a	0.003 ± 0.012 a	0.006 ± 0.038 a	0.021 ± 0.082 a	0.010 ± 0.049 a
	F-D	-0.1 ± 2.8 a	0.009 ± 0.009 a	0.001 ± 0.022 a	0.107 ± 0.086 a	0.009 ± 0.029 a
Yetuozai (2)						
Mean ± *SD*	E-D	0.1 ± 1.1 b	0.001 ± 0.007 a	-0.001 ± 0.008 a	0.013 ± 0.071 a	-0.002 ± 0.015 a
	F-D	1.8 ± 1.6 a	0.001 ± 0.007 a	-0.021 ± 0.020 b	0.135 ± 0.144 a	-0.020 ± 0.022 a
Shuhui 527/Yetuozai population (SY)						
Mean ± *SD*	E-D	0.3 ± 0.9 a	0.005 ± 0.017 a	0.001 ± 0.033 a	0.023 ± 0.042 a	0.008 ± 0.045 a
	F-D	0.6 ± 1.1 a	0.007 ± 0.010 a	-0.015 ± 0.035 a	0.102 ± 0.072 a	-0.007 ± 0.042 a
Range	E-D	-2.1–2.9	-0.037–0.097	-0.153–0.059	-0.102–0.131	-0.180–0.125
	F-D	-2.8–3.1	-0.015–0.040	-0.116–0.073	-0.084–0.406	-0.131–0.093
Minghui 86/Yetuozai population (MY)						
Mean ± *SD*	E-D	0.2 ± 1.2 b	0.001 ± 0.008 a	-0.007 ± 0.019 a	0.026 ± 0.044 b	-0.006 ± 0.025 a
	F-D	0.9 ± 1.0 a	0.004 ± 0.009 a	-0.027 ± 0.019 b	0.154 ± 0.112 a	-0.023 ± 0.026 b
Range	E-D	-2.4–1.6	-0.014–0.014	-0.043–0.044	-0.070–0.127	-0.057–0.057
	F-D	-1.3–3.0	-0.011–0.030	-0.065–0.024	0.054–0.839	-0.064–0.054

Yetuozai (1) and Yetuozai (2) represent the Yetuozai which was used for Shuhui 527/Yetuozai and Minghui 86/Yetuozai population in the experiment, respectively; E, Nutrient solution with 1/3 phosphorus of the control; F, Nutrient solution without phosphorus; RRL, Relative root length; RRW, Relative root dry weight; RSW, Relative shoot dry weight; RWRSR, Relative root-shoot ratio of dry weight; RTW, Relative total dry weight.

The values in this table are the difference value compared with treatment D (control). The different lowercase letter followed the values means significant difference among different treatments in the same materials.

QTL was simultaneously detected in these two populations, which indicated that there were significant genetic background effects for QTL mapping of yield and yield-related traits.

A total of 17 main-effect QTLs for yield and its related traits were identified under treatment A, including 12 in SY population and 5 in MY population. For SY population, there were two QTLs for PN, one for FGP, two for SNP, one for SF, four for TGW and two for GY. For MY population, one QTL for PN, two for SF and two for TGW were identified.

A total of 16 main-effect QTLs for yield and its related traits were identified under treatment B. Among them, three QTLs affecting PN were identified

Table 4. Identification of QTLs for yield traits in two populations under three treatments in Langfang, Hebei Province, China.

