RESEARCH NOTE

# Comparison of quantitative trait loci for rice yield, panicle length and spikelet density across three connected populations

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## Introduction

Enhancing crop yield is one of the top priorities in crop breeding programmes. Among various crop breeding efforts, improving plant architecture has been proved to be a successful strategy. Panicle architecture covers several aspects such as spikelet density (SD), panicle length (PL), panicle curvature and so on, which are inherited in a quantitative manner and typically controlled by a plurality of major and minor quantitative trait loci (QTLs). The advent of molecular markers and QTL analysis methods provide an opportunity for characterizing these traits of panicle shape. However, most QTL analyses have been focussing on traits that are components of grain yield and quality (Yu *et al*. 1997; Xing *et al*. 2002; Hittalmani *et al*. 2003; Yoon *et al*. 2006; Fu *et al*. 2010). Other traits, such as PL and SD, have received relatively less attention (Lin *et al*. 1996; Hittalmani *et al*. 2003; Yoon *et al*. 2006).

A population derived from diverse parental materials increases the probability of polymorphism, it, thus, ascertains systemic QTL information of a trait-controlled QTL among different populations. Global comparison of the effects of segregation of all QTL alleles was not possible because it has few connections among different populations. Developing connected populations (one common parent among populations) is an alternative approach by which identical allelic effects can be achieved from the same QTL over populations. This reduces the total number of parameters and consequently increases the power of QTL detection (Rebai and Goffinet 1993; Jannink and Jansen 2001; Blanc *et al*. 2006). In addition, the effects of segregating alleles were estimated simultaneously by executing a global comparison among several populations, and the most beneficial alleles can be selected by evaluating the effects and the action direction of the same QTL among several populations (Liu *et al*. 2010b). Therefore, developing connected populations is an effective method to use the germplasm with different genetic background.

In this study, three sets of RILs were developed from the crosses between Zhenshan 97 and Minghui 63 (PZM), Zhenshan 97 and Teqing (PZT), and Minghui 63 and Teqing (PMT), respectively. QTL analyses on PL, SD and YPP were conducted in PZT and PMT. Combining the QTL results of two populations with the QTL mapping results in the PZM (Xing *et al*. 2001, 2002), QTL comparison was executed through three connected populations.

## Materials and methods

### *Experimental populations and phenotypic measurements*

The PZM, PMT and PZT populations and their planting methods have been described by Xing *et al*. (2002) and Liu *et al*. (2010a, b), respectively. After ripening, 10 plants in the middle of two rows of each plot was harvested respectively to score the following traits: spikelets per panicle (SPP), panicle length (PL, cm), spikelet density (SD) and yield per plant (YPP, g). The average trait measurements across the two replicates within each year were collected for data analyses.

### *Genetic linkage map and data analysis*

A total of 694 simple-sequence repeat (SSR) markers that were well distributed in the whole genome were chosen to screen polymorphism among the parents, Zhenshan 97,

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Minghui 63 and Teqing. Markers designing, polymorphic SSR markers genotyping and genetic linkage map construction for PZT and PMT populations are described by Liu *et al*. (2010a,b).

Composite interval mapping (CIM) was performed using QTL CARTOGRAPHER 2.0 (Wang *et al*. 2001-2003) on Windows. Forward stepwise regression was used to find significant markers as cofactors. The experimentwise LOD threshold significance level is determined by computing 1000 permutations ($P < 0.05$) as implemented by Windows QTL CARTOGRAPHER. The threshold of LOD values range between 2.7 and 2.9.

### *Comparative QTL analysis*

Comparison of QTL was executed by combining the QTL results of PZM for YPP, PL and SD (Xing *et al*. 2001, 2002) with the QTL identified in this study. The comparative method is as follows: the physical locations of flanking markers of QTL is ascertained from the database (http://www.gramene.org); the QTL identified in different populations for the same trait are considered to be identical if their 1-LOD confidential intervals overlap; the allele with the largest increasing effects at a given QTL is considered as beneficial allele among the three parents.

