RESEARCH ARTICLE

Mapping Quantitative Trait Loci Associated with Toot Traits Using Sequencing-Based Genotyping Chromosome Segment Substitution Lines Derived from 9311 and Nipponbare in Rice (Oryza sativa L.)

Yong Zhou1☯, Guichun Dong1☯, Yajun Tao1, Chen Chen1, Bin Yang1, Yue Wu1, Zefeng Yang1, Guohua Liang1, Baohe Wang2*, Yulong Wang1*

1 Jiangsu Key Laboratory of Crop Genetics and Physiology/Co-Innovation Center for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, China, 2 Lixiahe Agricultural Research Institute of Jiangsu Province, Yangzhou 225007, China

☯ These authors contributed equally to this work.
* ylwang@yzu.edu.cn (YLW); wangbhyznks@126.com (BW)

Abstract

Identification of quantitative trait loci (QTLs) associated with rice root morphology provides useful information for avoiding drought stress and maintaining yield production under the irrigation condition. In this study, a set of chromosome segment substitution lines derived from 9311 as the recipient and Nipponbare as donor, were used to analysis root morphology. By combining the resequencing-based bin-map with a multiple linear regression analysis, QTL identification was conducted on root number (RN), total root length (TRL), root dry weight (RDW), maximum root length (MRL), root thickness (RTH), total absorption area (TAA) and root vitality (RV), using the CSSL population grown under hydroponic conditions. A total of thirty-eight QTLs were identified: six for TRL, six for RDW, eight for the MRL, four for RTH, seven for RN, two for TAA, and five for RV. Phenotypic effect variance explained by these QTLs ranged from 2.23% to 37.08%, and four single QTLs had more than 10% phenotypic explanations on three root traits. We also detected the correlations between grain yield (GY) and root traits, and found that TRL, RTH and MRL had significantly positive correlations with GY. However, TRL, RDW and MRL had significantly positive correlations with biomass yield (BY). Several QTLs identified in our population were co-localized with some loci for grain yield or biomass. This information may be immediately exploited for improving rice water and fertilizer use efficiency for molecular breeding of root system architectures.
Introduction

Rice (*Oryza sativa* L.) is one of the most important food sources. With the booming population around the world, we have to produce 40% more rice to reduce the food crisis [1]. Rice has the greatest water requirement of all cereal crops, requiring 3000~5000 liters of water per kilogram of grain produced in flooded fields. Rice plants often experience drought in environments when rainfall is not sufficient to maintain flooded paddy conditions. As an important organ of the plant, the roots are involved in the acquisition of water and nutrients, and in the synthesis of plant hormones [2]. In previous studies, a strong correlation was found between root morphology and grain yield or biomass yield [3,4]. Therefore, study on rice root is of meaningful. Root morphology breeding is thought to be an important strategy to achieve a new breakthrough of rice high breeding in the future [5].

Root morphology includes root length, root number, root thickness, root weight, root vitality and total absorption area, etc. All these physiological and morphological traits of roots affect shoot growth [6]. For example, the maximum root length determines the efficiency of water and nutrition uptake, while root number, root thickness, and root length density determine the intensity of colonization of the soil profile [7]. Generally speaking, thick roots can decrease the risk of cavitations and facilitate water flux [8]. Several studies indicated that root biomass is strongly correlated with aboveground biomass [2]. Root oxidation activity is regarded as an important index of root physiological activity [2,9,10]. Root vitality represents the strength of metabolism, which further determines the growth of leaf and the level of grain yield, and root total absorption area reflects the ability of nutrition utilization. As a result, the rice root traits have been widely studied from the perspective of genetics and physiology.

Mutants of root traits are well materials for study on root development. Genetic approaches in a series of root mutants, such as crl1, crl4/Osgnom1, wox11, Oscand1, and Osfh1 have contributed to our understanding of the genetic mechanisms underlying root growth and development [11–17]. In addition, transgenic studies have also provided evidence that several genes are involved in rice root development, such as *OsARF12*, *OsPID1*, *OsNAC6*, and *IAA3* [18–21]. These cloning of genes associated with root morphology provide a theoretical basis for root growth. However, these mutants are difficult for breeding, because most of them have obvious negative effects on grain yield or plant growth. Most agronomic traits, including those of the root, are quantitative traits. Many QTLs associated with root morphological traits have been characterized. Using different populations, more than 600 QTLs have been mapped. Champoux et al. firstly reported QTLs associated with five root parameters, including maximum root length, root dry weight per tiller, root/shoot ratio, deep root dry weight per tiller and root thickness, using the 203 recombinant inbred lines (RILs) derived from *indica* cultivar Co39 and *japonica* cultivar Moroberekan [22]. Subsequently, Price and Tomos mapped QTLs for eight root growth characteristics using an F2 population derived from two drought-resistant rice varieties, Bala and Azucena [23]. Yadav et al. identified QTLs related to root traits using a doubled haploid (DH) population [24]. Price et al. identified 24 regions containing QTLs for different root traits in 140 RILs derived from Bala and Azucena [25]. Venuprasad et al. tagged several QTL associated with root morphological traits from the doubled haploid population of IR64 and Azucena [26]. Courtois et al. located QTLs related to several constitutive root traits, including maximum root length, root thickness and root dry weight in various layers in 125 RILs of IAC165 and Co39 [27]. Zheng et al. mapped QTLs related to root traits and screened two candidate genes from expressed sequence tags (ESTs) and cDNA-amplification length polymorphisms (AFLP) clones [28]. Yue et al. employed 180 RILs developed from Zhenshan 97 and IRAT109, and investigated 36 QTLs for five root traits under control, and 38 for seven root traits under drought stress conditions [29]. Courtois et al. detected 51 unique loci...
associated with root traits adopting genome-wide association mapping using 167 *japonica* accessions [30].

