Genetic analysis of root vascular traits in a population from two temperate japonica rice ecotypes

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ABSTRACT
The genetic basis for root vascular traits in rice, despite its direct impacts on root axial and radial hydraulic conductivity, has not been widely studied compared with deep rooting traits. We used five phenotyping datasets (i.e. from maturity stage grown in upland field in 2013, and from vegetative and maturity stages grown in upland and lowland fields in 2019) to quantify the genotypic variations and genomic regions of root vascular traits in a temperate japonica mapping population (from lowland Otomemochi (OTM) and upland Yumenohatamochi (YHM)). YHM had larger stele transversal area (STA) and total late metaxylem area (LMXA), as well as higher deep root ratio and total root length at deeper layers (>30 cm) than OTM. Root vascular traits were significantly different among progenies in each dataset, and the size of genotype-by-environment interactions was comparable. Root vascular traits were not positively correlated with deep rooting traits. From the multi-environment analysis of all five datasets, four key genomic regions related to STA in both joint and separate analyses were detected on chromosome 2 (RM3703-RM6379, RM6933-RM3857), chromosome 4 (RM1388-RM5503) and chromosome 12 (RM247-RM155), with the first and third collocated with deep rooting traits. QTL-by-environment interaction was comparable to the main additive effect. This study is the first report on genomic regions of root vascular traits in a japonica mapping population.

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Introduction

The increasing scarcity of freshwater calls for crop improvements which would reduce water consumption in agriculture. Genetic modification of root traits would be important as more extensive downward development of roots (deep-rooting traits) would increase extractable soil water, and the size of the root vascular system (root vascular traits) would change axial and radial hydraulic conductivity in water transport (Comas et al., 2013).

Various root vascular traits (including the phenology of cortex, stele, and metaxylem) have been the subjects of previous anatomical studies (Lynch et al., 2014; Uga et al., 2008; Yang et al., 2019). The radial anatomy of a typical rice root consists of three layers, epidermis, cortex, and stele (Morita & Nemoto, 1995; Rebourillat et al., 2009). Stele is the vascular cylinder that contains xylems, which transport water from root to shoot. Stele transversal area (STA) and its ratio to root transversal area (%STA) varied between dryland (e.g. wheat) and aquatic (e.g. rice) crops (Kadam et al., 2015; McDonald et al., 2002), as well as between upland and lowland genotypes (Kondo et al., 2000; Matsuo et al., 2009), and are suggested to contribute to the adaptation of the plant to its respective growing condition. Under water deficit conditions, STA usually increased, associated with better water acquisition by enhancing root water conductivity and tensile strength (Chimungu et al., 2015; Henry et al., 2012). Multi-environment analyses also showed that larger STA of a near-isogenic line resulted in higher leaf water potential, harvest index, and grain yield (Deshmukh et al., 2017; Phoura et al., 2020).

Similarly, %STA in both Henry et al. (2012) and Kadam et al. (2015) was reported to increase when exposed to water limiting conditions, supposedly to retain more water and reduce radial conductance, rather than decrease to prevent axial oxygen loss. This trend was also observed in woody, herbaceous, and grass species (Rieger & Litvin, 1999; Yamauchi et al., 2021) despite their range of anatomical features, suggesting a possible common response to water stress.

Xylem characteristics (number of vessels, total, and single area) have a direct effect on root axial conductivity. Since the fourth power of vessel diameter theoretically determines hydraulic conductivity of a vessel as per Poiseuille’s law (Steudle & Peterson, 1998), the size of metaxylems, their number, and probably STA would be important factors determining axial water conductivity through vascular bundles. A small change in the vessels’ (e.g. stele, xylem) diameter would greatly affect their conductivity. In rice, metaxylem size, in response to water deficit conditions, may increase (de Bauw et al., 2019; Ouyang et al., 2020) or decrease (Henry et al., 2012; Kadam et al., 2015). The increase in metaxylem size and number was proposed to improve axial water conductance and leaf water potential, while the decrease was to prevent the risk of embolism inside the vessels. This indicates the conceivable importance of root vascular traits for plant adaptation and improvement under water-limiting conditions.

Root vascular traits are shown to vary highly among rice varieties, as well as between rice and other crops (Kadam et al., 2015; McDonald et al., 2002; Uga et al., 2009). Traditional upland japonica rice varieties often have the largest stele, stele ratio, and late metaxylem diameter, followed by modern upland japonica, aus, and indica varieties (Gowda et al., 2011; Kondo et al., 2000; Uga et al., 2009; Phoura, 2019). Quantitative trait loci (QTLs) for root vascular traits so far have only been reported in a mapping population from lowland indica ‘IR64’ and tropical japonica ‘Kinandang Patong’ with one fine-mapped QTL for STA, Sta1 (Uga et al., 2008, 2010), but no more recent studies are available. Varieties with different genetic backgrounds may contain different QTLs for root vascular traits. Within temperate japonica, root traits such as gross morphology and root thickness are known to be variable (Ikeda et al., 2007; Manabe et al., 2005). Compared to rice, more QTLs for root vascular traits have been successfully identified in maize, wheat, and barley (Burton et al., 2015; Mano & Omori, 2008, 2009; Oyiga et al., 2020; Sharma et al., 2010).

Studies on root vascular traits have been less reported than those of deep rooting traits, probably due to the tedious and time-consuming processes of root sectioning, microscopic observation, and image analysis (Burton et al., 2012), and also due to the direct crop physiological relation of the latter to soil water extraction (e.g. Robertson et al., 1993; Yoshida & Hasegawa, 1982). Since root thickness and gross root morphology are often closely associated, as deep rooting varieties often have thicker roots (Phoura, 2019), root vascular traits may be linked to deep rooting traits (Eissenstat, 1992; Gowda et al., 2011). However, the width of the cortex may be independent of the size of the stele (de Bauw et al., 2019; Yamauchi et al., 2021), and, hence, greater root vascular traits may not always lead to thicker roots or be tightly linked with deeper rooting traits (Hendel et al., 2021; Kadam et al., 2017). In addition to the direct measurement of root morphology at depth, Kato et al. (2006) suggested root growth angle (RGA) can be a useful estimation for deep rooting, showing a positive correlation between the frequency of higher root growth angle and deep root development in upland fields. The correlation between RGA and root distribution below 30 cm has also been reported in
maize and rice (Araki et al., 2000; Ramalingam et al., 2017; Uga et al., 2011). While using rice accessions can provide insights into the genetic variation of the traits, mapping populations allows us to assess the interrelationship of the genetic control of root vascular traits and deep root traits.

In this study, we aimed to (1) identify QTLs of root vascular traits in a temperate japonica rice population, (2) evaluate the interactions of the root vascular traits and their QTLs with years, growth stages, and field environments, (3) determine the relation between root vascular traits and deep root traits. To the best of our knowledge, this study is the first to analyze root vascular traits and deep root traits and their respective QTLs in a temperate japonica mapping population.

Materials and methods

The field experiments were conducted during the summer season of 2013 (Experiment 1) and 2019 (Experiment 2) at the Institute of Sustainable Agroecosystem Services (ISAS), The University of Tokyo, Nishitokyo, Japan (latitude 35°43 N, longitude 39°32 E). Experiment 1 was conducted on an upland field, whereas Experiment 2 was conducted on both upland and paddy fields. The fields are empirically known to be homogeneous in fertility and physical property. The soil at the experimental site is volcanic ash soil with silty Kanto loam type (Humic Andosol). The topsoil layer (0–35 cm) is a dark humic silty loam, and the subsoil layer (below 35 cm) is a red-brown silty clay loam (Yamagishi et al., 2003). The surface soil (0–10 cm depth) before the experiment in 2019 had pH 6.6 and 6.2, electrical conductivity (EC) 0.12 and 0.05 dS m⁻¹, cation exchange capacity (CEC) 31.8 and 29.2 meq 100 g⁻¹, inorganic nitrogen 0.53 and 3.46 mg 100 g⁻¹, and phosphoric acid absorption coefficient 2.463 and 2.457 mg 100 g⁻¹ for the upland and lowland fields, respectively. The bulk density of the soil in the same site was reported as 0.90 ± 0.05 g cm⁻³ (n = 9) (Deshmukh et al., 2017).

