qDTY3.1, a major drought tolerant locus of APO promotes early flowering in the genetic background of a local cultivar improved white ponni

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Research Article

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Abstract

Advancements in molecular genetics led to identification of genomic regions in rice associated with performance under drought conditions which enabled designing molecular breeding strategies for development of drought tolerant rice genotypes. However, physiological and molecular basis of most of these QTLs are not yet studied. In this study, a recombinant inbred population (RIL) developed between Improved White Ponni (a drought susceptible rice variety) and Apo (a drought tolerant cultivar) was genotyped using SSR markers linked to major drought tolerant QTLs. Evaluation of RILs for days to flowering and subsequent marker–trait association analysis revealed that qDTY3.1, a major drought tolerant loci was found to be associated with earliness with an R² value of 0.66. Further analysis revealed that qDTY3.1 was found to be co-localized or located near a major heading date locus Hd6 (30.4–35.6 Mb; RM3199–RM3329) on chromosome 3. Further experiments for developing back cross progenies harboring the two loci in different combinations are in progress to dissect out the interaction between the qDTY3.1 and Hd6.

Key words

Rice, Drought, SSR Marker, Validation

Introduction

Rice is the important food crop for more than half of the world’s population. Abiotic stresses like drought and submergence severely affects rice production and productivity (Vikram et al., 2011). Among the various abiotic stresses, drought affects 23 million hectares of rainfed rice (Serraj et al., 2011) and thus causing severe threat to agricultural production (Pandey and Shukla 2015). When drought coincides with flowering and grain filling, it leads to drastic reduction in yield (Then et al., 2011). Developing drought tolerant rice varieties remains hampered due to complexity of tolerance mechanisms and lack of reliable phenotyping procedures. The identification of major QTLs for grain yield under drought—qDTY12.1 (Bernier et al., 2007), qDTY2.1 and qDTY3.1 (Venuprasad et al., 2009), and qDTY1.1 (Vikram et al., 2011) in rice using modern molecular biology tools has provided new opportunities to breeders to develop varieties tolerant against drought.

However, many of the QTLs reported to be associated with drought tolerance are 1) detected based on single marker analysis; 2) large in their size (>2 Mb interval) and 3) physiological and molecular basis are not yet understood and hence making it difficult to put them into practical breeding programs. Several attempts have been made at IRRI, Philippines to unravel physiological basis of these major effect QTLs and it has been reported that qDTY3.2 had major influence on days to flowering. In the present study, attempts were made through phenotyping and genotyping of a RIL population (F3) derived between a popular long duration rice variety Improved White Ponni and a drought tolerant cultivar Apo to test the association between a major loci qDTY3.1 and days to flowering.

Materials and Methods

A set of recombinant inbred lines (F3) derived between Improved White Ponni and Apo was used in this study. Improved White Ponni is a popular long duration variety possessing high yield and superior grain quality. Whereas Apo is a drought tolerant upland cultivar using which major QTLs viz., qDTY1.1, qDTY1.2, qDTY 2.1, qDTY3.1, qDTY6.1 and qDTY12.1 were mapped (Venu Prasad et al., 2009; 2012a and 2012b). True F1s were identified and forwarded to F2 generation using Improved White ponni as a recurrent and Apo as a donor parent. True F1S were identified harboring qDTY3.1 and forwarded to F2 generation. In F3 generation, leaf samples were collected from 109 individual
progenies and used for extraction of genomic DNA through modified CTAB protocol as described by Murray and Thompson (1980). Isolated genomic DNA was quantified using Spectrophotometer (Nano Drop) and were diluted to final concentration of 50ng/ul, and stored under refrigerator condition at -40 °C.

A polymorphic SSR marker RM520 (30.9 Mb on chromosome 3) tightly linked to qDTY3.1 was used for genotyping of 109 F3 progenies along with the parents. PCR reactions were carried out using DNA sample with the concentration of 50ng/ul, PCR buffer consist of 1.5mM MgCl2, 0.4mM dNTPs, 10µM primers and 1.5U of Taq DNA polymerase were utilized. PCR reaction were performed in thermal cycler (DNA Engine, BioRad, USA) programmed as initial denaturation at 95°C for 5 min, 35 cycles of 95 °C for 30sec, 55 °C for 30 sec and 72 °C for 30 sec followed by final extension of one cycle at 72 °C for 10 min. PCR amplified products were subjected to gel electrophoresis using 3.5% agarose, stained with Ethidium Bromide and visualized with UV trans-illuminator. Observations on days to first flowering (emergence of spikelets of primary panicle outside the boot leaf sheath) and days to 50% flowering (when more than 50% of the panicles in a progeny started anthesis) were recorded.

Simple linear regression analysis (Zongo et al., 2017) were carried out with the following equation y = b0 + b1x + e (y- phenotypic value, b0 – population mean, b1x – function of molecular marker and e = error) using marker and phenotypic value of a particular trait. The potential relationship can be judged by the phenotypic variance of R² value (Boranayaka et al., 2018).

