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ISSN: 0975-928X

Volume: 10

Number:1

EJPB (2019) 10(1):155-159

DOI: 10.5958/0975-928X.2019.00018.8

Electronic Journal of Plant Breeding, 10 (1): 155 - 159 (Mar 2019) DOI: 10.5958/0975-928X.2019.00018.8
ISSN 0975-928X

Research Article

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

K. Shamini, R. Sudhagar, M. Raveendran* and A. John Joel
Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore
*Department of Biotechnology, Centre for Plant Molecular Biotechnology and Bioinformatics, Tamil Nadu Agricultural University, Coimbatore
E-Mail: raveendrantnau@gmail.com

(Received: 01 Mar 2019; Revised: 10 Mar 2019; Accepted: 15 Mar 2019)

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 R^2 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 (F_3) 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 (F_3) 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 F_2 generation using Improved White ponni as a recurrent and Apo as a donor parent. True F_{1S} were identified harboring *qDTY3.1* and forwarded to F_2 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 ^{0}C.

A polymorphic SSR marker RM520 (30.9 Mb on chromosome 3) tightly linked to *qDTY3*.1 was used for genotyping of 109 F_3 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 $MgCl_2$, 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 95ºC for 5 min, 35 cycles of 95 ºC for 30sec, 55 ºC for 30 sec and 72 ºC for 30 sec followed by final extension of one cycle at 72 ºC 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 fallowing 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 R^2 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, F_3 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 F_3 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 F_3 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. F_3 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 (R^2 – 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 *Hd6* on promoting earliness in flowering and drought tolerance.

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

QTL	Chr. No	Linked Marker	Marker interval	Physical position (Mb)	Reference
qDTY3.1	3	RM520	RM520 – RM16030 (30.9 – 32.5 Mb)	30.9	Venu Prasad *et al.* (2009)

Table 2. Performance of parents and F_3 lines (Improved white ponni x Apo) for days to heading traits

Genotypes	Allelic Pattern (*qDTY3*.1-RM520)	Days to first flowering (Days)	Days to fifty percent flowering (Days)
IWP	AA	117	122
APO	BB	108	112
F_3 progenies shown homozygous for IWP allele	AA	96-113	103-119
F_3 progenies shown homozygous for Apo allele	BB	92-111	96-118

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

Table 3. Marker trait association by regression analysis

Trait	Marker	Chromosome	Position	R^2 value
Days to first flowering	RM520	3	30.9	0.656
Days to fifty percent flowering	RM520	3	30.9	0.657

 DOI: 10.5958/0975-928X.2019.00018.8

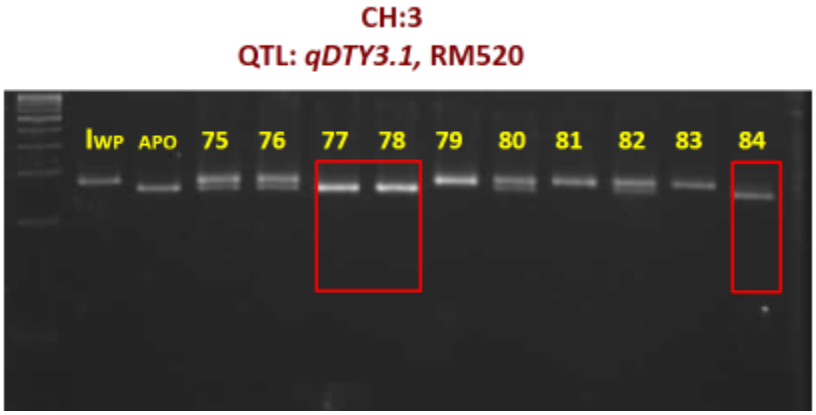

Fig.1. Foreground selection of F_3 population (Improved White ponni x Apo) using SSR primer (RM520) linked to the target QTL (*qDTY3.1*)

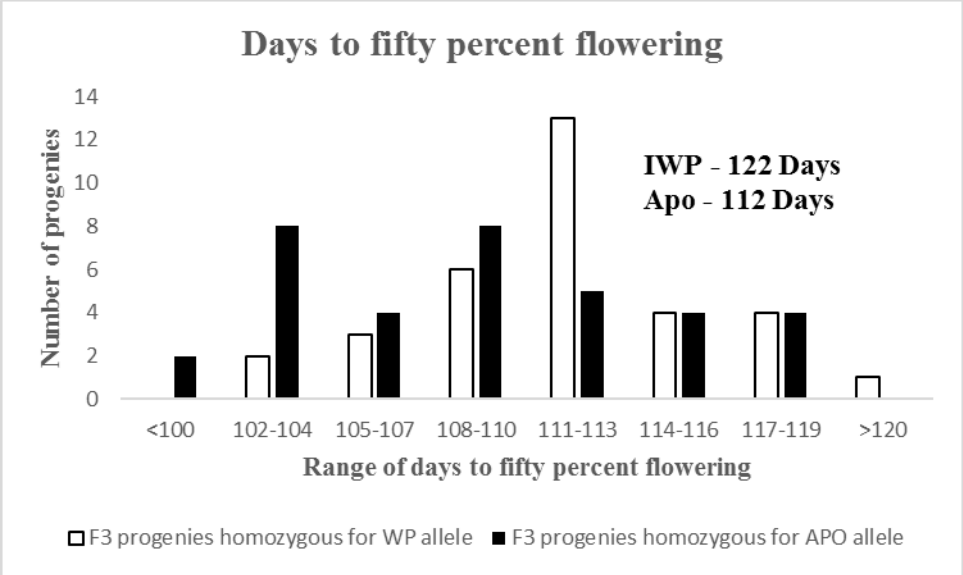

Fig.2. Frequency distribution of F_3 homozygous progenies for Improved white ponni and Apo allele for the trait days to fifty percent flowering

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