So far, only four QTLs for rice root morphology have been fine mapped. *Sta1*, a QTL determining stele transversal area, was delimited in a 359-Kb interval on rice chromosome 9 using BC₂F₃ and BC₂F₄ populations [31]. *qRL6.1*, a major QTL for root elongation, was fine mapped into a 337-Kb region using a CSSL and its derived population [32]. *Dro1*, controlling rice deep root, was narrowed into a 608.4-Kb segment [33]. Recently, Uga et al. identified *qSOR1*, a major QTL involved in soil-surface rooting in paddy fields and located it to an 812-kb interval using seven BC₂F₃ recombinant lines[34]. More recently, *Dro1* was cloned into a 6-kb interval [35].

Advanced populations, such as chromosome segment substituted lines (CSSLs), have same genetic background with recurrent parent, except the donor segments. In order to analyze the genetic basis of rice root development and detecting favorable genes related with root traits, we identified 38 QTLs related to seven root traits using a set of resequencing-genotyped CSSLs under hydroponic conditions. These results provide a valuable contribution to the genetic analysis of root morphology.

**Materials and Methods**

We state clearly that no specific permissions were required for these locations.

**Plant materials**

A set of 128 CSSLs with a *japonica* cultivar, Nipponbare, as the donor and an *indica* cultivar, 9311, as the recurrent parent was generated as previously reported [36]. Each CSSL line was genotyped, and a high quality physical map of ultrahigh-density single nucleotide polymorphisms (SNPs) based on whole-genome re-sequencing data was constructed. Every CSSL had approximately 60,000 SNPs. On the basis of the physical locations and genotypes of these SNPs, each CSSL was genotyped, and a physical map of the 128 CSSLs was constructed [36]. In this study, the CSSLs population was employed for QTLs mapping of rice root traits and other related traits. However, the phenotypic data of three CSSLs were not obtained because of abnormal growth.

**Hydroponic culture**

The experiment was carried out in Yangzhou University, Yangzhou, China (latitude 32°24′ N, longitude 119°26′ E) in summer in 2012. The CSSLs and their parents were grown for this experiment under hydroponic conditions, as previously described [37]. All rice seedlings were solution grown in eight concrete ponds with the inner dimensions of 880.0 cm long by 130.0 cm wide by 50 cm deep. Iron tubes at the bottom of the ponds connected the ponds to one another. The ponds were covered with concrete planks (135.0 cm long by 16.7 cm wide by 2.5 cm in deep), and each plank contained 14 holes (4 cm in diameter) for seedling fixation. Plump seeds were surface-sterilized in a 2.5% NaClO solution for 15 min, and then washed three times with distilled water. Seeds were germinated in an illuminated incubator at 30°C. Rice seedlings were transplanted from the soil seed bed 30 days after germination, and fixed into the holes with a sponge, one hole for one seedling. Fifty-six seedlings were prepared for each line in a randomized complete design with two replicates.

The nutrient solution was the mixture of the Epsino nutrient solution and the Arnon microelement nutrient solution. The mixed nutrient solution was filled into the ponds at transplanting and refreshed every 10 days. The pH value of nutrient solution was monitored daily and maintained between 5.5 and 6.5 by adding diluted H₂SO₄. A pump was used to keep
the solution continuously cycling during the whole growth period of the rice materials, to maintain the uniformity of pH and nutrient concentration in solution ponds and to improve the O2 supply.

Evaluation of phenotypes

Plants were sampled at the mature stage. Seven root traits, including root number (RN), total root length (TRL), root dry weight (RDW), maximum root length (MRL), root thickness (RTH), total absorption area (TAA) and root vitality (RV), were evaluated. For RN, only the first ramification was counted. For MRL, the length of the root was measured from the base of the plant to the tip of the longest root. TRL was the total length of the whole roots and calculated as MRL x RN/2. RTH was the average diameter of roots at the middle position of the root per plant. For RW, after deactivating enzymes at 105°C for 10 min, the roots were dried at 75°C to constant weight, and then weighed. For RV, we adopt the method of α-NA to measure root vitality [38]. The measure of TAA was by reference to the method of Xiao et al. [39].

We also measured several yield traits, including panicle number per plant (PN), number of spikelets per panicle (SPP), grain weight (GW), seed setting rate (SSR), biomass yield (BY) and grain yield (GY) during the mature stage. PN was the number of effective panicles with 10 or more grains. SPP was measured as the total number of spikelets of the whole plant divided by its total number of panicles. Grain weight was calculated on the basis of 100 grains and converted to 1000-grain weight. SSR was calculated as the number of filled grains per panicle divided by the total number of spikelets per panicle. For BY, after deactivating enzymes at 105°C for 10 min, the aboveground organ were dried at 75°C to constant weight, and then weighed. For GY, total grains per plant were dried naturally after harvesting and stored at room temperature for at least 1 month before weighing.