Treatment	Population	Trait	QTL	Marker	Chromosome	Position (cM)	Bin	Probability	R^2 (%)	Additive
A	SY	PN	qPN5.7	RM26	5	118.8	5.7	0.017	10.2	0.47
A	SY	PN	qPN11.3	RM536	11	55.1	11.3	0.005	13.0	0.29
A	MY	PN	qPN5.3	RM169	5	58.0	5.3	0.032	8.5	-0.30
A	SY	FGP	qFGP11.6	RM254	11	110.0	11.6	0.017	9.7	-8.00
A	SY	SNP	qSNP6.6	RM5463	6	124.4	6.6	0.016	9.8	10.48
A	SY	SNP	qSNP11.6	RM254	11	110.0	11.6	0.018	9.5	-9.11
A	SY	SF	qSF5.5	RM164	5	78.7	5.5	0.015	10.0	1.52
A	MY	SF	qSF4.1	RM335	4	22.0	4.1	0.014	11.0	-2.53
A	MY	SF	qSF12.1	RM20A	12	3.2	12.1	0.006	13.8	-4.51
A	SY	TGW	qTGW4.1	RM335	4	22.0	4.1	0.025	8.6	-1.05
A	SY	TGW	qTGW5.5	RM164	5	78.7	5.5	0.040	7.2	0.63
A	SY	TGW	qTGW6.6	RM5463	6	124.4	6.6	0.008	11.9	-1.00
A	SY	TGW	qTGW9.3	RM219	9	11.7	9.3	0.017	9.5	-0.83
A	MY	TGW	qTGW1.10	RM200	1	143.2	1.10	0.031	8.7	0.55
A	MY	TGW	qTGW3.12	RM514	3	216.4	3.12	0.010	12.0	0.64
A	SY	GY	qGY6.5	RM541	6	76.0	6.5	0.009	11.0	1.15
A	SY	GY	qGY11.6	RM254	11	110.0	11.6	0.018	9.5	-1.06
B	SY	PN	qPN1.7	RM9	1	92.4	1.7	0.047	6.8	0.32
B	SY	PN	qPN11.3	RM536	11	55.1	11.3	0.003	14.4	0.33
B	SY	PN	qPN12.2	RM155	12	20.9	12.2	0.004	14.4	0.25
B	SY	FGP	qFGP11.6	RM254	11	110.0	11.6	0.007	12.2	-8.56
B	MY	FGP	qFGP12.4	RM511	12	59.8	12.4	0.007	13.3	14.57
B	SY	SNP	qSNP11.6	RM254	11	110.0	11.6	0.006	12.7	-9.26
B	MY	SNP	qSNP12.4	RM511	12	59.8	12.4	0.003	16.1	18.81
B	SY	SF	qSF1.7	RM9	1	92.4	1.7	0.022	8.9	-2.30
B	SY	TGW	qTGW4.1	RM335	4	22.0	4.1	0.005	12.8	-1.32
B	SY	TGW	qTGW6.6	RM5463	6	124.4	6.6	0.002	15.1	-1.17
B	SY	TGW	qTGW7.1	RM481	7	3.2	7.1	0.046	6.7	0.90
B	SY	TGW	qTGW9.3	RM219	9	11.7	9.3	0.014	10.0	-0.88
B	MY	TGW	qTGW1.10	RM200	1	143.2	1.1	0.002	16.5	0.69
B	SY	GY	qGY6.5	RM541	6	76.0	6.5	0.024	8.5	0.88
B	SY	GY	qGY11.6	RM254	11	110.0	11.6	0.013	10.4	-0.98
C	SY	PN	qPN1.2	RM283	1	31.4	1.2	0.001	16.5	-0.42
C	SY	PN	qPN1.7	RM9	1	92.4	1.7	0.005	13.0	0.54
C	SY	PN	qPN5.7	RM26	5	118.8	5.7	0.007	12.6	0.65
C	SY	PN	qPN6.1	RM589	6	3.2	6.1	0.008	11.4	0.33
C	SY	PN	qPN11.3	RM536	11	55.1	11.3	0.007	11.8	0.35
C	MY	PN	qPN5.3	RM169	5	58.0	5.3	0.015	11.0	-0.44
C	SY	SNP	qSNP6.6	RM5463	6	124.4	6.6	0.020	9.2	9.87
C	SY	SNP	qSNP11.6	RM254	11	110.0	11.6	0.039	7.3	-7.75
C	SY	SF	qSF1.7	RM9	1	92.4	1.7	0.003	14.9	-3.42
C	SY	SF	qSF5.5	RM164	5	78.7	5.5	0.018	9.4	1.62
C	MY	SF	qSF4.1	RM335	4	22.0	4.1	0.043	7.6	-2.06
C	SY	TGW	qTGW4.1	RM335	4	22.0	4.1	0.007	12.3	-1.08
C	SY	TGW	qTGW5.5	RM164	5	78.7	5.5	0.004	13.7	0.74
C	SY	TGW	qTGW6.6	RM5463	6	124.4	6.6	0.004	13.7	-0.92
C	SY	TGW	qTGW7.1	RM481	7	3.2	7.1	0.032	7.7	0.80
C	SY	TGW	qTGW9.3	RM219	9	11.7	9.3	0.013	10.1	-0.74
C	MY	GY	qGY1.10	RM200	1	143.2	1.1	0.003	15.8	1.42

A, Normal fertilization treatment in normal soil; B, Normal fertilization treatment in barren soil; C, Low-phosphorus stress in barren soil; MY, Minghui 86/Yetuozai; SY, Shuhui 527/Yetuozai; PN, Panicle number per plant; FGP, Filled grain number per panicle; SNP, Spikelet number per panicle; SF, Spikelet fertility; TGW, 1000-grain weight; GY, Grain yield per plant; R^2, Proportion of phenotypic variance explained by the QTL effect.