Plant materials

A recombinant inbred mapping population (OY population) of the F₈ generation from a cross between two temperate japonica rice ecotypes, Otomemochi (OTM) and Yumehatamochi (YHM) (Manabe et al., 2005), was used for the experiments. OTM is a lowland japonica with short stature, thinner and shallower roots, early maturity, and susceptible to drought, while the upland japonica YHM is late maturing and drought tolerant with a deeper, thicker root system (Hirasawa et al., 1998). Ninety progenies in 2013, originally planted with 97 but seven failed to grow until maturity stage (Experiment 1) and only 67 progenies available in 2019 (Experiment 2) (30 progenies unexpectedly lost viability) were phenotyped together with their parents OTM and YHM.

Experimental design

In Experiment 1, an 11 × 11 row-column design was used with 11 replications of the two parents and a single replication for the 97 progenies (121 plots in total). A single plant was grown in each plot of 30 cm × 60 cm. In Experiment 2, a 9 × 10 row-column design with two blocks was used in each of the upland and lowland fields. In each block, two parents were repeated at least once in each row and column (10 replications). Five plants were grown in a single row in each plot with a distance of 30 cm. The row width or plot-to-plot distance was 45 cm in the upland field, and 60 cm in the lowland field, respectively (Supplementary Figure S1).

Growth conditions

In Experiment 1, the seeds were soaked in cups on 24 April 2013 and planted in the nursery on 2 May and seedlings were transplanted on 11 June with one plant in 30 cm × 60 cm with the root basket (to be described later). Top-dressing N, P₂O₅, and K₂O were applied at a rate of 6, 8, and 9 g/m², respectively, as basal fertilizers, together with 100 g/m² of calcium silicate. Supplementary irrigation was provided. Minimum and maximum daily air temperatures, total daily solar radiation, and daily rainfall were measured by a weather station (WatchDog 2900ET, Spectrum Technologies Inc., Aurora, IL, USA) installed at the side of the field. The daily minimum and maximum air temperatures were 21.3 ± 3.2°C (mean ± standard deviation) and 30.5 ± 4.2°C, respectively, in 2013. Daily solar radiation was 16.3 ± 6.4 MJ m⁻² in 2013. Total seasonal rainfall equaled 636 mm in 2013.

In Experiment 2, the seeds were soaked in cups on 17 May 2019 and transferred to nursery beds on 22 May. Seedlings were transplanted one seedling per hill to the lowland field on 12 June and to the upland field on 18 June. The delay was to ensure plants in the rainfed field could survive the transplant shock. During the first week following the transplanting, the rainfed field was kept saturated with manual irrigation to help with shock recovery. As for the lowland field, standing water was constantly kept at approximately 3 cm from the transplant date until the end of August. Basal fertilisers of N, P₂O₅ and K₂O were applied at the rate of 6, 10, and 10 g/m², respectively. Manual weed management was done
for both upland and lowland fields; herbicide was applied only for the lowland. Minimum and maximum daily air temperatures, total daily solar radiation, and daily rainfall were recorded by ISAS weather station sited near the field. The daily minimum and maximum air temperatures were 19.0 ± 4.5°C and 27.3 ± 4.9°C, respectively. The average daily solar radiation was 13.6 ± 6.9 MJ m⁻². Total seasonal rainfall was 1334 mm with two exceptional records on September 9 (106.5 mm) and October 12 (320.5 mm) due to typhoons.

**Measurements**

In Experiment 1, a single plant per genotype was sampled at maturity to determine root vascular traits (explained below) and a modified basket method was used for the determination of root growth angle (categorized as a deep rooting trait). In Experiment 2, three plants per genotype were sampled at 34 days after transplanting (i.e. vegetative stage) as well as at harvesting (i.e. maturity stage) to determine root vascular traits, and a core sampling method to determine root length along with the depth profile (categorized as a deep rooting trait). Details of the modified basket and core sampling method are described in the ‘Deep rooting traits’ subsection.

**Root vascular traits**

The basal parts of the roots were immersed in FAA solution (5% formalin, 5% acetic acid, 45% ethanol, and 45% H₂O) in Experiment 1 and Farmer’s solution (25% acetic acid, 75% ethanol) in Experiment 2 until dissection. In Experiment 1, three roots with medium diameters by visual observation per plant were selected, cut at ~3 cm from the base with a plant microtome (MTH-1, Nippon Medical & Chemical Instruments Co., Ltd., Osaka, Japan). In Experiment 2, three roots from the lower 3rd node from the top were selected and free-hand sectioned at ~3 cm from the base using double-edged stainless-steel blades (Uga et al., 2008). In both experiments, dissected samples were stained with Toluidine Blue 0.5% and then observed at 4X and 10X magnification under a fluorescence and phase-contrast microscope (BX51, Olympus, Hicksville, NY, USA). Horizontal cross-section images were captured by CellSens standard software (Olympus) and measured for root transversal area (RTA) and stele transversal area (STA), as well as total late metaxylem area (LMXA) and number (LMXN) using Polygon tool of Image J version 1.51 t (National Institute of Mental Health, Bethesda, Maryland, USA) (Schneider et al., 2012). RTA was measured as the total area bordered by the epidermis, while STA was by the endodermis. The single late metaxylem area (sLMXA), as well as the ratio of STA to RTA (%STA), of LMXA to STA (%LMXA), were calculated as follows:

\[
sLMXA = \frac{LMXA}{LMXN}
\]

\[
%STA = \frac{STA}{RTA} \times 100
\]

\[
%LMXA = \frac{LMXA}{STA} \times 100
\]

Three images obtained from three root cuts were used to calculate the average values for the individual root vascular trait as mentioned above (Figure 1).

![Figure 1. A typical cross-sectional image of a matured upland root at ~3 cm from root base at 4X magnification of a phase-contrast microscope.](image-url)
Deep rooting traits
In Experiment 1, root growth angle was evaluated by a modified basket method (Ramalingam et al., 2017). A hemispherical basket (top and bottom diameters of 15 and 8.6 cm with height of 9.5 cm) filled with soils and planted with the seedling of each genotype in the center was buried in the ground so as the surfaces of the basket and the ground were levelled. The root growth angle of a plant was divided into four categories from the horizontal line, namely, 0–25° (shallowest zone close to horizontal direction), 25–45°, 45–65°, and 65–90° (most vertical direction towards deeper zone), and numbers of roots penetrating the mesh of each category of the basket were recorded at maturity at each quarter of the basket as 4 replicated readings. Roots from 45–90° were defined as deep roots.

In Experiment 2, soil cores (5 cm in diameter, 30 cm in depth) were collected using a liner core sampler (DIK-s110C, Daiki Rika Kogyo Co., Saitama, Japan) at maturity, down to 30 cm in the lowland field and 60 cm in the upland field. Three cores per genotype were taken at ca. 7.5 cm away from the plant base. The collected cores were stored at 4°C, which were cut into the three 10 cm-long segments, washed, and sieved through first a 2 mm steel mesh to separate roots from the soil, and then through a 0.5 mm mesh for fine root fragments. The picked-up root pieces were arranged on a transparent plate and scanned with an Epson Expression 11000XL scanner set in professional mode, positive film, 8-bit grayscale at 600 dpi (Kamoshita et al., 2019). The obtained images were then analyzed by WinRhizo Pro (Regent Instruments) for total root length.

Statistical analysis
Phenotypic analysis
Phenotypic data of 90 progenies were analyzed by REML for row-column designs by GenStat (18th Edition, VSN International, 2015) in Experiment 1 (Mace et al., 2012). The common phenotypic data of 67 progeny across four environments were analyzed including genotype-by-environment interaction (G × E) in Experiment 2. The common phenotypic data of 61 progeny throughout all the 5 environments were subject to the multi-environment analysis. Coefficients of variation were calculated for each parent and progeny. Broad-sense heritability (h²) was also estimated (Kamoshita et al., 2002a), where σ² was the mean square of G while σ² was the residual mean square, and the number of replications k:

\[ h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_e / k) \]

Phenotypic correlations between root vascular traits and deep rooting traits were also analyzed.