QTLs analysis

QTLs analysis was conducted according to a previously published method [36]. The multiple linear model was used as the main effect model. The mean value of the i-th line of population is defined as $y_i$, the mean of the population is defined as the $b_0$ in the whole genome, overall number of bins is defined as $m$, the main effect related to bin $k$ is defined as $b_k$, donor parent bin denotes $x_{ik} = 1$, recurrent parent bin denotes $x_{ik} = -1$. $e_i$ denotes the residual error:

$$y_i = b_0 + \sum_{k=1}^{m} b_k x_{ik} + e_i$$

Results

Phenotypic variation

The phenotypic value of the seven root traits of the parents and CSSL population were summarized in Table 1 and Fig 1. The two parents, 9311 and Nipponbare, showed highly significant differences in all the root traits examined. 9311 produced more, longer and heavier roots than Nipponbare (Table 1). Extensive variations and a normal distribution among CSSLs were observed for the most traits, which is in accord with the characteristics of quantitative traits (Fig 1). Except MRL, the mean phenotypic values of other six root morphology in the CSSLs population were closer to those of parent 9311 (Table 1). We can explain that the CSSLs have the 9311 genetic backgrounds.

Correlations among root morphological traits. Table 2 showed the pair-wise correlation coefficients among the seven root traits. Three among them, RN, TRL and RDW, had significant
positive correlation with each other, indicating that these three traits promote each other. In addition, MRL had significant positive correlation with TRL. TRL had significant positive correlation with RDW, RTH and MRL. Positive correlation was also found between RTH and RV. Except this, a negative correlation was found between RN and MRL.

We also carried out the correlation analysis between the seven root traits and yield traits (Table 3). BY had significant positive correlation with TRL, RDW and MRL, respectively. Positive correlations were also found between GY and TRL, RTH and MRL. These data suggested that these three root traits are closely related to grain and biomass yield. Positive correlations were also found between TRL and PN, RTH and SSR, GW and MRL, SSR and TAA, respectively.

QTLs identification for root morphological traits

Thirty-eight QTLs were initially detected for the seven root traits on all chromosomes, except chromosome 12.

TRL. Six QTLs controlling TRL were finally detected. These QTLs were qTRL2.1, qTRL5.1, qTRL6.1, qTRL8.1, qTRL10.1 and qTRL11.1, which were located in X73 on chromosome 2, X205.

Table 1. Mean values and ranges of the seven root traits in the parents and CSSLs.

| Traits   | CSSLs  | 9311   | Parents |
|----------|--------|--------|---------|
| RN       | 153.17 ± 13.39 | 123.78–189.00 | 164.00 ± 5.69 | 125.67 ± 9.84 |
| TRL (cm) | 3730.69 ± 658.02 | 1486.67–5100.00 | 3800.00 ± 1652.27 | 3030.00 ± 984.23 |
| RDW (g)  | 2.56 ± 0.30  | 1.79–3.43 | 2.65 ± 0.28 | 1.78 ± 0.24 |
| RTH (mm) | 1.18 ± 0.12  | 0.93–1.48 | 1.08 ± 0.08 | 0.93 ± 0.19 |
| MRL (cm) | 46.50 ± 8.57 | 29.67–69.00 | 37.00 ± 1.00 | 47.83 ± 8.80 |
| TAA (m²) | 19.00 ± 1.62 | 15.50–24.73 | 20.90 ± 3.47 | 24.63 ± 5.60 |
| RV (ug g⁻¹) | 1326.19 ± 554.00 | 532.04–6337.74 | 1878.91 ± 318.27 | 760.72 ± 110.96 |

doi:10.1371/journal.pone.0151796.t001

Fig 1. Distributions of chromosome segment substitution lines for the seven root traits.

doi:10.1371/journal.pone.0151796.g001
on chromosome 5, X242 on chromosome 6, X292 on chromosome 8, X347 on chromosome 10 and X388 on chromosome 11, respectively. The QTL with the largest effect was mapped to X388 and occupied to the physical position of 26,318,602 bp to 30,828,668 bp (Table 4).

**RDW.** Six QTLs associated with RDW were located on chromosomes 3, 6, 8 and 11, respectively. These QTLs were qRDW3, qRDW6, qRDW8, qRDW8, qRDW8, qRDW11, which were located in X137 on chromosome 3, X233 on chromosome 6, X278 on chromosome 8, X284 on chromosome 8, X286 on chromosome 8 and X377 on chromosome 11, respectively. The QTL with the largest effect was mapped to X233 and occupied to the physical position of 7,814,673 bp to 9,668,398 bp (Table 5).

**MRL.** Similarly, we detected eight QTLs controlling MRL under hydroponic conditions. These QTLs were qMRL5, qMRL6, qMRL7, qMRL8, qMRL9, qMRL9, qMRL10 and qMRL11, which were located in X207 on chromosome 5, X233 on chromosome 6, X262 on

| Bin       | QTLs  | Interval / bp       | Size of the interval / bp | Chr. | Partial R-Square | F value |
|-----------|-------|---------------------|---------------------------|------|------------------|---------|
| X73       | qTRL2.1 | 19526907–19710693   | 183786                  | 2    | 5.88%            | 9.79    |
| X205      | qTRL5.1 | 19013592–19052307   | 38715                   | 5    | 6.86%            | 10.67   |
| X242      | qTRL6.1 | 21140686–22187608   | 1046922                 | 6    | 6.24%            | 11.27   |
| X292      | qTRL8.1 | 11500666–14235575   | 2734909                 | 8    | 4.18%            | 8.66    |
| X347      | qTRL10.1 | 17928505–18055332  | 126827                  | 10   | 5.57%            | 10.45   |
| X388      | qTRL11.1 | 26318602–30828668   | 4510066                 | 11   | 14.01%          | 20.21   |

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

doi:10.1371/journal.pone.0151796.t004
chromosome 7, X291 on chromosome 8, X316 on chromosome 9, X318 on chromosome 9, X352 on chromosome 10 and X388 on chromosome 11, respectively. The QTL with the largest effect was mapped to X207 and occupied to the physical position of 19,287,670 bp to 19,403,538 bp (Table 6).