The significant level is $P < 0.01$. The positive additive effect represents favorable allele is from donor parent, and the negative one represents the allele is from recurrent parent.
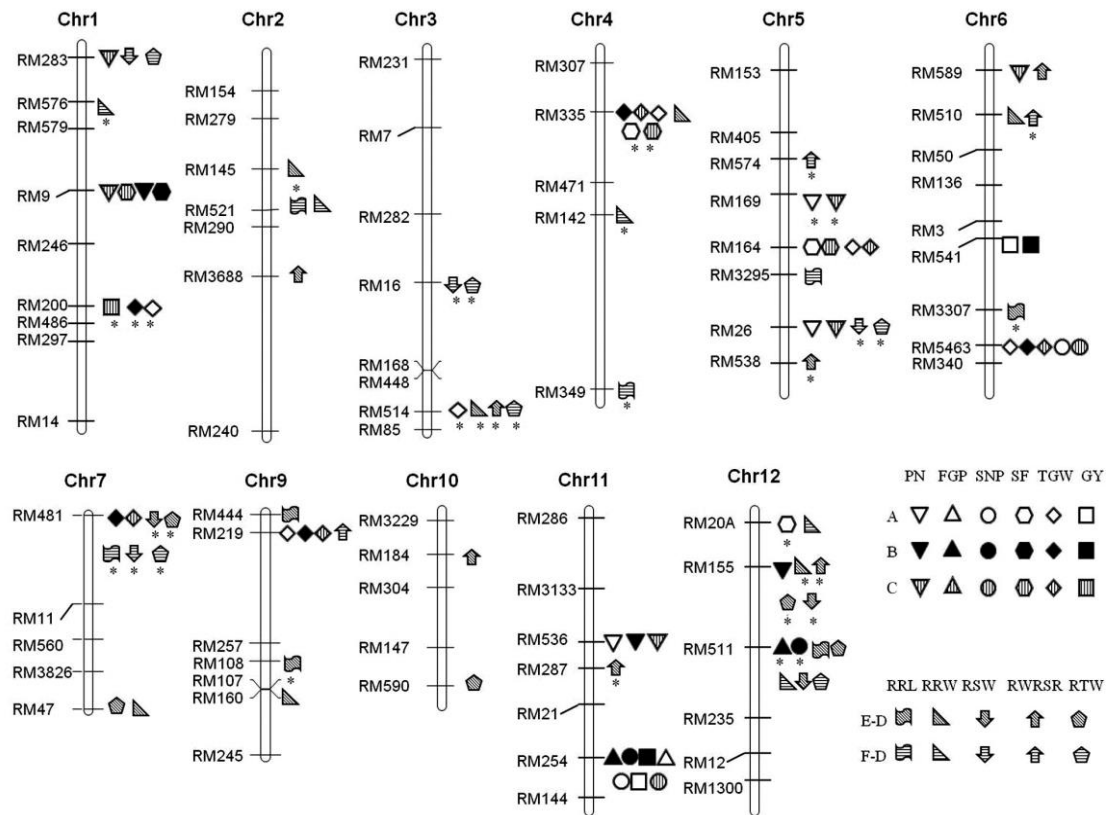
Fig. 1. Identified QTLs for measured traits in two introgressed line populations under two environments.

A, Normal fertilization treatment in normal soil; B, Normal fertilization treatment in barren soil; C, Low-phosphorus stress in barren soil; PN, Panicle number per plant; FGP, Filled grain number per panicle; SNP, Spikelet number per panicle; SF, Spikelet fertility; TGW, 1000-grain weight; GY, Grain yield per plant; RRL, Relative root length; RRW, Relative root dry weight; RSW, Relative shoot dry weight; RTW, Relative total dry weight; RWRSR, Relative root-shoot ratio of dry weight; D, Control; E, Nutrient solution with 1/3 phosphorus of the control; F, Nutrient solution without phosphorus; Chr, Chromosome.