Results and Discussion
Rice is one of the major cereals in Asia which remain at the top in both production and consumption (Hussain et al., 2014). Rice production is often hampered by several biotic/abiotic stresses and drought stands at the top among all the abiotic stresses in limiting rice yields especially under rainfed conditions (Prince et al., 2015). Rice crops depending on monsoon rains are frequently subjected to terminal drought and most of the existing varieties are highly sensitive to water deficit (Then et al., 2011). Developing early maturing rice varieties or varieties that can reduce their duration in response to drought will enable sustaining rice yields under drought. Several attempts have been made to unravel molecular genetic basis of drought tolerance traits in rice which led to identification of major loci governing performance under drought in a drought tolerant variety Apo (Venuprasad et al., 2009), Nagina 22 (Vikram et al., 2011) and Vandana (Solis et al., 2018). However, only few of them including qDTY1.1 and qDTY12.1 have been dissected out at physiological and molecular level and put into breeding applications. Several other QTLs including qDTY3.1 remain not fully understood at physiological and molecular levels.

In the present study, F3 populations derived from the cross between Improved White ponni and Apo were subjected to precision genotyping and phenotyping with a view to study the association between qDTY3.1 and days to flowering. A polymorphic marker RM520 located close to qDTY3.1 (30.9 Mb on chromosome 3) was selected for genotyping (Fig.1; Table 1). A total of 109 F3 progenies were genotyped using the marker RM520 which resulted in the identification of 32 progenies possessing homozygous IWP allele of RM520 and 34 progenies possessing homozygous Apo allele of RM520. Remaining 43 progenies were found to remain under heterozygous conditions.

Phenotyping of F3 population for days to first flowering and days to 50% flowering revealed that progenies exhibited continuous variation for the traits. IWP was found to reach first flowering in 117 days and 50% flowering in 122 days (Table 2). Drought tolerant Apo registered first flowering in 108 days and 50% flowering in 112 days. F3 progenies were found to reach first flowering ranging from 92 - 113 days and 50% flowering between 96 - 119 days (Table 2). Progenies exhibited clear transgressive segregation which revealed the involvement of several loci in controlling the days to flowering (Fig 2). Marker vs trait analysis using simple linear regression method (Boranayaka et al., 2018) revealed that RM520 was found to possess significant association (R² – 0.66) with days to first flowering and days to fifty percent flowering (Table 3). Further, in silico analysis of qDTY3.1 revealed its close proximity or co-localization a major heading date locus, with Hd6 spanning between 30.4 Mb to 35.6 Mb on Chromosome 3 (Hori et al., 2015). Scanning of target regions using more number of SSR markers will allow us to group the lines based on recombination events between qDTY3.1 and Hd6. Vikram et al. (2011) reported that qDTY3.1 did not exhibit any association with days to flowering under normal condition in a population developed by involving Swarna and Apo. In this study, qDTY3.1 exhibited moderate association with days to flowering which may be due to the favorable
interaction of Apo with the recipient genome IWP. However, further confirmatory experiments are needed for dissecting out the precise role of qDTY3.1 through development of backcross progenies of IWP harboring the QTL segment. This will also allow us to differentiate the effects of qDTY3.1 and Hdl6 on promoting earliness in flowering and drought tolerance.

References


Table 1. Description of target QTL \( (qDTY3.1) \) controlling yield under drought stress

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chr. No</th>
<th>Linked Marker</th>
<th>Marker interval</th>
<th>Physical position (Mb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>qDTY3.1</td>
<td>3</td>
<td>RM520</td>
<td>RM520 – RM16030</td>
<td>30.9</td>
<td>Venu Prasad et al. (2009)</td>
</tr>
</tbody>
</table>

Table 2. Performance of parents and F3 lines (Improved white ponni x Apo) for days to heading traits

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Allelic Pattern (qDTY3.1-RM520)</th>
<th>Days to first flowering (Days)</th>
<th>Days to fifty percent flowering (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IWP</td>
<td>AA</td>
<td>117</td>
<td>122</td>
</tr>
<tr>
<td>APO</td>
<td>BB</td>
<td>108</td>
<td>112</td>
</tr>
<tr>
<td>F3 progenies shown homozygous for IWP allele</td>
<td>AA</td>
<td>96-113</td>
<td>103-119</td>
</tr>
<tr>
<td>F3 progenies shown homozygous for Apo allele</td>
<td>BB</td>
<td>92-111</td>
<td>96-118</td>
</tr>
</tbody>
</table>

IWP – Improved White Ponni, AA – Homozygous for Improved White ponni allele, BB – Homozygous for Apo allele

Table 3. Marker trait association by regression analysis

<table>
<thead>
<tr>
<th>Trait</th>
<th>Marker</th>
<th>Chromosome</th>
<th>Position</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days to first flowering</td>
<td>RM520</td>
<td>3</td>
<td>30.9</td>
<td>0.656</td>
</tr>
<tr>
<td>Days to fifty percent flowering</td>
<td>RM520</td>
<td>3</td>
<td>30.9</td>
<td>0.657</td>
</tr>
</tbody>
</table>
Fig. 1. Foreground selection of F₃ population (Improved White ponni x Apo) using SSR primer (RM520) linked to the target QTL (qDTY3.1).

Fig. 2. Frequency distribution of F₃ homozygous progenies for Improved white ponni and Apo allele for the trait days to fifty percent flowering.