**RN.** Seven QTLs for RN were detected on chromosomes 1, 3, 5, 6, 9 and 11, respectively. These QTLs were $qRN1.1$, $qRN3.1$, $qRN3.2$, $qRN5.1$, $qRN6.1$, $qRN9.1$ and $qRN11.1$, which were located in X8 on chromosome 1, X131 on chromosome 3, X139 on chromosome 3, X221 on chromosome 5, X241 on chromosome 6, X321 on chromosome 9 and X380 on chromosome 11, respectively. The QTL with the largest effect was mapped to X207 and occupied to the physical position of 19,120,157 bp to 19,494,142 bp (Table 7).

**RTH.** Four putative QTLs associated with RTH were detected on chromosomes 1, 2, 5 and 10, respectively. These QTLs were $qRTH1.1$, $qRTH2.1$, $qRTH5.1$, and $qRTH10.1$, which were located in X24 on chromosome 1, X64 on chromosome 2, X193 on chromosome 5 and X347 on chromosome 10, respectively. The QTL with the largest effect was mapped to X347 and occupied to the physical position of 17,928,505 bp to 18,055,332 bp (Table 8).

### Table 5. QTLs mapping of RDW.

| Bins | QTLs | Interval / bp | Size of the interval / bp | Chr. | Partial R-Square | F value |
|------|------|---------------|--------------------------|------|------------------|---------|
| X137 | $qRDW3.1$ | 22449287–22467112 | 17825 | 3 | 6.15% | 10.78 |
| X333 | $qRDW6.1$ | 7814673–9668398 | 1853725 | 6 | 9.15% | 12.49 |
| X278 | $qRDW8.1$ | 2797909–3363094 | 538176 | 8 | 7.37% | 11.96 |
| X284 | $qRDW8.2$ | 6251746–7203791 | 952045 | 8 | 8.27% | 12.31 |
| X286 | $qRDW8.3$ | 7817482–8945724 | 1128242 | 8 | 4.22% | 8.39 |
| X377 | $qRDW11.1$ | 7416940–10601700 | 3184760 | 11 | 5.01% | 9.39 |

### Table 6. QTLs mapping of MRL.

| Bins | QTLs | Interval / bp | Size of the interval / bp | Chr. | Partial R-Square | F value |
|------|------|---------------|--------------------------|------|------------------|---------|
| X207 | $qMRL5.1$ | 19287670–19403538 | 115868 | 5 | 9.22% | 12.59 |
| X333 | $qMRL6.1$ | 7814673–9668398 | 1853725 | 6 | 3.42% | 6.98 |
| X262 | $qMRL7.1$ | 8527898–8572465 | 44567 | 7 | 4.68% | 8.61 |
| X291 | $qMRL8.1$ | 90684529–11500666 | 1816137 | 9 | 6.67% | 9.76 |
| X316 | $qMRL9.1$ | 798035–7638718 | 9 | 7.06% | 12.21 |
| X318 | $qMRL9.2$ | 9688069–11709751 | 2021682 | 9 | 3.82% | 8.26 |
| X352 | $qMRL10.1$ | 18855550–20084853 | 1229303 | 10 | 7.13% | 11.29 |
| X388 | $qMRL11.1$ | 26318602–30828668 | 4510066 | 11 | 3.95% | 7.66 |

### Table 7. QTLs mapping of RN.

| Bins | QTLs | Interval / bp | Size of the interval / bp | Chr. | Partial R-Square | F value |
|------|------|---------------|--------------------------|------|------------------|---------|
| X8   | $qRN1.1$ | 12001587–12038149 | 36562 | 1 | 6.31% | 8.96 |
| X131 | $qRN3.1$ | 15131566–15146848 | 15282 | 3 | 3.62% | 6.95 |
| X139 | $qRN3.2$ | 23156128–24417950 | 1261822 | 3 | 6.36% | 9.68 |
| X221 | $qRN5.1$ | 25834805–26310200 | 475395 | 5 | 5.40% | 9.34 |
| X241 | $qRN6.1$ | 20704751–21140686 | 435935 | 6 | 5.39% | 8.71 |
| X281 | $qRN9.1$ | 14738433–15449101 | 710668 | 9 | 4.29% | 7.85 |
| X380 | $qRN11.1$ | 19120157–19494142 | 373985 | 11 | 7.14% | 9.53 |
TAA. Two QTLs for TAA were detected on chromosomes 2 and 11, respectively. These QTLs were \( q\text{TAA}2.1 \) and \( q\text{TAA}11.1 \), which were located in \( X_{66} \) on chromosome 2 and \( X_{387} \) on chromosome 11, respectively. The QTL with the largest effect was mapped to \( X_{387} \) and occupied to the physical position of 25,559,185 bp to 26,317,711 bp (Table 9).