* below the icon represents the QTL identified in Minghui 86/Yetuozai population, otherwise, in Shuhui 527/Yetuozai population.

and their favorable alleles were from the donor Yetuozai. Loci near RM254 on chromosome 11 and RM511 on chromosome 12 for FGP were identified in SY and MY populations, respectively. Five QTLs for TGW and two for GY were identified.

Sixteen main-effect QTLs for yield and its related traits were identified under treatment C. Among them, six QTLs affecting PN were identified. On average, they explained 12.7% of the phenotypic variations. Two QTLs for SNP, *qSNP6.6* and *qSNP11.6*, were identified, which explained 9.2% and 7.3% of the phenotypic variations, respectively. Three QTLs for SF, *qSF1.7*, *qSF5.5* and *qSF4.1*, were identified, which explained 14.9%, 9.4% and 7.6% of the phenotypic variations, respectively. Five QTLs for TGW located on chromosomes 4, 5, 6, 7 and 9, respectively, were identified in SY population and explained an average of 11.5% of the phenotypic variation. One QTL for GY, *qGY1.10*, was identified, which explained 15.8% of the phenotypic variations.

More than 79.6% of the QTLs were detected under two or more treatments. In particular, the QTLs associated with SNP, SF and TGW expressed stably and had consistent effects under different treatments. According to the additive effect, they could be divided into two types. The first type included nine QTLs that had favorable alleles from the donor. These QTLs had effects on PN, FGP, SF, TGW and GY, including eight QTLs in SY population and one QTL in MY population. The second type included nine QTLs that had favorable alleles from the recurrent parent. The QTLs affecting PN, SNP, SF, TGW and GY included seven QTLs in SY population and two in MY population.

QTL detection in Beijing

The QTLs detected in the two IL populations for LPT-related traits (RRL, RRW, RWRSR, RSW and RTW) are shown in Table 5 and Fig. 1. Totally, 48 main-effect QTLs affecting LPT-related traits were identified. These QTLs can independently explain 7.7%–16.6% of the phenotypic variations, and 21

(43.8%) of them had favorable alleles from the donor. For SY population, 26 QTLs affecting LPT-related traits were identified. These QTLs were distributed on chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 12. For MY population, 22 QTLs affecting LPT-related traits were identified. These QTLs were distributed on chromosomes 1, 2, 3, 4, 5, 6, 7, 9 and 12.

A total of 26 main-effect QTLs for LPT-related traits were identified under treatment E-D, including 12 in SY population and 14 in MY population. For SY population, two QTLs for RRL, four for RRW, three for RWRSR, one for RSW and two for RTW were detected. For MY population, two QTLs for RRL, three for RRW, four for RWRSR, two for RSW and three for RTW were detected.

A total of 22 main-effect QTLs for LPT-related traits were identified under treatment F-D, including 9 in SY population and 13 in MY population. For SY population, two QTLs for RRL, three for RRW, one for RWRSR, one for RSW and two for RTW were

Table 5. Identification of QTLs for measured traits in two populations in Beijing, China.

