18. Molecular mapping of QTLs associated with whole plant Water Use Efficiency (WUE)

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With a substantial area of rice cultivation concentrated in upland and rain-fed lowlands, water availability is the greatest cause for low yields (Greenland 1984, Sharma and Datta 1994). Improving drought tolerance through introgressing relevant physiological trait is expected to boost productivity. Among such traits, WUE, the amount of biomass produced per unit water transpired is regarded as most relevant. The significant genetic variability in WUE in various crop species (Sheshshayee et al. 2003) including rice (Impa et al. 2005) that can be exploited through breeding enhances the optimism in achieving the crop improvement. Nevertheless breeding efforts were not largely initiated owing mainly to the tedium involved in measuring the trait. This lacuna has been significantly overcome with the discovery that carbon isotope discrimination is strongly linked to WUE in C₃ species (Farquhar and Richards 1984, Farquhar et al. 1989). Δ¹³C has also been established as a surrogate for the season long WUE in rice as well (Impa et al. 2005). This high throughput technique has hence provided an accurate tool in identifying QTLs conditioning the complex trait like WUE. QTL have been identified for WUE mainly based on measurement of Δ¹³C in several crop species such as tomato (Martin et al. 1989), soybean (Mian et al. 1996) and barley (Handley et al. 1994) etc. However, there have been no such reports of identifying QTLs for WUE measured on a season long scale besides surrogates like Δ¹³C.

The present study envisaged mapping of QTLs for WUE and its associated physiological traits at whole plant level. A mapping population of 39 Doubled haploid lines from the cross CT9993-5-1-M (an upland japonica type) and IR62266-42-6-2 (an indica type) was used for this purpose.

These DH lines were grown in carbonized rubber containers measuring 0.41 m (L) x 0.21 m (B) x 0.67 m (H) filled with a potting mixture of wetland soil and farmyard manure in 3:1 proportion. The containers were irrigated twice daily to keep the soil at field capacity. All nutrients were added as per the recommendation to raise healthy and uniform plants. Plastic pieces were spread on the soil surface to minimize direct soil evaporation. To minimize the direct soil evaporation further a set of bare containers (without plants) was maintained to measure the soil evaporation. Water use efficiency was determined gravimetrically as per Impa et al, 2005. Each of the containers was weighed daily using a load cell hanging balance (ATCO Balances, India). The difference in weight on subsequent days was corrected by adding equal volume of water. The water thus added during the experimental period between 55 Days after sowing (DAS) and 89 DAS was summed up to arrive at the total evapo-transpiration. The
cumulative water transpired (CWT) was computed by subtracting the water added to bare containers. Total plant leaf area and total biomass (BM) were determined at the beginning and end of the experiment (55 to 89 DAS). Leaf area was determined using a leaf area meter (ΔAT Devices, Burwell, England, UK). The soil was washed carefully to remove the roots. And all the plant parts (leaves, stem and roots) were separately oven dried at 70°C for three days and dry weights were recorded. Using this primary values several growth parameters and WUE were computed. The ratio of biomass increment over the experimental period to the CWT was computed to arrive at WUE. Similarly the ratios of total biomass and CWT to LAD (LAD (cm² days) = [(LA₈₉ + LA₅₅)/2] * 35) were computed as measures of NAR and MTR respectively. The dried leaf samples were powdered and used for the determination of ¹³C composition using an IRMS (Delta plus, Thermo Electron, Bremen, Germany) interfaced with an elemental analyzer (NA1112, Carlo Erba, Italy) via a continuous flow device. Δ¹³C was computed using the following notation (Farquhar et al. 1989) (Δ¹³C = a- p/(1+ p/1000)).

The Doubled Haploid lines varied significantly for WUE and associated traits (Table 1). As predicted by theory Δ¹³C and WUE were inversely correlated (r = -0.47; p<0.01; n=39).

The genetic polymorphism among the DHLs was assessed using RAPD and AFLP technologies. The resultant polymorphic loci (115 RAPD and 89 AFLP) were used to construct the genetic linkage map using MAPMAKER/EXP3.0 with a threshold LOD 3.0. The markers were grouped into 12 linkage groups. QTL mapping was done using MAPMAKER/QTL1.0. QTLs associated with WUE and linked traits are presented in Table 2. Two QTLs were identified for WUE which mapped on to linkage group 1 and 4. QTL, qWUE-4 on linkage group 4 with LOD 3.3 explained a maximum phenotypic variance of 37.30%. Since the QTL detected had a total length of 40.8 cM further fine mapping of this region is essential before using the markers in breeding or to identify novel candidate genes.

QTLs were also identified for other associated physiological traits. Two QTLs were identified for BM one each on chromosome 2 and 6. QTL for BM, flanked by markers OPX4_1 and AT_CTG2 with a LOD score of 3.12 explained phenotypic variance of 39.30%. Three QTLs were identified for CWT and two QTLs for MTR. The overlapping QTL identified for CWT and MTR flanked by AFLP markers AT_CTG2 and AG_CTC31 mapped adjacent to the BM QTL, qBM-6 on linkage group 6. Overlapping QTL was also identified for BM and CWT on linkage group 2 bracketed by RAPD markers OPW16_3 and OPW16_4 explaining a phenotypic variance of 33.6 for BM and 45.9% for CWT with LOD of 2.49 and 3.89 respectively. This overlapping of QTLs arises due to pleiotrophic effect which is evidenced from the genotypic correlation between the BM and CWT (r = 0.79). One QTL for Δ¹³C was mapped on linkage group 6.
Separate QTLs were also identified for $\Delta^{13}$C and WUE, not withstanding the significant correlation between the traits. QTLs identified in this study can be fine mapped and utilized as an indirect selection criterion for drought tolerance in marker assisted selection.