RV. Five putative QTLs associated with RV were detected on chromosomes 3, 4 and 5 respectively. These QTLs were \( q\text{RV}3.1 \), \( q\text{RV}4.1 \), \( q\text{RV}5.1 \), \( q\text{RV}5.2 \) and \( q\text{RV}5.3 \), which were located in \( X_{134} \) on chromosome 3, \( X_{160} \) on chromosome 4 and \( X_{218}, X_{219}, X_{225} \) on chromosome 5, respectively. The QTL with the largest effect was mapped to \( X_{160} \) and occupied to the physical position of 16,578,162 bp to 26,317,711 bp (Table 10).

Among these QTLs, we found that some bins controlling one trait was simultaneously detected for other traits. \( q\text{TRL}10.1 \) and \( q\text{RTH}10.1 \) were simultaneously located in bin \( X_{347} \). \( q\text{TRL}11.1 \) and \( q\text{MRL}11.1 \) were detected in \( X_{388} \). \( q\text{RDW}6.1 \) and \( q\text{MRL}6.1 \) were detected in \( X_{233} \).

**Discussion**

Rice has the greatest water requirement, compared with other cereals [40]. Rice production also needs a supply of exogenous nutrients, especially nitrogen fertilizer [41]. Rice roots play an important role in water and nutrient uptake [42]. More water and nutrient uptake of rice depend on faster and more extensive root growth. [43]. Additionally, some studies found that rice root traits have positive relationship with grain yield. [44]. Among different rice varieties, there are large variation associated with root traits.[45]. Generally speaking, indica rice varieties have fewer roots, smaller root absorbing areas, lower root absorbing area density, lower root/shoot ratio, larger root volume and root length, and show an earlier decline in root parameters than japonica rice variety. The upland rice variety has a greater rooting capacity, root length, root absorbing area density and root/shoot ratio [46].
The development of crop cultivars with thicker and deeper roots is expected to increase water and nutrient availability. Therefore, improving our understanding of the genetics of rice roots could have a significant impact on yield production and water saving. Most of the root traits showed polygenic inheritance. A more thorough knowledge of genetic mechanism of root traits is very important for root breeding program, and there are few examples of the introduction in breeding programs of root traits [5,42,47]. Although hundreds of QTLs associated with rice root traits have been found by different populations, including F2, DHs, and mostly RILs, only four QTLs have been fine mapped.

Primary populations such as F2, DHs and RILs are very easy to develop; however, due to their genetic background noise, they are difficult for further study [48]. Therefore, advanced mapping populations, like CSSLs, have been developed. CSSLs have same genetic background with female, except the donor segments. Thus, CSSLs make QTLs analysis easier than before and many QTLs of important agronomic traits have been detected in this way [36,49–55]. In our previous work, we developed a set of 128 CSSLs generated using an indica cultivar, 9311, as the recurrent and a japonica cultivar, Nipponbare, as the donor. We detected accurately the lengths of the substituted segments and provided more accurate background information using the resequencing-based map [36].

In this study, 38 QTLs associated with root morphology were identified using the CSSLs population mentioned above. Among these QTLs, qRV4.1 explained the largest variance of phenotypic effect (37.08%). qMRL6.1 explained the least variance of phenotypic effect (3.42%). Four QTLs identified for all root traits was found for more than 10% phenotypic variation explained by a single QTL. Several of them were located on the same chromosome segment reported in previous studies (Fig 2). For example, qRTH1.1 was on bin X24 close to the QTL for

![Fig 2. Bin allocations on the rice molecular linkage map of the QTLs identified in this study and other nine mapping populations evaluated for root traits. Acronyms on the left of the chromosomes represent QTLs identified in this study and acronyms to the right of the chromosomes represent QTLs identified in the other nine mapping populations. Vertical parentheses spanning two bins indicate QTLs that could not be assigned to a single bin. The colors of acronyms for root traits represent the nine populations in which such traits were identified.](http://example.com/fig2.png)
maximum root thickness detected in the Bala × Azu cross [56]. qRN6.1 was identified on bin X241 close to the QTL for lateral root number detected in the IR1552 × Azu cross [28]. qMRL7.1, identified on bin X262, was locating on a overlapping region containing a pleiotropic QTL for seven root traits on chromosome 7 [57]. qRN9.1, locating on bin X321, was very close to a fine mapped QTL, Sta1, which determines the stele transversal area on chromosome 9 [31]. Additionally, qMRL8.1 on bin X291, qRTH5.1 on X193 and qRDW8.1 on X378 co-localized on the same region with qRL8.1, qRT5.1 and qRDW8.1, associated with root length, root thickness and dry root weight, respectively, using an ARB25 × Pusa Basmati 1460 F2:3 population [58].

A well-developed root system increases biomass and yield in different treatments and cultivars in paddy fields [6]. We are interested in whether or not the QTLs for root traits are located on the overlapping region containing QTLs regulating grain yield and biomass. Actually, several QTLs associated with grain yield and biomass yield found in other studies also close to the bins controlling root traits found in our research. For example, qMRL11.1 was located on the chromosome segment containing a QTL for shoot biomass detected in a DH population from the cross of CT9993 × IR62266 [9]. qRTH1.1 on bin X24 and qRDW3.1 on X137 had the similar positions with two QTLs, yd1b and yd3, respectively, which were involved in grain yield found in RILs from the cross of Zhenshan97 and Minghu63 [59]. Both of qRV4.1 on bin X160 and qYLD4-1, a QTL for grain yield per plant identified in the DH population from IR64 and Azucena [60], was located in a overlapping region. In addition, qRTH2.1 on bin X64 shared same region with two QTLs, DTY2.1 [61] and qDTY2.1 [62], regulating grain yield under drought conditions. This result implied that qRTH2.1 maybe a valuable loci to improve the ability to avoid drought stress and beneficial for stable rice production.