## Results

### *Variation of traits in two populations*

Descriptive statistics of PL, SD and YPP traits of PZT, PMT and three parents are given in table 1. Three parents present distinct characteristics, i.e. Teqing has large SD with short PL, Minghui 63 has small SD with long PL, whereas Zhenshan 97 has small SD and short PL. Except a transgressive segregation of SD observed in PMT towards low value, transgressive segregation for all traits is observed in both directions in two populations (table 1).

### *Genetic linkage map*

The polymorphic rates are respectively 32.8% and 33.4% between PZT, and PMT, among all the tested 694 SSR markers. In order to develop the genetic linkage maps for populations PZT and PMT, 176 and 133 genomewide evenly distributed SSR markers were chosen to genotype the populations. The PZT genetic map spans 1432.1 cM and the average genetic distance between neighbouring markers is of 8.1 cM. While the PMT genetic map spans 1371.4 cM and the average genetic distance between neighbouring makers is 10.4 cM.

### *QTL analysis*

In PZT, five YPP QTLs are detected on chromosomes 1, 5, 6, 7 and 11; the Teqing allele has an increase in yield. All the five QTLs are detected only in one year (figure 1; table 2).

For PL trait, four QTLs are detected on chromosomes 3, 6 and 10. Except *ZPL3* in which Teqing allele increases PL, the PL is detected within two years, whereas the other QTLs were detected in only one year and Zhenshan 97 allele increases the PL (figure 1; table 2).

Five SD QTLs were detected on chromosomes 1, 5, 7 and 11. *ZSD1*, *ZSD5* and *ZSD7a* are identified in two years, but *ZSD7b* and *ZSD12* in one environment only. The Zhenshan 97 allele of *ZSD5* increases the trait value and the Teqing allele of the other QTL increases the phenotype value (figure 1; table 2).

In PMT, four YPP QTLs were detected on chromosomes 4, 6, 9 and 11; *MYP4* and *MYP6* increase the yield by Minghui 63 allele, whereas *MYP9* and *MYP11* increase the yield by Teqing allele. All the four QTLs were detected in only one year (figure 1; table 2).

For PL trait, five QTLs were detected on chromosomes 2, 5, 6, 9 and 12. QTLs *MPL2* and *MPL9*, identified in one year, increased the trait value by Teqing alleles, whereas the other three QTLs, each identified in both two years, increase the trait value by Minghui 63 alleles (figure 1; table 2).

**Table 1.** Descriptive statistics of PL, SD and YPP traits of PZT, PMT and three parents.

| | | PZT | | PMT | | Parents | | |
|---|---|---|---|---|---|---|---|---|
| Trait | Year | Range | Mean±SD | Range | Mean±SD | Teqing | Zhenshan 97 | Minghui 63 |
| PL (cm) | 2004 | 18.4–25.7 | 21.8 ± 1.2 | | | 20.7 | 20.5 | |
| | 2005 | | | 18.5–28.5 | 24.0 ± 1.7 | 21.3 | | 25.2 |
| | 2006 | 19.1–26.2 | 22.1 ± 1.4 | 19.2–27.5 | 23.5 ± 1.5 | 19.4 | 21.2 | 25.6 |
| SD | 2004 | 3.8–12.0 | 8.2 ± 1.4 | | | 10.4 | 5.0 | |
| | 2005 | | | 4.3–9.7 | 6.6 ± 1.2 | 9.9 | | 5.5 |
| | 2006 | 3.6–10.5 | 7.4 ± 1.3 | 4.1–10.1 | 6.7 ± 1.2 | 10.2 | 5.2 | 5.3 |
| YPP (g) | 2004 | 15.0–41.0 | 28.5 ± 5.1 | | | 35.9 | 17.4 | |
| | 2005 | | | 5.7–56.1 | 26.7 ± 6.5 | 36.7 | | 31.7 |
| | 2006 | 6.7–47.3 | 22.2 ± 6.0 | 10.4–37.6 | 21.1 ± 4.5 | 36.2 | 18.6 | 28.1 |