Treatment	Population	Trait	QTL	Marker	Chromosome	Position (cM)	Bin	Probability	R^2 (%)	Additive
E-D	SY	RRL	qRRL9.3	RM444	9	3.3	9.3	0.014	11.5	0.397
E-D	SY	RRL	qRRL12.4	RM511	12	59.8	12.4	0.009	13.0	0.289
E-D	MY	RRL	qRRL6.6	RM3307	6	113.4	6.6	0.007	14.4	-0.403
E-D	MY	RRL	qRRL9.7	RM108	9	73.3	9.7	0.034	11.4	0.353
E-D	SY	RRW	qRRW4.1	RM335	4	21.5	4.1	0.019	10.9	0.009
E-D	SY	RRW	qRRW6.3	RM510	6	20.8	6.3	0.035	8.8	-0.007
E-D	SY	RRW	qRRW7.7	RM47	7	90.0	7.7	0.036	8.8	-0.009
E-D	SY	RRW	qRRW9.7	RM160	9	82.4	9.7	0.030	9.9	0.006
E-D	MY	RRW	qRRW2.3	RM145	2	49.8	2.3	0.028	9.6	0.002
E-D	MY	RRW	qRRW3.12	RM514	3	216.4	3.12	0.049	7.9	-0.002
E-D	MY	RRW	qRRW12.2	RM155	12	20.9	12.2	0.016	11.5	0.003
E-D	SY	RSW	qRSW12.4	RM511	12	59.8	12.4	0.037	8.4	-0.010
E-D	MY	RSW	qRSW7.1	RM481	7	3.2	7.1	0.032	9.2	-0.004
E-D	MY	RSW	qRSW12.2	RM155	12	20.9	12.2	0.040	8.5	0.007
E-D	SY	RTW	qRTW7.7	RM47	7	90.0	7.7	0.039	8.7	-0.024
E-D	SY	RTW	qRTW12.4	RM511	12	59.8	12.4	0.024	10.2	-0.014
E-D	MY	RTW	qRTW7.1	RM481	7	3.2	7.1	0.038	8.7	-0.006
E-D	MY	RTW	qRTW10.6	RM590	10	117.2	10.6	0.046	8.0	-0.010
E-D	MY	RTW	qRTW12.2	RM155	12	20.9	12.2	0.035	9.0	0.010
E-D	SY	RWRSR	qRWRSR2.5	RM3688	2	88.2	2.5	0.020	10.8	-0.019
E-D	SY	RWRSR	qRWRSR6.1	RM589	6	3.2	6.1	0.022	10.3	-0.017
E-D	SY	RWRSR	qRWRSR10.5	RM184	10	58.0	10.5	0.008	13.4	0.015
E-D	MY	RWRSR	qRWRSR3.12	RM514	3	216.4	3.12	0.019	11.0	-0.015
E-D	MY	RWRSR	qRWRSR5.8	RM538	5	132.7	5.8	0.046	8.0	0.021
E-D	MY	RWRSR	qRWRSR11.4	RM287	11	68.6	11.4	0.048	7.9	0.012
E-D	MY	RWRSR	qRWRSR12.2	RM155	12	20.9	12.2	0.005	15.0	0.007
F-D	SY	RRL	qRRL2.4	RM521	2	62.2	2.4	0.005	14.5	0.392
F-D	SY	RRL	qRRL5.6	RM3295	5	91.2	5.6	0.003	16.6	-0.489
F-D	MY	RRL	qRRL4.8	RM349	4	146.8	4.8	0.006	15.1	0.835
F-D	MY	RRL	qRRL7.1	RM481	7	3.2	7.1	0.015	12.1	0.166
F-D	SY	RRW	qRRW2.4	RM290	2	66.0	2.4	0.047	8.5	0.004
F-D	SY	RRW	qRRW12.1	RM20A	12	3.2	12.1	0.049	7.7	0.003
F-D	SY	RRW	qRRW12.4	RM511	12	59.8	12.4	0.007	14.1	-0.004
F-D	MY	RRW	qRRW1.3	RM576	1	51.0	1.3	0.047	8.2	0.003
F-D	MY	RRW	qRRW4.4	RM142	4	68.5	4.4	0.016	11.5	-0.003
F-D	SY	RSW	qRSW1.2	RM283	1	31.4	1.2	0.036	8.5	0.012
F-D	MY	RSW	qRSW3.8	RM16	3	131.5	3.8	0.028	9.7	-0.012
F-D	MY	RSW	qRSW5.7	RM26	5	118.8	5.7	0.039	8.7	0.002
F-D	MY	RSW	qRSW7.1	RM481	7	3.2	7.1	0.006	14.6	-0.009
F-D	SY	RTW	qRTW1.2	RM283	1	31.4	1.2	0.039	8.4	0.015
F-D	SY	RTW	qRTW12.4	RM511	12	59.8	12.4	0.034	8.9	-0.017
F-D	MY	RTW	qRTW3.8	RM16	3	131.5	3.8	0.021	10.6	-0.017
F-D	MY	RTW	qRTW3.12	RM514	3	216.4	3.12	0.043	8.2	-0.007
F-D	MY	RTW	qRTW5.7	RM26	5	118.8	5.7	0.032	9.4	0.001
F-D	MY	RTW	qRTW7.1	RM481	7	3.2	7.1	0.005	15.4	-0.010
F-D	SY	RWRSR	qRWRSR9.3	RM219	9	11.7	9.3	0.044	8.0	0.024
F-D	MY	RWRSR	qRWRSR5.2	RM574	5	41.0	5.2	0.006	14.7	0.056
F-D	MY	RWRSR	qRWRSR6.3	RM510	6	20.8	6.3	0.012	12.6	0.043