In this study, a total of 38 QTLs associated with root morphology were identified using a resequencing-genotyped CSSLs population under hydroponic culture conditions. Six of the detected QTLs were narrowed in very small regions. For instance, qTRL5.1 on bin X205, qRDW3.1 on bin X137, qMRL7.1 on bin X262, qRN1.1 on bin X8, qRN3.1 on bin X131, and qRV3.1 on bin X134 were delimited into chromosome segments less than 100-Kb in physical distance. These results would be helpful for cloning of these QTLs and improving rice water and fertilizer use efficiency by molecular breeding of root system architectures.

Supporting Information
S1 File. Original data of root traits.
(XLS)

Author Contributions
Conceived and designed the experiments: YZ GD GL BW YLW. Performed the experiments: YT CC BY YW. Analyzed the data: YZ ZY. Contributed reagents/materials/analysis tools: GD YLW ZY. Wrote the paper: YZ.

References
1. Khush GS. What it will take to Feed 5.0 Billion Rice consumers in 2030. Plant Molecular Biology. 2005; 59: 1–6. PMID: 16217597
2. Yang CM, Yang LZ, Yang YX, Zhu OY. Rice root growth and nutrient uptake as influenced by organic manure in continuously and alternately flooded paddy soils. Agri Water Manag. 2004; 70: 67–81.
3. Chloupek O, Forster BP, Thomas WT. The effect of semi-dwarf genes on root system size in field-grown barley. Theor Appl Genet. 2006; 112: 779–786. PMID: 16425022
4. de Dorlodot S, Forster B, Pages L, Price A, Tuberosa R, Draye X. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci. 2007; 12: 474–481. PMID: 17822944

5. Wu WM, Cheng SH. Significance and prospects of breeding for root system in rice (Oryza sativa). Chin J Rice Sci. 2005; 19: 174–180.

6. Arai-Sanoh Y, Takai T, Yoshinaga S, Nakano H, Kondo M, Uga Y. Deep rooting conferred by DEEPER ROOTING 1 enhances rice yield in paddy fields. Sci Rep. 2014; 4: 5563. doi: 10.1038/srep05563 PMID: 24988911

7. Courtois B, Ahmadi N, Khowaja F, Price AH, Rami JF, Frourin J et al. Rice Root Genetic Architecture: Meta-analysis from a Drought QTL Database. Rice. 2009; 2: 115–128.

8. Yamagao EB, Ingram KT, Real JG. Root xylem influence on the water relations and drought resistance of rice. J Exp Bot. 1992; 43: 925–932.

9. Kamoshita A, Zhang J, Siopongco J, Sarkarung S, Nguyen HT, Wade LJ. Effects of phenotyping environments on identification of QTL for rice root morphology under anaerobic conditions. Crop Sci. 2002; 42: 255–265. PMID: 11756283

10. Samejima H, Kondo M, Ito O, Nozoe T, Shinano T, Osaki M. Root-shoot interaction as a limiting factor of biomass productivity in new tropical rice lines. Soil Sci Plant Nutr. 2004; 50: 545–554.

11. Wang XF, He FF, Ma XX, Mao CZ, Hodgman C, Lu CG, et al. OsCAND1 is required for crown root emergence in rice. Mol Plant. 2011; 4: 289–299. doi: 10.1093/mp/sqq068 PMID: 20978084

12. Liu S, Wang J, Wang L, Wang X, Xue Y, Wu P, et al. Adventitious root formation in rice requires OsG-NOM1 and is mediated by the OsPINs family. Cell Res. 2009; 19: 1110–1119. doi: 10.1038/cr.2009.70 PMID: 19546891

13. Huang J, Kim CM, Xuan YH, Liu J, Kim TH, Kim BK et al. Formin homology 1 (OsFH1) regulates root-hair elongation in rice (Oryza sativa). Planta. 2013; 237: 1227–1239. doi: 10.1007/s00425-013-1938-8 PMID: 2334469

14. Zhao Y, Hu Y, Dai M, Huang L, Zhou DX. The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. Plant Cell. 2009; 21: 736–748. doi: 10.1105/tpc.108.061655 PMID: 19258439

15. Inukai Y, Sakamoto T, Ueguchi-Tanaka M, Shibata Y, Gomi K, Umemura I, et al. Crown rootless1, which is essential for crown root formation in rice, is a target of an AUXIN RESPONSE FACTOR in auxin signaling. Plant Cell. 2005; 17: 1387–1396. PMID: 15829602

16. Kitomi Y, Ogawa A, Kitano H, Inukai Y. CRL4 regulates crown root formation through auxin transport in rice. Plant Root. 2008; 2: 19–28.