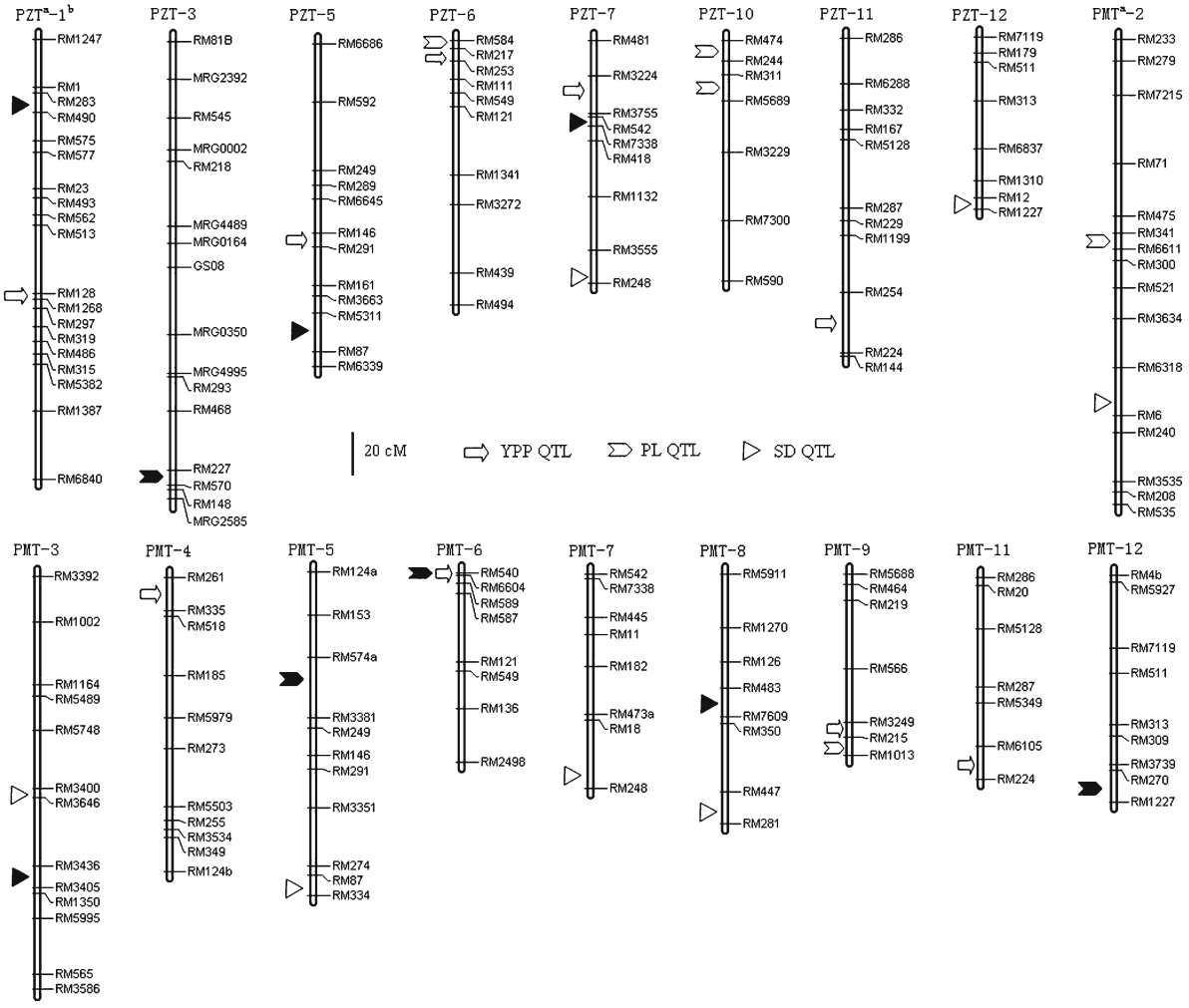


**Figure 1.** Molecular linkage map showing the position of QTL for yield per plant, panicle length and spikelet density identified in PZT and PMT. The black and white arrows indicate that the QTL was detected in both environments and single environment, respectively. [a]PZT and PMT indicate that the RIL population derived from crosses between Zhenshan 97 and Teqing, and Minghui 63 and Teqing, respectively; [b] the chromosome no.