Table 1. Genetic variability in Water Use Efficiency (WUE), Total transpiration (CWT), Dry matter accumulation (BM) and Functional Leaf area (LAD), Net Assimilation Rate (NAR) and Mean Transpiration rate (MTR), among the selected DHLs of the cross CT9993 x IR62266

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>Range</th>
<th>CD_{95}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWT (kg pot⁻¹)</td>
<td>15.34</td>
<td>8.29-24.73</td>
<td>0.195</td>
</tr>
<tr>
<td>BM (g pot⁻¹)</td>
<td>42.74</td>
<td>18.34-87</td>
<td>0.399</td>
</tr>
<tr>
<td>LAD (dm³days)</td>
<td>655</td>
<td>374.01-1161.33</td>
<td>207.13</td>
</tr>
<tr>
<td>WUE (g kg⁻¹)</td>
<td>2.88</td>
<td>1.28-4.46</td>
<td>0.056</td>
</tr>
<tr>
<td>NAR (g dm⁻³day⁻¹)</td>
<td>66.34</td>
<td>24.58-118.10</td>
<td>0.893</td>
</tr>
<tr>
<td>MTR (mL dm⁻³day⁻¹)</td>
<td>23.99</td>
<td>11.28-40.39</td>
<td>0.411</td>
</tr>
<tr>
<td>$\Delta^{13}$C (%)</td>
<td>21.55</td>
<td>19.82-22.91</td>
<td>0.178</td>
</tr>
</tbody>
</table>

Table 2. QTLs associated with WUE and related physiological traits

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL name</th>
<th>Interval</th>
<th>Linkage Group</th>
<th>Length (cM)</th>
<th>LOD</th>
<th>$R^2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WUE</td>
<td>qWUE-1</td>
<td>OPQ9_1 - OPS18_1</td>
<td>1</td>
<td>10.0</td>
<td>2.23</td>
<td>25.40</td>
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<td>WUE</td>
<td>qWUE-4</td>
<td>OPN12_2 - OPX13_2</td>
<td>4</td>
<td>40.8</td>
<td>3.34</td>
<td>37.30</td>
</tr>
<tr>
<td>BM</td>
<td>qBM-6</td>
<td>OPX16_3 - OPW16_4</td>
<td>2</td>
<td>19.2</td>
<td>2.49</td>
<td>33.60</td>
</tr>
<tr>
<td>BM</td>
<td>qBM-6</td>
<td>OPX4_1 - AT_CTG2</td>
<td>6</td>
<td>3.5</td>
<td>3.12</td>
<td>39.20</td>
</tr>
<tr>
<td>CWT</td>
<td>qCWT-1</td>
<td>OPV17_2 - OPR3_1</td>
<td>1</td>
<td>18.9</td>
<td>2.76</td>
<td>34.00</td>
</tr>
<tr>
<td>CWT</td>
<td>qCWT-2</td>
<td>OPW16_3 - OPW16_4</td>
<td>2</td>
<td>19.2</td>
<td>3.89</td>
<td>45.90</td>
</tr>
<tr>
<td>CWT</td>
<td>qCWT-6</td>
<td>AT_CTG2 - AG_CTC31</td>
<td>6</td>
<td>26.6</td>
<td>2.13</td>
<td>37.60</td>
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<tr>
<td>LAD</td>
<td>qLAD-1</td>
<td>OPQ9_2 - OPY17_2</td>
<td>1</td>
<td>22.5</td>
<td>2.48</td>
<td>37.90</td>
</tr>
<tr>
<td>MTR</td>
<td>qMTR-2</td>
<td>OPW16_3 - OPW16_4</td>
<td>2</td>
<td>19.2</td>
<td>2.12</td>
<td>28.10</td>
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<tr>
<td>MTR</td>
<td>qMTR-6</td>
<td>AT_CTG2 - AG_CTC31</td>
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<td>26.6</td>
<td>3.03</td>
<td>49.60</td>
</tr>
<tr>
<td>NAR</td>
<td>qNAR-6</td>
<td>AT_CTG2 - AG_CTC31</td>
<td>6</td>
<td>26.6</td>
<td>3.3</td>
<td>42.30</td>
</tr>
<tr>
<td>$\Delta^{13}$C</td>
<td>Qd13C-6</td>
<td>OPN9_3 - AG_CTC3</td>
<td>6</td>
<td>22.5</td>
<td>2.4</td>
<td>31.50</td>
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</tbody>
</table>
Fig. 1. Rice linkage map showing the position of QTLs associated with WUE related physiological traits (LOD>2.0). (The legends represent the symbols used for denoting QTLs of different traits: Water Use Efficiency (WUE), Total transpiration (CWT), Dry matter accumulation (BM) and Functional Leaf area (LAD), Net Assimilation Rate (NAR) and Mean Transpiration rate (MTR)).

References