D, Control; E, Nutrient solution with 1/3 phosphorus as the control; F, Nutrient solution without phosphorus; MY, Minghui 86/Yetuozai; SY, Shuhui 527/Yetuozai; RRL, Relative root length; RRW, Relative root dry weight; RSW, Relative shoot dry weight; RTW, Relative total dry weight; RWRSR, Relative root-shoot ratio of dry weight; R^2, Proportion of phenotypic variance explained by the QTL effect.

The significant level is $P < 0.05$. The positive additive effect represent favorable alleles were from donor parent, and the negative one represents the allele is from recurrent parent.

detected. For MY population, two QTLs for RRL, two for RRW, two for RWRSR, three for RSW and four for RTW were detected.

Eight QTLs were simultaneously detected under low P stress across two environments (Tables 4 and 5), including loci near RM283 on chromosome 1 associated with PN, RSW and RTW, near RM335 on chromosome 4 associated with SF, TGW and RRW, near RM26 on chromosome 5 associated with PN, RSW and RTW, near RM589 on chromosome 6 associated with PN and RWRSR, near RM481 on chromosome 7 associated with TGW, RRL, RSW and RTW, near RM219 on chromosome 9 associated with TGW and RWRSR, near RM155 on chromosome 12 associated with PN, RRW, RWRSR, RSW and RTW, as well as locus near RM511 on chromosome 12 associated with SNP, FGP, RRL, RRW, RSW and RTW.

DISCUSSION

Backcross breeding and phenotypic selection is effective for the improvement of a complex target trait (Xu et al, 2005; Zhang et al, 2007; He et al, 2010; Chen et al, 2011; Meng et al, 2013). In this study, the two selected IL populations were developed through large-scale backcross breeding and directional phenotypic selection. The results suggest that all the progenies showed transgressive segregation of LPT, indicating that the donor parent carried favorable 'hidden genes' of LPT. The ILs from SY population had superior yield performances than Shuhui 527 under treatment B, and they had similar yield level with Shuhui 527 under treatment C in Langfang. The ILs from MY population had significantly better yield performances than Minghui 86 under three treatments. The results demonstrate that ILs from MY population had higher average selection efficiency in response to low P stress than ILs from SY population in Langfang.

In addition, based on the yield performance under low P stress in Langfang, one IL from SY population and thirteen ILs from MY population were identified with significantly higher GY than their recurrent parents (Table 6). Compared with Shuhui 527, line BL66 from SY population under low P showed

Table 6. Trait performance of two IL populations with significant higher yield than the recurrent parents selected under low phosphorus stress conditions in Langfang, Hebei Province and Hefei, Anhui Province, China.