QTL analysis
The mean values of root vascular traits and deep rooting traits obtained from the ANOVA were used for QTL analysis; separate analysis of each of the five datasets with 90 progenies for the 1st dataset (Experiment 1) and with 67 for the 2nd to 5th datasets (Experiment 2)) and the multi-environment analysis across all the five datasets with 61 progenies that detected QTLs jointly as well as in each single environment. In a separate analysis, putative QTLs were detected by using composite interval mapping (CIM) with Haldane map function with 106 markers of QTL Cartographer version 2.5 (Wang et al., 2012). The setting was at 0.05 significance level, 1000 permutation times, 10 cm window size, and 0.5 cm walk speed. In the multi-environmental analysis, multiple-trait CIM (MT-CIM) function (Yu et al., 2016) was used to investigate QTL-by-environment interaction. The threshold for putative CIM and single environment MT-CIM QTLs was 2.5 LOD, whereas a LOD threshold of 3.0 was used for putative QTLs of the multi-environment MT-CIM (i.e. detected in joint analysis). These QTL analyses used the genetic map of the OY population generated at the Plant Biotechnology, Institute, Ibaraki Agricultural Center using 106 SSR markers that cover all 12 chromosomes (Manabe et al., 2005).

Results
Phenotypic variation
Root vascular traits
In Experiment 1, RTA of YHM was twice that of OTM, while for STA and LMXA, the sizes were thrice larger in YHM than in OTM (Table 1). The differences were smaller in %STA and sLMXA (approximately 1.5 times). The two parents showed almost no difference in %LMXA. Except for %LMXA, the genetic variation was significant between parents and among progenies for all other traits. STA had a high positive correlation with LMXA (r = 0.85*** and RTA (r = 0.78***) (data not shown).

In Experiment 2, YHM also had significantly larger root vascular traits than OTM at both stages under both field conditions, except for %LMXA at maturity (Table 2). The difference was large for STA, RTA, LMXA, and sLMXA but smaller for %LMXA. STA, LMXA, and RTA of all genotypes had larger values at the maturity than at the vegetative stage (e.g. double to triple). RTA of both parents was larger under lowland than upland. Environmental variation (E) and G × E interaction were significant for progenies for most traits at both stages and both environments, except for LMXN, LMXA (E), and %LMXA (G × E) at the vegetative stage. Genotypic variation was significant for all traits at both stages and both
environments (except for %LMXA at maturity) with an average broad-sense heritability of 0.57. Broad-sense heritability for all traits tended to be higher at the vegetative stage than at maturity except for LMXN. STA had high positive correlations with LMXA (0.76*** – 0.93***). LMXN (0.52*** – 0.66*** at both growth stages in both fields and with RTA (0.63*** – 0.82*** (data not shown).

**Deep rooting traits**

In Experiment 1, the root number (RN) of each growth angle and their proportion to total root number (PRN) varied between the two parents and among the progenies (Table 3). RN and PRN (45–90°) were higher in YHM, while for shallower angle (0–45°), the values of OTM were higher, especially at the angle of 0–25°.

In Experiment 2, at maturity, YHM tended to have larger total root length (TRL) in 20–30 cm depth under the lowland field, and in 20–60 cm depth under the upland field, compared to OTM, though not significant (Table 4). The progenies were varied in TRL with significant genotypic variation in each depth from 0 cm to 50 cm depth and significant G × E for the top 30 cm layer. In the lowland field, a fraction of roughly 5% of OTM’s root length was recorded at depths deeper than 20 cm, while the two upper layers had similar length, accounted for 47.5% for each; on the contrary, YHM distributed 54%, 36%, and 10% of its TRL in each layer from 0 cm to 30 cm under lowland. Under upland field, OTM distributed 63%, 34%, and 3% of its TRL from top (0–10 cm) to bottom (30–60 cm) layer, while YHM did more evenly at 45%, 47%, and 8% levels.

**The relation between root vascular traits and deep rooting traits**

In Experiment 1, no positive correlation was found between deep root growth angle traits and large root vascular traits. In contrast, LMXA and sLMXA were weakly positively correlated with RN and PRN of shallow roots (i.e. RN and PRN of 0–25° root, RN of 0–45° root) (Table 5). RTA, STA, LMXA, and sLMXA were negatively correlated with PRN 65–90°.

In Experiment 2, no significant correlation was found between root vascular and deep rooting traits at both vegetative and maturity stages. At the vegetative stage, TRL had weak negative correlations with STA, LMXA under lowland and with STA, %STA, LMXA, LMXA under upland (Table 6). At maturity, most correlations between TRL and root vascular traits in the lowland were negligible, although TRL at 0–10 cm increment depth was weakly correlated to %LMXA and LMXN. For the upland condition, TRL at 0–10 cm depth was weakly negatively correlated to %STA and %LMXA and TRL at 20–30 cm depth negatively with LMXN.

**QTL analysis**

**Separate analysis of individual dataset**

**Root vascular traits**

In Experiment 1, nine QTLs for root vascular traits were detected on four chromosomes (e.g. Chr. 1, 4, 6, 8) (Table 7). The QTL for %STA on Chr. 1 contributing 11.0% to the trait was found collocated with a QTL for LMXN (18.1%) near RM8147. On Chr. 4, a region near RM3288 was related to both RTA (14.9%) and sLMXA (20.0%).

In Experiment 2, in total 15 QTLs for root vascular traits were found by analysing four datasets of two growth stages and two field environments by CIM (Table 8). A QTL for %STA was found under upland maturity in RM3785-RM3288 on chromosome 4. RM5508-RM1364 no chromosome 7 contained QTLs for STA and LMXA at maturity under upland condition. There were a few overlapping QTLs across the stages and the environments.

**Deep rooting traits**

In Experiment 1, QTLs for RN and PRN in 45–65° and 65–90°, and PRN in 45–90° (deep root ratio) were identified as deep rooting traits (Table 9). The two deep root ratio QTLs were in RM2811-RM5953 on chromosome 4 with its positive allelic contribution from OTM and in RM3183-RM6734 on chromosome 6 with its allelic contribution from YHM.

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**Table 1. Root vascular traits for Otomemochi (OTM) and Yumenohatamochi (YHM), and their 90 progenies in Experiment 1.**

| Genotype | RTA(×10^2 μm^2) | STA(×10^2 μm^2) | %STA | %LMXA | LMXN | LMXA(×10^2 μm^2) | sLMXA(×10^2 μm^2) |
|----------|-----------------|-----------------|------|-------|------|-----------------|-------------------|
| OTM      | 625 ± 15        | 30 ± 0.8        | 4.88 ± 0.09 | 17.76 ± 0.4 | 3.98 ± 0.13 | 5.41 ± 0.2 | 1.36 ± 0.03 |
| CV (%)   | 8                | 10              | 7    | 8     | 12   | 9               |                   |
| YHM      | 1208 ± 32       | 87 ± 4.7        | 7.33 ± 0.4 | 18.24 ± 0.4 | 7.43 ± 0.14 | 15.8 ± 0.6 | 2.14 ± 0.06 |
| CV (%)   | 9                | 19              | 21   | 8     | 6    | 14              | 11                |
| G (parents) | ***             | ***             | ***  | ns    | ***  | ***             | ***               |
| Progenies | 751 ± 88        | 43 ± 7          | 5.79 ± 0.8 | 18.21 ± 2.4 | 5.05 ± 0.8 | 7.77 ± 1.6 | 1.54 ± 0.2 |
| CV (%)   | 12               | 17              | 14   | 13    | 14   | 21              | 17                |
| LSD      | 166             | 13              | 1.5  | 4.4   | 1.4  | 3               | 0.5               |
| G        | ***             | ***             | ***  | ***   | ***  | ***             | ***               |