17. Zhao H, Ma T, Wang X, Deng Y, Ma H, Zhang RS, et al. OsAUX1 controls lateral root initiation in rice (Oryza sativa L.). Plant Cell Environ. 2015; 38: 2208–2222. doi: 10.1111/pce.12467 PMID: 25311360

18. Qi Y, Wang S, Shen C, Zhang S, Chen Y, Xu Y, et al. OsARP12, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (Oryza sativa). New Phytol. 2011; 193: 109–120. doi: 10.1111/j.1469-8137.2011.03910.x PMID: 21973088

19. Chung PJ, Kim YS, Jeong JS, Park SH, Nahm BH, Kim JK. The histone deacetylase OshDAC1 epigenetically regulates the OsNAC6 gene that controls seedling root growth in rice. Plant J. 2009; 59: 764–776. doi: 10.1111/j.1365-313X.2009.03908.x PMID: 19453457

20. Morita Y, Kyozuka J. Characterization of OsPID, the rice ortholog of PINOID, and its possible involvement in the control of polar auxin transport. Plant Cell Physiol. 2007; 48: 540–549. PMID: 17303594

21. Nakamura A, Umemura I, Gomi K, Hasegawa Y, Kitano H, Sazuka T, et al. Production and characterization of auxin-insensitive rice by overexpression of a mutated rice IAA protein. Plant J. 2006; 46: 297–306. PMID: 16623891

22. Champoux MC, Wang G, Sarkarung S, Mackill DJ, O’Toole JC, Huang N, et al. Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet. 1995; 90: 969–961. doi: 10.1007/BF0022910 PMID: 24173051

23. Price AH, Tomos AD. Genetic dissection of root growth in rice (Oryza sativa L.). II. Mapping quantitative trait loci using molecular markers. Theor Appl Genet. 1997; 95: 143–152.

24. Yadav R, Courtois B, Huang N, McLaren G. Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. Theor Appl Genet. 1997; 94: 619–632.

25. Price A, Steele KA, Moore BJ, Jones RGW. Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes. II. Mapping quantitative trait loci for root morphology and distribution. Field Crops Res. 2002; 76: 25–43.
26. Venuprasad R, Shashidhar HE, Hittalmani S, Hemamalini GS. Tagging quantitative trait loci associated with grain yield and root morphological traits in rice (Oryza sativa L.) under contrasting moisture regimes. Euphytica. 2002; 128: 293–300.

27. Courtois B, Shen L, Petalcorin W, Carandang S, Mauleon R, Li Z. Locating QTLs controlling constitutive root traits in the rice population IAC 165×Co39. Euphytica. 2003; 134: 335–345.

28. Zheng BS, Yang L, Zhang WP, Mao CZ, Wu YR, Yi KK. Mapping QTLs and candidate genes for rice root traits under different water-supply conditions and comparative analysis among three populations. Theor Appl Genet. 2003; 107: 1505–1515. PMID: 12920516

29. Yue B, Xue W, Xiong L, Yu X, Luo L, Cui K, et al. Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics. 2006; 172: 1213–1228. PMID: 16272419

30. Courtois B, Audebert A, Dardou A, Roques S, Ghneim-Herrera T, Droc G, et al. Genome-wide association mapping of root traits in a japonica rice panel. PLoS One. 2013; 8: e78037. doi: 10.1371/journal.pone.0078037 PMID: 24223758

31. Uga Y, Okuno K, Yano M. Fine mapping of Sta1, a quantitative trait locus determining stele transversal area, on rice chromosome 9. Mol Breed. 2010; 26: 533–538

32. Obara M, Tamura W, Ebihara T, Yano M, Sato T, Yamaya T. Fine-mapping of qRL6.1, a major QTL for root length of rice seedlings grown under a wide range of NH4(+) concentrations in hydroponic conditions. Theor Appl Genet. 2010; 121: 535–547. doi: 10.1007/s00122-010-1328-3 PMID: 20390245

33. Uga Y, Okuno K, Yano M, Dro1, a major QTL involved in deep rooting of rice under upland field conditions. J Exp Bot. 2011; 62: 2485–2494. doi: 10.1093/jxb/erq429 PMID: 21212298

34. Uga Y, Hanzawa E, Nagai S, Sasaki K, Yano M, Sato T. Identification of qSOR1, a major rice QTL involved in soil-surface rooting in paddy fields. Theor Appl Genet. 2012; 124: 75–86. doi: 10.1007/s00122-011-1688-3 PMID: 21894467

35. Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, et al. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet. 2013; 45: 1097–1102. doi: 10.1038/ng.2725 PMID: 23913002

36. Xu J, Zhao Q, Du P, Xu C, Wang B, Peng Q, et al. Developing high throughput genotyped chromosome segment substitution lines based on population whole-genome re-sequencing in rice (Oryza sativa L.). BMC Genomics. 2010; 11: 656. doi: 10.1186/1471-2164-11-656 PMID: 21106060

37. Dong GC, Wang Y, Yu XF, Zhou J, Peng B, Li JQ, et al. Differences of nitrogen uptake and utilization of conventional rice varieties with different growth duration. Sci Agri Sin. 2011; 44: 4570–4582

38. Zhang JDL, G.P; Shi Y.N. Experimental Method for Plant Physiology. Nanchang: Jiangxi People's Publishing House; 1982. pp. 52–57

39. Xiao L.T, Wang SG. Plant Physiology Experiment Technology. Beijing: China Agriculture Press; 2005. pp. 61–62

40. Bouman B, Barker R, Humphreys E, Tuong TP, Atlin GN. Rice: Feeding the billions. Colombo, Sri Lanka: IWM, Water for food, water for life: A comprehensive assessment of water management in agriculture; 2007.