Seven SD QTLs were detected on chromosomes 2, 3, 5, 7, and 8. Except *MSD5*, all QTLs increase the trait value by Teqing allele. QTLs *MSD3b*, *MSD7* and *MSD8b* were detected in two years, whereas the other QTLs in one year (figure 1; table 2).

### *QTL comparison across three connected populations*

One QTL (*ZSD7b/MSD7/dn7b*) is commonly identified in three populations and 12 QTLs were commonly detected in two populations. Eight QTLs for each trait is detected only in one population. Hence, a total of 37 QTLs were identified among three populations.

YPP QTLs, 5, 4 and 5 were detected in PZT, PMT and PZM, respectively. Of them, 3, 1 and 2 QTLs in PZT, PMT and PZM were commonly identified in two populations (table 3). *ZYP1* and *ZYP6* of PZT commonly detected in PZM are named as *yd1a* and *yd6*. The Teqing allele increases 1.23 and 2.35 g, respectively, whereas Minghui 63 allele increases 1.2 and 1.29 g respectively. It suggests that Teqing allele is the most beneficial allele at loci of *ZYP1/yd1a* and *ZYP6/yd6*. *ZYP11* of PZT is commonly detected in PMT named as *MYP11*, and the yield of Teqing allele increases in both populations, which suggests it as the most beneficial allele at locus of *ZYP11/MYP11*.

A total of five PL QTLs were commonly identified in two populations. Of them, three QTLs, *MPL2/pl2*, *MPL5/pl5* and *MPL9/pl9*, were commonly detected in PMT and PZM where as Minghui 63 and Teqing alleles were the most beneficial allele in *MPL5/pl5* and the other loci. One QTL, *ZPL6/MPL6*, is commonly detected in PZT and PMT and the detected Minghui 63 and Zhenshan 97 alleles were more beneficial than Teqing allele; *ZPL10b/pl10a* is commonly detected in PZT and PZM where Zhenshan 97 allele

**Table 2.** QTL identified for YPP, PL and SD from PZT and PMT.

| Population | Trait | QTL | Interval | Chr. | LOD | ADD[a] | Var.[b](%) |
|---|---|---|---|---|---|---|---|
| PZT | YP (g) | *ZYP1* | RM128–RM1268 | 1 | 2.9/– | 1.23/– | 5.7/– |
| | | *ZYP5* | RM146–RM291 | 5 | –/3.3 | –/2.96 | –/7.8 |
| | | *ZYP6* | RM217–RM253 | 6 | –/3.2 | –/2.35 | –/6.3 |
| | | *ZYP7* | RM3755–RM3224 | 7 | 2.8/– | 1.18/– | 5.3/– |
| | | *YPP11* | RM224–RM254 | 11 | –/3.2 | –/2.90 | –/10.5 |
| | PL (cm) | *ZPL3* | RM227–RM570 | 3 | 7.0/3.1 | 0.58/0.39 | 22.3/6.0 |
| | | *ZPL6* | RM584–RM217 | 6 | –/7.7 | –/–0.63 | –/14.5 |
| | | *ZPL10a* | RM244–RM474 | 10 | 3.4/– | –0.34/– | 7.42/– |
| | | *ZPL10b* | RM5689–RM311 | 10 | –/3.1 | –/–0.38 | –/5.6 |
| | SD | *ZSD1* | RM490–RM283 | 1 | 11.6/11.4 | 0.66/0.65 | 20.0/23.5 |
| | | *ZSD5* | RM5311–RM87 | 5 | 2.9/2.9 | –0.37/–0.34 | 6.2/6.5 |
| | | *ZSD7a* | RM7338–RM542 | 7 | 7.8/6.4 | 0.52/0.45 | 12/10.9 |
| | | *ZSD7b* | RM248–RM3555 | 7 | 5.9/– | 0.45/– | 9.1/– |
| | | *ZSD12* | RM1227–RM12 | 12 | 5.1/– | 0.4/– | 7.5/– |
| PMT | YPP (g) | *MYP4* | RM518–RM185 | 4 | –/3.7 | –/–1.49 | –/10.3 |
| | | *MYP6* | RM540–RM6604 | 6 | 2.8/– | –1.43/– | 5.6/– |
| | | *MYP9* | RM3249–RM215 | 9 | 2.7/– | 1.49/– | 5.9/– |
| | | *MYP11* | RM6105–RM224 | 11 | –/4.2 | –/1.32 | –/8.7 |
| | PL (cm) | *MPL2* | RM341–RM6611 | 2 | –/4.3 | –/0.43 | –/8.4 |
| | | *MPL5* | RM574a–RM3381 | 5 | 6.4/4.1 | –0.61/–0.51 | 11.8/11.1 |
| | | *MPL6* | RM540–RM6604 | 6 | 9.5/3.4 | –0.65/–0.37 | 13.2/6.0 |
| | | *MPL9* | RM215–RM1013 | 9 | 6.8/– | 0.60/– | 10.9/– |
| | | *MPL12* | RM270–RM1227 | 12 | 6.8/4.3 | –0.54/–0.50 | 9.1/10.2 |
| | SD | *MSD2* | RM6318–RM6 | 2 | –/5.2 | –/0.42 | –/11.8 |
| | | *MSD3a* | RM3400–RM3646 | 3 | 4.9/– | 0.33/– | 7.7/– |
| | | *MSD3b* | RM3405–RM3436 | 3 | 5.9/3.8 | 0.37/0.33 | 10.2/7.6 |
| | | *MSD5* | RM87–RM334 | 5 | –/4.5 | –/–0.34 | –/8.4 |
| | | *MSD7* | RM18–RM248 | 7 | 5.3/3.5 | 0.46/0.29 | 16.5/5.8 |
| | | *MSD8a* | RM281–RM447 | 8 | 4.0/– | 0.33/– | 8.1/– |
| | | *MSD8b* | RM7609–RM483 | 8 | 3.1/4.0 | 0.28/0.33 | 6.0/7.9 |