RTA: root transversal area; STA: stele transversal area; %STA: (STA×100)/RTA; LMXN: late metaxylem number; LMXA: total late metaxylem area; sLMXA: single late metaxylem area (LMXA/LMXN); G: genotypic variation; LSD: least significant difference; CV (Progenies) was average of coefficient of variation for each progeny. *** and ns show significance at P < 0.001 and not significant, respectively. Mean ± standard error (parents) or ± standard deviation (progenies).
Table 2. Root vascular traits for Otomemochi (OTM) and Yumenohatamochi (YHM), and their 67 progenies at vegetative and maturity stages, under upland and lowland fields in Experiment 2.

|                          | UP (×10³ µm²) | LL | UP (×10³ µm²) | LL | %STA | %LMXA | LMKN | LMXA (×10³ µm²) | sLMXA (×10³ µm²) |
|--------------------------|---------------|----|---------------|----|------|------|------|----------------|------------------|
| **Vegetative stage**     |               |    |               |    |      |      |      |                |                  |
| OTM                      | 347 ± 14      | 404 ± 9.9 | 10 ± 0.5      | 15 ± 0.3 | 2.8 ± 0.1 | 3.6 ± 0.00 | 17.1 ± 0.1 | 18.2 ± 0.4 | 0.0 ± 0.1 | 0.0 ± 0.0 | 0.6 ± 0.0 | 0.8 ± 0.03 |
| CV (%)                   | 13            | 11  | 14            | 10  | 10   | 1.9  | 2.7  | 8.1           | 17.0             |
| YHM                      | 472 ± 72      | 443 ± 39 | 23 ± 5        | 26 ± 8 | 5.0 ± 0.63 | 5.8 ± 1.4 | 23.3 ± 2.4 | 20.1 ± 2.8 | 5.0 ± 1  | 5.0 ± 1  | 5.5 ± 1.5 | 5.1 ± 1.2 |
| CV (%)                   | 15            | 19  | 22            | 33  | 13   | 23   | 10   | 14            | 20               |
| Progenies                | 455 ± 89      | 506 ± 150 | 23 ± 5.8      | 19 ± 5.6 | 5.0 ± 0.7 | 3.9 ± 0.7 | 17.8 ± 1.9 | 20.4 ± 2.0 | 4.0 ± 0.6 | 4.0 ± 0.6 | 4.1 ± 1.2 | 3.9 ± 1.2 |
| CV (%)                   | 24            | 291 | 22            | 24  | 11   | 12   | 13   | 12            | 16               |
| LSD                      | 189           | 291 | 9.6           | 8.9  | 1.1  | 1    | 43   | 45            | 1.2             |
| h²                       | 0.56          | 0.66  | 0.58          | 0.58 | 0.58 | 0.6  | 0.63 | 0.63          |                  |
| G (%)                    | 22.3***       | 36.7*** | 27.2***       | 21.8*** | 27.9*** | 36.5*** | 33.7*** |                  |
| E (%)                    | 1.9***        | 6.6*** | 29.6***       | 15.1*** | 0.2***  | 0.6*** | 0.7*  |                  |
| G × E (%)                | 20.8***       | 18.6*** | 18.8***       | 15.2*** | 18.2*   | 21.2*** | 19.3*** |                  |
| **Maturity stage**       |               |    |               |    |      |      |      |                |                  |
| OTM                      | 579 ± 32      | 857 ± 75 | 26 ± 0.3      | 22 ± 1.4 | 4.6 ± 0.3 | 2.6 ± 0.1 | 21.9 ± 1.0 | 18.1 ± 0.6 | 4.0 ± 0.2 | 4.0 ± 0.2 | 5.7 ± 0.2 | 4.0 ± 0.4 |
| CV (%)                   | 20            | 33  | 3.6           | 25   | 22   | 12   | 18   | 13            | 13               |
| YHM                      | 1111 ± 46     | 1551 ± 66 | 85 ± 4.6      | 65 ± 2.2 | 7.8 ± 0.5 | 4.2 ± 0.2 | 20.1 ± 0.1 | 20.2 ± 0.3 | 7.0 ± 0.3 | 7.0 ± 0.2 | 17.1 ± 0.9 | 13.1 ± 0.6 |
| CV (%)                   | 795 ± 182     | 1164 ± 220 | 45 ± 11       | 41 ± 6.6 | 5.9 ± 1.1 | 3.9 ± 0.6 | 19.5 ± 2.0 | 20.0 ± 2.4 | 5.0 ± 0.8 | 5.0 ± 0.6 | 8.8 ± 2.5 | 7.6 ± 1.4 |
| Progenies                | 795 ± 182     | 1164 ± 220 | 45 ± 11       | 41 ± 6.6 | 5.9 ± 1.1 | 3.9 ± 0.6 | 19.5 ± 2.0 | 20.0 ± 2.4 | 5.0 ± 0.8 | 5.0 ± 0.6 | 8.8 ± 2.5 | 7.6 ± 1.4 |
| CV (%)                   | 19            | 17  | 19            | 17   | 16   | 12   | 11   | 11            | 14               |
| LSD                      | 314           | 404  | 16.4          | 12.2  | 1.8  | 0.7  | 3.9  | 4             | 1.1             |
| h²                       | 0.43          | 0.60  | 0.55          | 0.54 | 0.54 | 0.62 | 0.55 | 0.46          |                  |
| G (%)                    | 14.3***       | 25.8*** | 27.1***       | 22.7*** | 24.8*** | 23.3*** | 17.2*** |                  |
| E (%)                    | 32.8***       | 7.7*** | 52.4***       | 1.3*** | 0.6  | 4.3*** | 4.2*** |                  |
| G × E (%)                | 18.8***       | 17.0*** | 8.8***       | 19.3*** | 15.0*** | 18.6*** | 20.2*** |                  |

RTA: root transversal area; STA: stele transversal area; %STA: (STA × 100)/RTA; LMNA: late metaxylem number; LMXA: total late metaxylem area; sLMXA: single late metaxylem area (LMXA/LMKN); UP: upland; LL: lowland; G: genotypic variation; E: environmental variation; G × E: genotype–environment interaction; LSD: least significant difference at 0.05; **, ***: ns show significance at P < 0.05, 0.01, 0.001, and not significant, respectively; h²: broad-sense heritability; CV (Progenies): the average CV of all progenies. Mean ± standard error (parents) or ± standard deviation (progenies).
Table 3. Root numbers (RN) of each growth angle and their proportion (PRN) to the total root number for Otomemochi (OTM), Yumenoahatomichi (YHM), and their 90 progenies in Experiment 1.

| Traits | OTM | YHM | G | Progenies | G |
|--------|-----|-----|---|-----------|---|
| Total RN | 65.71 ± 22.15 | 72.85 ± 19.08 | *** | 68.46 ± 28.74 | *** |
| RN 0–25° | 21.50 ± 10.26 | 10.77 ± 10.30 | *** | 17.59 ± 13.05 | *** |
| RN 25–45° | 19.36 ± 7.01 | 20.85 ± 6.89 | ns | 19.65 ± 10.52 | *** |
| RN 45–65° | 18.93 ± 8.74 | 32.38 ± 10.61 | *** | 23.01 ± 12.75 | *** |
| RN 65–90° | 5.93 ± 2.97 | 8.85 ± 3.80 | *** | 8.21 ± 4.26 | *** |
| RN 0–45° (shallow RN) | 40.86 ± 16.15 | 31.62 ± 15.30 | ** | 37.23 ± 20.80 | *** |
| RN 45–90° (deep RN) | 24.86 ± 10.40 | 41.2 ± 12.38 | *** | 31.22 ± 15.38 | *** |
| PRN 0–25° | 0.30 ± 0.16 | 0.14 ± 0.12 | *** | 0.24 ± 0.16 | *** |
| PRN 25–45° | 0.28 ± 0.14 | 0.29 ± 0.11 | ns | 0.27 ± 0.13 | *** |
| PRN 45–65° | 0.26 ± 0.13 | 0.44 ± 0.14 | *** | 0.33 ± 0.17 | *** |
| PRN 65–90° | 0.09 ± 0.09 | 0.13 ± 0.08 | * | 0.13 ± 0.10 | *** |
| PRN 0–45° (shallow PRN) | 0.58 ± 0.21 | 0.43 ± 0.16 | *** | 0.51 ± 0.21 | *** |
| PRN 45–90° (deep PRN) | 0.35 ± 0.17 | 0.57 ± 0.16 | *** | 0.45 ± 0.20 | *** |

G: genotypic variation; *, **, *** show significance at P < 0.05, 0.01, 0.001, and not significant, respectively.