Type	Population	Material	Environment	HD (d)	PH (cm)	PN	PL (cm)	FGP	SNP	SF (%)	TGW (g)	GY (g)	BI (g)	HI
Type 1	MY	BL151	Langfang, 2013	**118.7**	**109.3**	5.1	**27.4**	204.6	230.2	88.5	29.0	18.6	43.0	0.43
			Hefei, 2012	94.0	**119.4**	6.9	26.8	**109.7**	160.6	68.3	**29.1**	21.7		
	MY	BL158	Langfang, 2013	115.7	105.8	6.2	**27.5**	175.7	**235.0**	74.6	**29.3**	23.8	51.6	0.46
			Hefei, 2012	93.0	106.4	7.7	23.6	107.6	135.4	**79.7**	**31.6**	25.8		
	MY	BL165	Langfang, 2013	**119.3**	100.6	6.7	25.5	170.4	234.6	73.1	20.6	19.5	46.7	0.42
			Hefei, 2012	**90.0**	115.4	8.3	24.9	92.3	126.9	72.5	**30.1**	22.8		
	MY	BL167	Langfang, 2013	115.3	101.2	6.3	25.2	143.6	203.8	70.5	**28.5**	20.9	41.1	**0.51**
			Hefei, 2012	94.0	107.7	8.3	24.2	105.2	140.6	74.9	25.9	22.7		
	MY	BL170	Langfang, 2013	116.7	108.1	4.7	**27.6**	**239.0**	**272.6**	87.7	26.9	18.7	37.3	0.50
			Hefei, 2012	93.0	120.4	6.0	23.7	97.7	125.2	78.0	27.6	16.2		
	MY	BL197	Langfang, 2013	117.7	93.2	4.5	27.2	170.0	214.8	79.1	25.3	18.5	37.1	0.50
			Hefei, 2012	92.0	108.0	7.7	25.9	92.0	150.1	61.4	**30.3**	21.4		
	MY	BL198	Langfang, 2013	117.3	100.4	5.8	**27.4**	202.8	**256.5**	77.9	28.0	22.4	**45.7**	0.49
			Hefei, 2012	92.0	111.9	**5.3**	24.3	82.5	142.3	58.9	28.5	12.5		
Type 2	SY	BL66	Langfang, 2013	116.7	105.9	6.7	24.9	180.5	221.2	81.6	29.2	**29.2**	**60.0**	0.49
			Hefei, 2012	92.0	109.5	7.4	24.4	**116.8**	148.8	78.6	**30.9**	26.5		
	MY	BL152	Langfang, 2013	**119.0**	101.9	6.2	25.7	216.4	248.9	87.0	25.7	26.3	49.9	0.53
			Hefei, 2012	94.0	111.5	7.8	26.7	96.2	142.1	67.9	**30.0**	22.4		
	MY	BL162	Langfang, 2013	117.3	**107.8**	5.7	**29.0**	211.3	245.3	86.2	**27.0**	26.2	51.0	0.51
			Hefei, 2012	94.0	109.7	6.4	27.7	**113.6**	157.8	71.9	28.5	20.4		
	MY	BL172	Langfang, 2013	116.7	**111.0**	5.7	27.2	139.5	169.2	82.5	27.8	18.2	37.0	0.49
			Hefei, 2012	91.0	109.6	8.7	24.7	87.2	108.9	**80.0**	**30.0**	22.7		
	MY	BL190	Langfang, 2013	117.3	**110.3**	5.1	**27.6**	171.9	208.8	82.6	29.5	23.0	**46.9**	0.49
			Hefei, 2012	91.0	**115.2**	8.4	24.9	103.3	140.6	73.6	**30.6**	26.3		
	MY	BL192	Langfang, 2013	**120.3**	97.2	4.5	25.5	174.3	217.4	79.7	26.3	19.5	40.3	0.48
			Hefei, 2012	**95.0**	102.4	8.0	23.0	85.7	131.5	65.2	28.7	19.6		
	MY	BL195	Langfang, 2013	118.0	106.9	5.1	28.2	150.1	225.4	66.4	**27.8**	17.6	34.6	0.51
			Hefei, 2012	93.0	**115.7**	6.2	25.2	84.0	138.8	60.4	**29.6**	15.6		
	Shuhui 527		Langfang, 2013	116.3	98.1	4.6	25.6	130.3	162.1	80.6	27.6	16.2	34.1	0.48
			Hefei, 2012	92.4	99.3	8.0	24.7	97.7	127.9	77.2	29.9	21.3		
	Minghui 86		Langfang, 2013	113.7	92.7	4.3	23.4	120.8	159.5	75.8	23.8	9.1	24.3	0.37
			Hefei, 2012	93.8	106.5	8.1	26.3	82.8	143.7	61.3	27.8	16.3		

MY, Minghui 86/Yetuozai; SY, Shuhui 527/Yetuozai; HD, Heading date; PH, Plant height; PN, Panicle number per plant; PL, Panicle length; FGP, Filled grain number per panicle; SNP, Spikelet number per panicle; SF, Spikelet fertility; TGW, 1000-grain weight; GY, Grain yield per plant; BI, Biomass; HI, Harvest index.
The bold values represent the significant difference at $P < 0.05$ between ILs and recurrent parent based on the t-test.

Table 7. Validation of five QTLs identified from Shuhui 527/Yetuozai population in Langfang (2013) and Hefei (2012) in China.