[a]Additive effect, positive additive indicates that the Teqing allele increased the trait in two population; [b]percentage of total phenotypic variance explained by the QTL.

**Table 3.** Comparison of QTL across three connected RIL populations.

| | | | PZT (2004/2006) | | | PMT (2005/2006) | | | PZM (1997/1998) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Chr. | BA[a] | QTL | Interval | A[b] | QTL | Interval | A[b] | QTL | Interval | A[b] |
| YPP | 1 | TQ | *ZYP1* | RM128–RM1268 | 1.23/– | | | | *yd1a* | C922–RG101 | –/1.2 |
| | 6 | TQ | *ZYP6* | RM217–RM253 | –/2.35 | | | | *yd6* | RZ667–RG424 | –/1.29 |
| | 11 | TQ | *ZYP11* | RM224–RM254 | –/2.90 | *MYP11* | RM6105–RM224 | –/1.32 | | | |
| PL | 2 | TQ | | | | *MPL2* | RM341–RM6611 | –/0.43 | *pl2* | R712–RZ324 | –0.21 |
| | 5 | MH | | | | *MPL5* | RM574a–RM3381 | –0.61/–0.51 | *pl5* | RG360–c734b | 0.28 |
| | 6 | ZS or MH | *ZPL6* | RM584–RM217 | –/–0.63 | *MPL6* | RM540–RM6604 | –0.65/–0.37 | | | |
| | 9 | TQ | | | | *MPL9* | RM215–RM1013 | 0.60/– | *pl9* | RG570–RG667 | –0.42 |
| | 10 | ZS | *ZPL10b* | RM5689–RM311 | –/–0.38 | | | | *pl10a* | RM239–C1633 | –0.26 |
| SD | 1 | TQ | *ZSD1* | RM490–RM283 | 0.66/0.65 | | | | *dn1a* | G359–RG532 | 0.26 |
| | 3 | TQ | | | | *MSD3a* | RM3400–RM3646 | 0.33/– | *dn3* | C1087–RZ403 | –0.28 |
| | 5 | ZS or MH | *ZSD5* | RM5311–RM87 | –0.37/–0.34 | *MSD5* | RM87–RM334 | –/–0.34 | | | |
| | 7 | TQ | *ZSD7a* | RM7338–RM542 | 0.52/0.45 | | | | *dn7a* | C1023–R1440 | 0.43 |
| | 7 | TQ | *ZSD7b* | RM248–RM3555 | 0.45/– | *MSD7* | RM18–RM248 | 0.46/0.29 | *dn7b* | R1245–RM234 | –0.16 |