Table 4. Total root length (TRL) at 0–60 cm for Otomemochi (OTM), Yumenoahatomichi (YHM), and their 67 progenies at maturity in Experiment 2.

| Trait | Condition | OTM | CV (%) | YHM | CV (%) | ANOVA (parent) | Progenies | CV (%) | LSD | h² | ANOVA |
|-------|-----------|-----|--------|-----|--------|---------------|-----------|--------|-----|----|------|
| TRL0-10 (cm) | Upland | 281 ± 20 | 27 | 304 ± 26 | 33 | E*** | 167 ± 100 | 50 | 154 | 0.48 | G***, E***, G × E*** |
| | Lowland | 245 ± 1.5 | 48 | 290 ± 37 | 68 | 322 ± 135 | 30 | 182 | | |
| TRL10-20 (cm) | Upland | 98 ± 1.8 | 7 | 204 ± 18 | 32 | E** | 116 ± 83 | 33 | 120 | 0.45 | G***, E***, G × E*** |
| | Lowland | 241 ± 55 | 86 | 195 ± 15 | 28 | 189 ± 74 | 31 | 120 | | |
| TRL20-30 (cm) | Upland | 54 ± 1.4 | 10 | 108 ± 14 | 48 | E*** | 37 ± 42 | 69 | 67 | 0.48 | G***, G × E*** |
| | Lowland | 29 ± 3.3 | 41 | 52 ± 8.6 | 62 | 37 ± 37 | 59 | 82 | | |
| TRL30-40 (cm) | Upland | 11 ± 3.4 | 344 | 43 ± 6.5 | 57 | G** | 18 ± 28 | 87 | 39 | | G*** |
| | Lowland | 0.09 ± 0.02 | 100 | 9.2 ± 2.1 | 87 | G** | 9.0 ± 28 | 66 | 12 | G*** |
| TRL50-60 (cm) | Upland | 0.07 ± 0.02 | 100 | 1.2 ± 0.3 | 100 | G** | 3.7 ± 4.9 | 73 | 12 | G** |

G: genotypic variation, E: environmental variation, G × E: genotype–environment interaction, h²: broad-sense heritability, CV of progenies: the average of all progenies CV, LSD: least significant difference at 0.05, *, **, ***; ns show significance at P < 0.05, 0.01, 0.001, and not significant, respectively. Mean ± standard error (parents) or ± standard deviation (progenies).

Table 5. Correlation coefficients between root vascular traits and deep rooting traits among the 90 progenies of the OY population in Experiment 1.

| Trait | RA | STA | %STA | %LMXA | LMXN | LMXA | sLMXA |
|-------|----|-----|------|--------|------|------|--------|
| RN 0–25° | 0.12 | 0.18 | 0.06 | 0.07 | 0.12 | 0.27** | 0.21* |
| RN 25–45° | 0.07 | 0.07 | 0.06 | 0.06 | 0.07 | 0.00 | 0.09 |
| RN 45–65° | −0.01 | −0.06 | 0.02 | −0.02 | −0.07 | −0.08 | 0.03 |
| RN 65–90° | 0.17 | 0.19 | 0.08 | 0.07 | 0.17 | −0.17 | −0.09 |
| Total RN | 0.05 | 0.05 | 0.07 | 0.03 | 0.10 | 0.12 | 0.09 |
| PRN 0–25° | 0.11 | 0.15 | 0.03 | 0.05 | 0.10 | 0.08 | 0.13 |
| PRN 25–45° | 0.11 | 0.19 | 0.06 | 0.04 | 0.16 | 0.24* | 0.15 |
| PRN 45–65° | 0.10 | 0.07 | 0.05 | 0.02 | 0.01 | 0.08 | 0.13 |
| PRN 65–90° | −0.06 | −0.08 | 0.03 | −0.02 | −0.10 | −0.07 | 0.01 |
| PRN 0–45° | −0.16 | −0.19 | −0.02 | −0.05 | −0.14 | −0.24* | 0.19 |
| PRN 45–90° | −0.16 | −0.19 | −0.02 | −0.05 | −0.14 | −0.24* | 0.19 |

RTA: root transversal area; STA: stele transversal area; %STA: (STA × 100); RA: total late metaxytem number; LMXN: late metaxytem area; LMXA: single late metaxytem area (LMXA/LMXN); *, **: show significance at P < 0.05 and 0.01, respectively.

In Experiment 2, deep rooting traits were identified as TRL and %TRL at each depth at maturity, with seven QTLs detected in CIM analysis, of which two came from lowland and the rest were from upland condition (Table 10). The allelic contribution of the QTL for TRL at 50–60 cm depth layer in RM5503-RM3276 on chromosome 4 came from OTM.

Q × E interaction analysis by multiple trait analysis

The multiple-trait analysis of five environments of lowland (low), upland (up), vegetative stage (veg), and maturity stage (mat) from Experiment 1 (13) and Experiment 2 (19) identified 55 QTLs for root vascular traits covering 11 chromosomes with the Chromosome 2 containing the highest number of QTLs (15), while Chromosome 11 contained none (Supplementary Table 1).
Table 6. Correlation coefficients between root vascular traits and deep rooting traits at vegetative and maturity stages among the 67 progenies of OY population in Experiment 2.

|                         | RTA         | STA         | %STA        | %LMXA  | LMXN  | LMXA  | sLMXA |
|-------------------------|-------------|-------------|-------------|--------|-------|-------|-------|
| **Vegetative stage**    |             |             |             |        |       |       |       |
| TRL (lowland)           | −0.15       | −0.22*      | −0.07       | 0.07   | −0.10 | −0.19 | −0.18 |
| TRL (upland)            | −0.06       | −0.17       | −0.21*      | −0.06  | −0.17 | −0.17 | −0.08 |
| **Maturity stage**      |             |             |             |        |       |       |       |
| Lowland                 |             |             |             |        |       |       |       |
| TRL 0–10 cm             | 0.07        | 0.08        | −0.01       | −0.22* | −0.17 | −0.07 | 0.09  |
| TRL 10–20 cm            | 0.05        | −0.02       | −0.06       | −0.15  | −0.06 | −0.12 | −0.09 |
| TRL 20–30 cm            | 0.07        | 0.01        | −0.07       | 0.06   | −0.08 | 0.04  | 0.13  |
| Upland                  |             |             |             |        |       |       |       |
| TRL 0–10 cm             | 0.00        | −0.12       | −0.20*      | −0.18  | −0.14 | −0.20*| −0.13 |
| TRL 10–20 cm            | −0.01       | 0.00        | −0.06       | −0.05  | −0.16 | −0.03 | 0.09  |
| TRL 20–30 cm            | −0.12       | −0.12       | −0.07       | 0.04   | −0.18 | −0.11 | 0.01  |
| TRL 30–40 cm            | 0.03        | −0.08       | −0.14       | 0.03   | −0.04 | −0.07 | −0.02 |
| TRL 40–50 cm            | −0.06       | −0.07       | −0.02       | 0.12   | −0.07 | −0.03 | 0.02  |
| TRL 50–60 cm            | −0.09       | −0.16       | −0.08       | 0.15   | 0.00  | −0.08 | −0.12 |

RTA: root transversal area; STA: stele transversal area; %STA: (STA x 100)/RTA; LMXN: late metaxylem number; LMXA: total late metaxylem area; sLMXA: single late metaxylem area (LMXA/LMXN); TRL: total root length; *shows significant at P < 0.1.