Trait	Marker	Chromosome	Position (cM)	Bin	QTL	Treatment	Langfang, Hebei Province			Hefei, Anhui Province		
							Probability	R^2 (%)	Additive	Probability	R^2 (%)	Additive
TGW	RM335	4	22.0	4.1	*qTGW4.1*	A	0.025	8.6	-1.1	0.010	11.0	-1.2
						C	0.006	12.3	-1.1	0.002	16.0	-1.4
TGW	RM5463	6	124.4	6.6	*qTGW6.6*	A	0.008	11.9	-1.0	0.003	17.0	-1.2
						C	0.004	13.7	-0.9	0.020	9.0	-0.9
TGW	RM481	7	3.2	7.1	*qTGW7.1*	C	0.032	7.7	0.8	0.050	6.0	0.8
TGW	RM219	9	11.7	9.3	*qTGW9.3*	A	0.017	9.5	-0.8	0.010	12.0	-0.9
						C	0.013	10.1	-0.7	0.002	15.0	-1.0
GY	RM254	11	110.0	11.6	*qGY11.6*	A	0.018	9.5	-1.1	0.010	11.0	-2.3

A, Normal fertilization treatment under normal soil; C, Low phosphorus stress under barren soil; TGW, 1000-grain weight; GY, Grain yield per plant; R^2, Proportion of phenotypic variance explained by the QTL effect.

superior yield performances than Shuhui 527 under three treatments with an increase of 13.0 g in GY. The 13 superior lines from MY population showed increase of 11.9 g (131.1%) in GY compared with Minghui 86. Based on the yield performance of these ILs under three treatments, they were divided into two types. ILs in type I showed significantly higher yield than the recurrent parent under treatments C and A. ILs in type II showed significantly higher yield than the recurrent parent under two or three treatments. It is noteworthy that line BL195 had significantly higher yield than Minghui 86, and its yield components i.e. PN, FGP and TGW showed superior performances than Minghui 86 (Table 6). Interestingly, 10 of the 14 ILs had significantly higher yield than their recurrent parents except the lines BL170, BL192, BL195 and BL198 which was shown from the previous study in Hefei in 2012 (Table 6). Therefore, the ILs in type I can serve as useful breeding materials for developing rice varieties with LPT. The ILs in type II are very promising for the development of LPT varieties. In addition, one superior IL (BL166) from SY population and two ILs (BL158 and BL165) from MY population were identified. They had significantly lower RWRSR than their recurrent parents under low P stress in Beijing. Furthermore, these lines showed significantly better yield performances than their recurrent parents in Langfang. Thus, RWRSR can be used as an index for LPT screening.

In combination with the identified loci affecting yield traits under low P stress, we analyzed genotypes of the 14 superior ILs which had significant yield performances than their recurrent parents under low P stress in Langfang, aiming to further dissect the genetic basis of LPT. Line BL66 from SY population carried the alleles from the donor at loci near RM164,

RM26, RM589, RM541, RM481 and RM536. It is noteworthy that *qTGW7.1* near RM481 was also detected in the same population in Hefei in 2012 (Table 7), indicating that locus near RM481 was an important QTL for the further improvement of LPT in the recurrent parent Shuhui 527. Furthermore, line BL66 carried the alleles from Shuhui 527 at loci near RM283, RM335, RM5463, RM219 and RM254. QTLs near RM335, RM5463, RM219 and RM254 were also detected in the same population in our previous study, indicating that these four loci played an important role in yield stability of Shuhui 527 under low P stress. Lines BL152, BL160, BL162, BL167, BL170, BL172, BL190, BL192 and BL198 from MY population carried the alleles from the donor parent at the locus near RM200. The result suggests that the locus near RM200 was an important locus for the further improvement of LPT in the recurrent parent Minghui 86. Furthermore, lines BL151, BL152, BL160, BL162, BL165, BL167, BL170, BL172, BL192 and BL198 from MY population carried the alleles from Minghui 86 at loci near RM335 and RM169, indicating that these two loci can be used as important candidate loci for improving yield in rice molecular breeding.

These selected ILs used for QTL mapping can not only improve the efficiency of QTL mapping, but also served as useful materials for practical breeding (Li et al, 2005). Based on the results of QTL mapping for yield traits in IL populations and the analysis of favorable alleles from the donor parent, we can judge which chromosome regions need to be replaced or kept in order to improve target traits. The above results suggest that the strategy of combining backcross introgression with target traits selection is effective for the improvement of LPT and discovery of favorable genes/QTLs, and will provide important

information for further molecular design breeding.

ACKNOWLEDGEMENTS

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