41. Kraiser T, Gras DE, Gutierrez AG, Gonzalez B, Gutierrez RA. A holistic view of nitrogen acquisition in plants. J Exp Bot. 2011; 62: 1455–1466. doi: 10.1093/jxb/erq426 PMID: 21293977

42. Gowda VRP, Henry A, Yamauchi A, Shashidhar HE, Serraj R. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res. 2011; 122: 1–13.

43. Coudert Y, Perin C, Courtois B, Khong NG, Gantet P. Genetic control of root development in rice, the model cereal. Trends Plant Sci. 2010; 15: 219–226. doi: 10.1016/j.tplants.2010.01.008 PMID: 20153971

44. Lilley JM, Fukai S. Effect of timing and severity of water deficit on four diverse rice cultivars. 1. Rooting pattern and soil water extraction. Field Crops Res. 1994; 37: 205–213

45. O'Toole JC, Bland WL. Genotypic variation in crop plant root systems. Advances in Agronomy. 1987; 41: 91–145.

46. Dai GJ, Hua ZT, Chen WF, Xu ZJ, Wang YR. Comparison in root characteristics among japonica hybrid rice, japonica conventional rice, upland rice and indica rice varieties. J Shenyang Agri Uni. 2008; 39: 515–519.

47. Steele KA, Price AH, Wilcombe JR, Shrestha R, Singh BN, Gibbons JM, et al. QTLs associated with root traits increase yield in upland rice when transferred through marker-assisted selection. Theor Appl Genet. 2013; 126: 101–108. doi: 10.1007/s00122-012-1963-y PMID: 22968512

48. Yamamoto T, Lin H, Sasaki T, Yano M. Identification of heading date quantitative trait locus Hd6 and characterization of its epistatic interactions with Hd2 in rice using advanced backcross progeny. Genet. 2000; 154: 885–891. PMID: 10655238
49. Ye G, Liang S, Wan J. QTL mapping of protein content in rice using single chromosome segment substitution lines. Theor Appl Genet. 2010; 121: 741–750. doi: 10.1007/s00122-010-1345-2 PMID: 20473653

50. Wang J, Wan X, Crossa J, Crouch J, Weng J, Zhai H, et al. QTL mapping of grain length in rice (Oryza sativa L.) using chromosome segment substitution lines. Genet Res, 2006; 88: 93–104. PMID: 17125584

51. Marzougui S, Sugimoto K, Yamanouchi U, Shimono M, Hoshino T, Hori K, et al. Mapping and characterization of seed dormancy QTLs using chromosome segment substitution lines in rice. Theor Appl Genet. 2011; 124: 411–420. doi: 10.1007/s00122-011-1593-9 PMID: 21512773

52. Li M, Sun P, Zhou H, Chen S, Yu S. Identification of quantitative trait loci associated with germination using chromosome segment substitution lines of rice (Oryza sativa L.). Theor Appl Genet. 2011; 123: 893–902. doi: 10.1007/s00122-011-1753-y PMID: 22105913

53. Hao W, Zhu MZ, Gao JP, Sun SY, Lin HX. Identification of quantitative trait loci for rice quality in a population of chromosome segment substitution lines. J Integr Plant Biol. 2009; 51: 500–512. doi: 10.1111/j.1744-7909.2009.00822.x PMID: 19508361

54. Wan XY, Wan JM, Su CC, Wang CM, Shen WB, Li JM, et al. QTL detection for eating quality of cooked rice in a population of chromosome segment substitution lines. Theor Appl Genet. 2004; 110: 71–79. PMID: 15551043

55. Zhang H, Zhao Q, Sun ZZ, Zhang CQ, Feng Q, Tang SZ, et al. Development and high-throughput genotyping of substitution lines carrying the chromosome segments of indica 9311 in the background of japonica Nipponbare. J Genet Genomics. 2011; 38: 603–611. doi: 10.1016/j.jgg.2011.11.004 PMID: 22196403

56. MacMillan K, Enrich K, Plepho HP, Mullins CE, Price AH. Assessing the importance of genotype x environment interaction for root traits in rice using a mapping population II: conventional QTL analysis. Theor Appl Genet. 2006; 113: 953–964. PMID: 16896715

57. Liang YS, Wang HM, Gao ZQ, Lin ZC, Chen DB, Shen XH, et al. Locating QTLs controlling several adult root traits in an elite Chinese hybrid. Gene. 2013; 526: 331–335. doi: 10.1016/j.gene.2013.04.010 PMID: 23624393

58. Sandhu N, Jain S, Kumar A, Mehlia BS, Jain R. Genetic variation, linkage mapping of QTL and correlation studies for yield, root, and agronomic traits for aerobic adaptation. BMC Genet. 2013; 14: 104. doi: 10.1186/1471-2156-14-104 PMID: 24168061

59. Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q, et al. Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theoretical and applied genetics. 2002; 105: 248–257. PMID: 12582526

60. Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar HE, Zhuang JY, et al. Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. Theor Appl Genet. 2003; 107: 679–690. PMID: 12920521

61. Venuprasad R, Dalid CO, Del Valle M, Zhao D, Espiritu M, Sta Cruz MT et al. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. Theor Appl Genet. 2009; 120: 177–190. doi: 10.1007/s00122-009-1168-1 PMID: 19841886

62. Dixit S, Swamy BP, Vikram P, Ahmed HU, Sta Cruz MT, Amante M, et al. Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. Theor Appl Genet. 2012; 125: 155–169. doi: 10.1007/s00122-012-1823-9 PMID: 22361948