Table 7. Putative QTLs for root vascular traits detected in CIM in OY population in Experiment 1.

| Trait                  | Chr. | Nearest marker | Position (cm) | Interval | LOD  | R^2  | A    |
|------------------------|------|----------------|---------------|----------|------|------|------|
| RTA                    | 4    | RM3288         | 82.11         | RM3288-RM5503 | 3.9  | 14.9 | 52.75|
|                        | 8    | RM7356         | 104.51        | RM7356-RM3155 | 3.2  | 38.4 | −87.15|
| %STA                   | 1    | RM8147         | 41.01         | RM8147-RM6039 | 2.7  | 11.0 | 0.28 |
| %LMXA                  | 4    | RM5913         | 34.31         | RM5953-RM3643 | 3.0  | 39.2 | 1.53 |
|                         | 6    | RM3183         | 70.21         | RM7023-RM3183 | 6.7  | 39.8 | 1.58 |
| LMXN                   | 1    | RM8147         | 34.01         | RM8147-RM6039 | 4.6  | 18.1 | 0.34 |
| LMXA                   | 4    | RM5785         | 1.01          | RM7585-RM2811 | 2.5  | 14.7 | 0.76 |
| sLMXA                  | 4    | RM3288         | 84.61         | RM3288-RM5503 | 3.9  | 20.0 | 0.12 |
|                        | 4    | RM1113         | 116.61        | RM3335-RM1113 | 2.7  | 15.8 | −0.10|

RTA: root transversal area; STA: stele transversal area; %STA: (STA x 100)/RTA; LMXN: late metaxylem number; LMXA: total late metaxylem area; sLMXA: single late metaxylem area (LMXA/LMXN); Nearest marker: name of marker stayed closest to the QTL; position: distance from the end of the short arm, LOD: logarithm of the odds, A: the additive effect either from YHM (>0) or OTM (<0), R^2: percentage of phenotypic variation explained.

Table 8. Putative QTLs for root vascular traits detected by CIM in OY population in Experiment 2.

| Trait                  | Chr. | Marker         | Position (cm) | Interval | LOD  | R^2  | A    |
|------------------------|------|----------------|---------------|----------|------|------|------|
| **Vegetative stage**   |      |                |               |          |      |      |      |
| %STA                   | 1    | RM6324         | 2.21          | RM6324-RM8110 | 3.2  | 16.4 | 0.3  |
| %LMXA                  | 8    | RM5911         | 0.01          | RM5911-RM4085 | 2.6  | 13.8 | 0.7  |
| %LMXA                  | 8    | RM3374         | 33.81         | RM3374-RM7356 | 3.5  | 30.2 | −1.1 |
| **Vegetative stage**   |      |                |               |          |      |      |      |
| %STA                   | 6    | RM3183         | 63.21         | RM7023-RM3183 | 3.5  | 17.0 | −64.53|
| %LMXA                  | 10   | RM216          | 14.31         | RM216-RM467  | 2.5  | 26.4 | −0.39|
| LMXA                   | 1    | RM5919         | 92.31         | RM5919-RM3475 | 2.7  | 11.9 | 0.45 |
| LMXA                   | 2    | RM2770         | 11.51         | RM2770-RM3703 | 3.4  | 25.6 | −0.65|
| **Maturity stage**     |      |                |               |          |      |      |      |
| %LMXA                  | 7    | RM5508         | 70.06         | RM5508-RM1364 | 3.5  | 18.9 | 5.16 |
| %LMXA                  | 4    | RM3785         | 74.76         | RM3785-RM3288 | 2.6  | 12.2 | 0.39 |
| %LMXA                  | 1    | RM3494         | 128.61        | RM3494-RM6696 | 2.6  | 32.9 | −1.22|
| LMXA                   | 9    | RM409          | 47.41         | RM409-RM566  | 3.4  | 16.4 | −0.85|
| LMXN                   | 8    | RM5911         | 5.51          | RM5911-RM4085 | 2.8  | 29.7 | 0.44 |
| LMXN                   | 7    | RM5508         | 70.06         | RM5508-RM1364 | 3.4  | 18.3 | 1.13 |
| **Maturity stage**     |      |                |               |          |      |      |      |
| %LMXA                  | 10   | RM1374         | 65.01         | RM1374-RM4771 | 3.4  | 19.8 | −1.11|
| LMXN                   | 6    | RM4447         | 89.31         | RM4447-RM5509 | 2.9  | 25.4 | 0.32 |

RTA: root transversal area; STA: stele transversal area; %STA: (STA x 100)/RTA; LMXN: late metaxylem number; LMXA: total late metaxylem area; sLMXA: single late metaxylem area (LMXA/LMXN); Marker: name of marker closest to the QTL; position: distance from the end of the short arm, LOD: logarithm of the odds, A: the additive effect either from YHM (>0) or OTM (<0), R^2: percentage of phenotypic variation explained.

Four regions collocating putative QTLs for STA detected in both joint and single environment analysis were regarded as key genomic regions (Table 11): (1) A region near RM6911 on Chr. 2 collocating joint QTLs for STA, %STA, LMXN, and LMXA, and single environment QTLs for STA (upma13) and %STA (upveg19); (2) RM6933-RM3857 on Chr. 2 for STA, LMXA, sLMXA (joint analysis) and STA, LMXA, sLMXA (local analysis); (3) RM1388-RM5503
Table 9. Putative QTLs for deep root growth angle traits detected by CIM in OY population in Experiment 1.

| Trait     | QTL            | Ch  | Marker | Position(cM) | Interval | LOD  | A    | R²   |
|-----------|----------------|-----|--------|--------------|----------|------|------|------|
| PRN45-65  | Qtl_prn45-65_oy_1 | 1   | RM8110 | 27.31        | RM8110-RM8147 | 2.8  | -5.07| 15.4 |
| PRN45-90  | Qtl_prn45-90_oy_7 | 7   | RM3404 | 64.51        | RM1335-RM3404 | 3    | -1.47| 11.7 |
| PRN65-65  | Qtl_prn65-65_oy_2 | 2   | RM3857 | 137.01       | RM3857-RM6733 | 3.3  | 0.04 | 10.5 |
| PRN65-90  | Qtl_prn65-90_oy_4 | 4   | RM7585 | 4.81         | ~RM5953      | 5.4  | -0.06| 20.2 |
| PRN65-90  | Qtl_prn65-90_oy_6 | 6   | RM5814 | 120.51       | RM5509-RM3509 | 3.5  | -0.03| 15.1 |
| PRN65-90  | Qtl_prn65-90_oy_12 | 12  | RM247  | 1.51         | ~RM247       | 2.6  | 0.02 | 11.5 |
| PRN45-90  | qtl_prn45-90_oy_4 | 4   | RM7585 | 4.81         | RM7585-RM5953 | 4.2  | -0.06| 14.3 |
| PRN45-90  | Qtl_prn45-90_oy_6 | 6   | RM4447 | 74.81        | RM3183-RM6734 | 2.8  | 0.05 | 7.2  |

RN: root number; PRN: proportion of root number; Marker: name of marker closest to the QTL; position: distance from the end of the short arm, LOD: logarithm of the odds, A: the additive effect either from YHM (>0) or OTM (<0), R²: percentage of phenotypic variation explained.