The QTL information for YPP in PZM quoted from Xing *et al*. (2002), for SD and PL in PZM from Xing *et al*. (2001).
[a]The most beneficial alleles; TQ, MH and ZS represent Teqing, Minghui 63 and Zhenshan 97, respectively.
[b]Additive effect, positive additive effect indicates that the Minghui 63 allele increased the trait in PZM, the Teqing allele increased the trait in PZT, and the Teqing allele increased the trait in PMT.

increases yield in both populations. This suggests Zhenshan 97 allele is the most beneficial allele at that locus.

SD QTLs, 4 and 1 were commonly identified in two populations and three populations, respectively. *ZSD7b/MSD7/dn7b* were commonly detected in three populations; *ZSD1/dn1a* and *ZSD7a/dn7a* were commonly detected in PZT and PZM; *MSD3a/dn3* was commonly identified in PMT and PZM, and *ZSD5/MSD5* were commonly detected in PZT and PMT. Except the locus of *ZSD5/MSD5*, where Minghui 63 and Zhenshan 97 allele were more beneficial than Teqing allele, Teqing allele in all other QTL loci is the most beneficial one.

## Discussion

### *The ability of QTL detection*

Comparison of QTL across connected populations could improve the ability of QTL detection, by validating whether the QTL can be identified in similar region in two connected populations by the third population (Jannink and Jansen 2001; Blanc *et al*. 2006). Taking *ZYP1* of PZT and *yd1a* of PZM, both were detected in similar region. Teqing allele of *ZYP1* increases the yield by 1.23 g, compared to the Zhenshan 97 allele in PZT. Similarly, Minghui 63 allele of *yd1a* increases YPP by 1.2 g compared to the Zhenshan 97 allele in PZM. If QTL of *ZYP1* and *yd1a* are identical, Teqing allele may possibly increases no more than 0.3 g compared with that of Minghui 63 allele, which is too small to be significantly detected in PMT. As a matter of fact, it is validated by the fact that no yield QTL is detected in PMT, which suggests that *ZYP1* and *yd1a* are with the same QTL.

### *Ascertainment of beneficial allele*

A good strategy of multi allelic study and the most beneficial allele ascertainment is to compare QTL among connected populations which own a common parent between two populations proposed by Liu *et al*. (2010b). Identification of the beneficial allele would be useful for molecular design breeding. In this study, of all the 13 loci detected commonly in multi populations, Teqing allele is the best for trait expression at nine loci (table 3); Zhenshan 97 and Minghui 63 alleles are the best at locus of *ZPL10b/pl10a* and *MPL5/pl5*, respectively. The best alleles at loci of *ZPL6/MPL6* and *ZSD5/MSD5* are uncertain between two better alleles Minghui 63 and Zhenshan 97.

### *Panicle length and spikelet density affecting rice yield*

In this study, a significant positive correlation was observed between the two panicle architecture traits (SD and PL). But no QTL for SD and PL shares the same interval with yield QTL except *MPL6*. A possible reason might be that yield is a complex trait with a low heritability, which suggested yield was controlled by many minor QTLs frequently interacting with environments; and the LOD threshold value claiming a QTL in this study was higher stringent, leading to a failed detection of a small effect QTL. Basically, even though as yield component, few QTLs can be located in the same region of yield QTL (Hittalmani *et al*. 2003; Fu *et al*. 2010). Another reason is that yield components are often negative corrected. If one QTL or two closely linked QTLs involved such negative correlated trait, the genetics effect of these gene or genomic region on yield would cancelled each other, leading to a failed detection of QTL for yield.

Few studies have been conducted on SD, and little is known about its genetic basis at molecular level. However, it is speculated that genes/QTLs involved in SD and PL could also affect rice yield.

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