Table 10. Putative QTLs for deep rooting traits (total root length (TRL)) and their proportion at each depth (%TRL) at maturity stage by CIM in OY population in Experiment 2.

| Maturity stage – Upland condition  | Chr. | Marker | Position (cM) | Interval | LOD  | R²   | A    |
|------------------------------------|------|--------|---------------|----------|------|------|------|
| TRL10-20 cm                        | 3    | RM1332 | 5.51          | RM1332-RM3209 | 3.7  | 15.3 | 49.6 |
| TRL10-20 cm                        | 11   | RM287  | 72.61         | RM287-RM209  | 2.7  | 22.3 | -34.9|
| TRL20-30 cm                        | 3    | RM1332 | 5.51          | RM1332-RM3209 | 4.6  | 23.6 | 29   |
| TRL30-60 cm                        | 4    | RM5503 | 97.61         | RM5503-RM3276 | 2.5  | 12.3 | -1.8 |
| %TRL50-60 cm                       | 10   | RM222  | 0.01          | RM160-RM3808  | 2.8  | 16.2 | 16.5 |

| Maturity stage – Lowland condition | Chr. | Marker | Position (cM) | Interval | LOD  | R²   | A    |
|------------------------------------|------|--------|---------------|----------|------|------|------|
| %TRL10-20 cm                       | 8    | RM5911 | 0.01          | RM5911-RM4085 | 2.7  | 14.6 | -4.49|
| TRL20-30 cm                        | 9    | RM160  | 74.01         | RM160-RM3808 | 2.8  | 16.2 | 16.5 |

Marker: name of marker closest to the QTL; position: distance from the end of the short arm, LOD: logarithm of the odds, A: the additive effect either from YHM (>0) or OTM (<0), R²: percentage of phenotypic variation explained.

Discussion

In this study, QTLs for root vascular and deep rooting traits in vegetative and maturity stages in upland and lowland fields have been identified using a recombinant inbred population (OY) derived from two temperate japonica ecotypes, lowland rice OTM (Otomemochi) and upland rice YHM (Yumenohatamochi). To the best of our knowledge, this is the first comprehensive report on QTLs of root vascular traits at multiple measurement occasions (two growth stages, two growth conditions) from a temperate japonica cross. Other studies on rice root vascular traits were either limited to one growth stage or to mesocosm systems (Kadam et al., 2017; Uga et al., 2008).

Key genomic regions for root vascular traits

Root vascular traits showed significant genotypic variation among the progenies of OTM and YHM at both vegetative and maturity stages, with their sum of squares in general larger than those of field effect (upland or lowland) and genotype by field interaction (except for RDA and sLMXA at maturity in Experiment 2). The coefficient of variation of the phenotypic data of root vascular traits ranged from 11% to 27% and 11% to 21% at vegetative and maturity stages, respectively, with average broad-sense heritability being 0.60 and 0.54, respectively. The multi-environment analysis of five datasets showed significant genotypic variations of root vascular traits, contributing to 6.8–23.7% of the total phenotypic variation (Supplementary Table 3). In comparison, G × E interaction explained 17.9–21.9% of the total variation, suggesting substantial influences of phenotyping environments (i.e. field condition, growth stage) on genotypic assessment. Interactions between genotype and water regimes, and between genotype and stages were shown for root vascular traits (Ouyang et al., 2020; Phoura, 2019). Nonetheless, the genotypic differences were statistically significant, and the identified QTLs, particularly from the multi-environment analysis for root vascular traits (i.e. Table 11) would be credible, as discussed in the next paragraph.

Among the genomic regions related to root vascular traits identified in this temperate japonica mapping population, we regarded four regions on chromosome 2 (RM3703-RM6379, RM6933-RM3857), chromosome 4 (RM1388-RM5503) and chromosome 12 (RM247-RM1155) which collocated putative QTLs for STA detected in both joint and single environment analysis as key genomic regions. The region RM3703-RM6379 on chromosome 2 contained a QTL for STA (LOD peak near RM6911) in the multi-environment analysis with an insignificant LOD.
Table 11. Four key genomic regions (containing putative QTLs for STA in both joint and single environment analysis) for root vascular traits by multi-environment QTL analysis with references to root vascular traits and deep rooting traits QTLs by CIM and single environment MT-CIM analyses. Selected from the whole analysis in Supplementary Table 1.

| No | Trait     | Environment | Chr. | Marker | Position (cM) | LOD  | LOD (GxE) | A   | Separate, single environment QTLs |
|----|-----------|-------------|------|--------|---------------|------|-----------|-----|---------------------------------|
| 1  | %STA      | upveg19     | 2    | RM6911 | 35.71         | 4.63 | 1.81      | 0.29| -                               |
| 1  | %STA      | joint       | 2    | RM6911 | 35.71         | 5.34 | 3.23      | 0.17| -                               |
| 1  | LMXA      | joint       | 2    | RM6911 | 37.41         | 3.28 | 1.09      | 0.29| -                               |
| 1  | LMXN      | joint       | 2    | RM6911 | 37.21         | 4.49 | 1.89      | 0.16| -                               |
| 1  | STA       | upma13      | 2    | RM6911 | 37.41         | 2.98 | 1.43      | 3.12| -                               |
| 1  | STA       | joint       | 2    | RM6911 | 37.21         | 4.01 | 2.01      | 1.43| -                               |
| 2  | LMXA      | lowma19     | 2    | RM6933 | 125.21        | 4.43 | 2.59      | 0.44| -                               |
| 2  | LMXA      | joint       | 2    | RM6933 | 125.71        | 4.72 | 3.82      | 0.22| -                               |
| 2  | sLMXA     | lowma19     | 2    | RM6933 | 126.71        | 5.17 | 2.83      | 0.09| -                               |
| 2  | sLMXA     | joint       | 2    | RM6933 | 126.21        | 5.44 | 4.19      | 0.05| -                               |
| 2  | STA       | lowma19     | 2    | RM6933 | 125.21        | 3.66 | 2.36      | 1.93| -                               |
| 2  | STA       | joint       | 2    | RM6933 | 125.21        | 3.97 | 3.38      | 0.86| -                               |
| 3  | LMXA      | joint       | 4    | RM3288 | 82.11         | 4.16 | 1.87      | 0.32| -                               |
| 3  | LMXN      | joint       | 4    | RM3288 | 82.11         | 2.88 | 0.91      | 0.16| -                               |
| 3  | RTA       | joint       | 4    | RM3288 | 82.11         | 4.5  | 1.05      | 3.24| -                               |
| 3  | sLMXA     | lowveg19    | 4    | RM3288 | 82.11         | 2.67 | 1.37      | 0.09| -                               |
| 3  | sLMXA     | joint       | 4    | RM3288 | 82.11         | 3.66 | 2.18      | 0.04| -                               |
| 3  | STA       | lowveg19    | 4    | RM3288 | 82.11         | 3.59 | 2.05      | 3.69| -                               |
| 3  | STA       | joint       | 4    | RM3288 | 82.11         | 5.25 | 3.02      | 1.57| -                               |
| 4  | LMXN      | joint       | 12   | RM247  | 35.41         | 4.94 | 3.26      | 0.15| -                               |
| 4  | RTA       | lowveg19    | 12   | RM247  | 40.91         | 2.56 | 1.61      | 47.01| -                               |
| 4  | RTA       | joint       | 12   | RM247  | 39.41         | 3.02 | 2.24      | 17.99| -                               |
| 4  | STA       | lowveg19    | 12   | RM247  | 36.91         | 3.41 | 2.74      | 3.24| -                               |
| 4  | STA       | joint       | 12   | RM247  | 38.91         | 3.41 | 2.74      | 1.01| -                               |

RTA: root transversal area; STA: stele transversal area; %STA: (STA×100)/RTA; LMXN: late metaxylem number; LMXA: total late metaxylem area; sLMXA: single late metaxylem area (LMXA/LMXN); PRN: percentage of root number at angle; TRL: total root length; Marker: name of marker closest to the QTL; position: distance from the end of the short arm; LOD: logarithm of the odds; A: the additive effect either from YH (>0) or OTM (<0); R2: percentage of phenotypic variation explained; *: minor QTL from CIM or MT-CIM with LOD > 1.3 (Supplementary Table 2)
score for G × E, whose allelic contribution came from YHM. Uga et al. (2008) reported qSTA-2 and qLMXA-2 nearby this genomic region using a tropical japonica and indica population, with its positive allelic effect deriving from the thicker root parent, Kinandang Patong. Regardless of the genetic background, this genomic region (RM3703-RM6379) on chromosome 2 controls stele size of rice. Other studies also reported nearby QTLs for root thickness at 20–25 cm (Kamoshita et al., 2002a) and root penetration ability which may be interrelated with thickness of the root and/or stele (Ray et al., 1996; Zhang et al., 2001), with their allelic contributions all from the thicker root parents.

Another key region on chromosome 2 between RM6933-RM3857 contained the QTLs for STA, LMXA, and sLMXA from the multi-environment analysis with their positive allelic contribution came from YHM and had a significant LOD for G × E. From the checking of the regions between flanking markers for genes related to root development in Rice Annotation Project (https://rapdb.dna.affrc.go.jp) and the Overview of functionally characterized Genes in Rice Online database (http://qtdb.abr.affrc.go.jp/ogro), we found one gene for root hair development, OsRac3 (Kim et al., 2021) and one gene for adventitious root emergence and development, OsPIN1B (Xu et al., 2005) located nearby. This region is also linked to root morphological traits such as root thickness, number of penetrated roots, and nodal root number at depth (Kamoshita et al., 2002b; Price et al., 2000; Suralta et al., 2015) besides physiological and agronomical traits like osmotic adjustment (Nguyen et al., 2004), drought tolerance and tiller number (Kang et al., 2017), yield under cyclical stress (qDTY2.3 – Sandhu et al., 2014) or by meta-analysis (Kahani et al., 2021; Swamy et al., 2011). Brassinosteroid (BR), a site-specific hormone regulating cell division and differentiation, has been recently been reported to directly affect STA (Fridman et al., 2021). The expression of BR can enlarge or reduce STA depending on the location of its promoter. Fridman et al. (2021) showed that in the bri1 background, the expression of BRI1 in the stele increased STA and shrink STA if the expression was in the epidermis. As the expression of promoters for BR can contribute to STA, we checked for BR-related genes in the key regions and found that three genomic regions for BIN1, BIN2, and BZR1 promoters of BR were located near two key regions on Chr. 2 (Supplementary Table 4). This colocation suggests that the two QTLs for STA on Chr. 2 regulation on the trait may be related to BR. Further works should be continued to clarify the genetic basis of root vascular traits such as STA, %STA, LMXA, and LMXN.
The third key genomic region on chromosome 4 (RM1388-RM5503) contained the QTLs for STA, RTA, LMXN, LMXA, and sLMXA from the multi-environment analysis with their positive allelic contribution came from YHM. Near this chromosomal region of RM1388-RM5503, there had been reports of QTLs related to basal root thickness (Nguyen et al., 2004; Price et al., 2002), and their positive allelic contribution came from the thicker root parents (i.e. CT9993, Azucena). The putative genes regulating crown root development, D17 (Arite et al., 2012), cZOGT1, cZOGT2 (Kudo et al., 2012), and OsSaur18, an auxin-responsive gene (Kikuchi et al., 2003) were identified in this region (Supplementary Table 4). However, for root vascular traits, Sta1 on chromosome 9 in Uga et al. (2010) was not detected in our temperate japonica population, and our third region on chromosome 4 had not been identified in their study.

The multi-environment QTLs for STA and RTA, LMXN on chromosome 12 (RM155) located near qDTY12.1, a consistent QTL for grain yield in drought conditions (Bernier et al., 2007; Mishra et al., 2013) and OsPSTOL1, a gene for phosphorus uptake (Gamuyao et al., 2012; Wissuwa et al., 2002). Proliferation of crown roots with vascular traits such as larger STA, LMXN, and LMXA may be advantageous for water uptake under intermittent water replenish (Richards & Passioura, 1989), thus maintaining grain yield especially in drought cycles as applied by Bernier et al. (2007) and Mishra et al. (2013). STA was overall higher under low phosphorus conditions than under high phosphorus conditions (Vejchasarn et al., 2016).

**Relation between root vascular traits and deep rooting traits**

In this temperate japonica mapping population, no significant correlation between root vascular traits and deep rooting traits was detected. In a study of 274 indica rice genotypes, Kadam et al. (2017) reported no significant correlation between root vascular traits (e.g. stele and late metaxylem diameter) and root morphological traits (e.g. maximum root length, specific root length, root volume). In spring barley, Oyiga et al. (2020) also did not find any significant correlation between nodal root growth angle and root transversal area. On the contrary, Uga et al. (2009) reported significant correlations between root length index (the index for the length of the whole root system) and root vascular traits among 59 accessions. Hazman and Brown (2018) also found strong correlations of maximum root depth to RTA, STA, and LMXA in 11 Egyptian rice cultivars grown under well-watered conditions. The studied population was developed under well-irrigated lowland conditions during early generations from F₁ (T. Manabe, personal communication), which may have led to relatively limited variation in traits such as deep rooting traits which are essential for adaptation to upland environment.

Among the four key genomic regions for STA from the multi-environment analysis, two were collocated with the QTLs for deep rooting traits in this mapping population; (1) RM6933-RM6732 on chromosome 2 (STA, LMXN, sLMXA, and deep PRN), (2) RM1388-RM5503 on chromosome 4 (various root vascular traits and deep TRL). The region on chromosome 4 detected in this study was an important genomic region for root vascular traits collocated with various QTLs for different root and shoot traits, one of them was the QTL for deep root growth angle in the other population from Kinandang Patong and IR64 (Kitomi et al., 2015; Uga et al., 2013). Other studies also show a QTL cluster of root traits on chromosome 4 (Kamoshita et al., 2008; Ray et al., 1996). Interestingly, nearby the key region on chromosome 4, different production QTLs had been identified such as yield under water stress (Yadav et al., 2019), leaf nitrogen content (Takai et al., 2012), nai1 gene which controls leaf width and vascular bundle number (Cho et al., 2014), and SPIKE gene that increases spikelet number (Fujita et al., 2013). The collocation of the above shoot and root physical traits might indicate a possible usefulness for genomic selection of the key chromosome 4 region to improve rice adaptation and productivity.

The other key genomic region for root vascular traits was on chromosome 2 (as discussed in the previous section), collocating a QTL for PRN4566 from upma13 separate analysis. This region is near several deep rooting QTLs such as DRO4 (Kitomi et al., 2015) or maximum root length mgMRL_2-5 (Courtois et al., 2009). Khahani et al. (2021) and Suralta et al. (2015) showed the region RM6933-RM3857 may as well be related to the ratio of deep rooting and nodal root length and number below 30 cm. These collocations suggested this region may contain major QTLs for deep rooting traits, especially for root length at depth. Norton et al. (2008) also reported that this region is highly dense with root-trait QTLs with a maximum density of 23 QTLs.

These results indicate that deep rooting traits and root vascular traits were not necessarily co-localized (i.e. no major linkage or pleiotropy on chromosomes 2 and 12), suggesting that in this temperate japonica population, separate selection for each of the deep rooting traits and root vascular traits is possible as desired. This contrasts with empirical observations of the strong phenotypic correlations between these two group traits in rice varieties as is commonly contrasted between tropical japonica ecotype and lowland indica ecotype (Phoura, 2019; Uga et al., 2009).
Though using a limited number of DNA markers, this study could demonstrate several key genomic regions controlling root vascular traits through a series of five phenotype datasets. Based on the results of this study, the multi-environment QTL analysis is clearly advantageous over the separate approach due to its improved statistic power (Jiang & Zeng, 1995) when mapping the QTLs of traits of considerable size of $G \times E$ interaction like root vascular traits. For example, the multi-environment QTL analysis detected a QTL for STA in a single environment MT-CIM (upma13) as well as several root vascular trait QTLs (i.e. %STA, LMXN, LMXA) in the key region on chromosome 2, whereas the separate CIM analysis did not in our experiment. Phenotyped data of root vascular traits under multiple environmental conditions should be integrated considering both seedling and later growth stages and both upland and lowland conditions. Phenotyping precision for root vascular traits should be improved for more precise detection of QTLs and their effects. Rice is known to be the lowest in global crop water productivity among cereals (Foley et al., 2020), and to what extent genetic modification of its root vascular traits would improve rice’s water productivity should be further assessed.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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