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Quantitative trait loci analysis of stele traits of rice (*Oryza sativa*) under well-watered and drought conditions

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Abstract

Drought is a major yield-limiting factor for rice production. In drying soils, plants experience water scarcity and soil compaction. Penetration of deep roots in hard soil can improve crop productivity under drought. It has been shown that stele is an important anatomical predictor for root penetrability. Here, we studied genetic controls of root stele by quantitative trait loci analysis (QTL). One hundred and thirty-five chromosome segment substitutive lines derived from a cross between, KDML 105 and IR68586-F2-CA-31 (DH103) and IR68586-F2-CA-143 (DH212) were used as mapping population. Plants were grown in the field under well-watered and drought conditions. Under drought, rice decreased leaf weight by 16.63% but increased stele area by 20.21%. Stele traits were significantly correlated with root thickness in well-watered (r= 0.57) and drought conditions (r= 0.70). We identified 4 SNP markers on chromosomes 1, 4, and 9 which explained 9.47% to 11.36% of individual phenotypic variance. A marker on chromosomes 1 were found to be co-localized with previously described QTL for basal root thickness in double haploid rice mapping populations. These markers could be further developed to assist in rice breeding program.

***Keywords**:* Rice root, stele, drought

1. Introduction

It is predicted that by 2030 global food production has to be increased by at least 40% to sustain a dramatic increase of the world population (Pennisi, 2008). Rice is a major food crop, feeding more than half of the world population. Rice cultivation required a large amount of water compared with other cereal crops. Lowland rice takes approximately 3,000 liters of water per kg of grain production (Bouman *et al.*, 2007). Because of the climate change and unpredictable patterns of precipitation, drought widely spreads and becomes a major thread for rice production in many areas (Dai A, 2012). In Asia, at least 23 million hectares are potentially affected by drought (Asras *et al.*, 2001). Thailand is one the most important rice exporters of the world and much of the cultivating areas relies on rain, making it highly susceptible to drought (Haefele and Bouman, 2009).

Root is the main organ for water uptake. Several lines of evidence demonstrate that phenotypic variations for root traits associate with the adaptation of plants under drought. In rice, root thickness, rooting depth, root length density, and root pulling resistance have been shown to encourage drought avoidance by facilitating deep soil exploration for water under drought (Nguyen *et al*., 1997). Like other agronomical traits, drought tolerance is typically controlled by many genes (Fukai and Cooper, 1995). Genetic controls of root traits have been studied mainly through quantitative trait loci (QTL) analysis, and a wide range of the QTLs associated with small-medium effects on root biomass, root length and root number has been identified in rice. To date, QTLs linked to drought tolerance have been mapped in at least 15 different populations (Kamoshita *et al*., 2008). For example, DEEPER ROOTING 1 (DRO1 gene), a QTL for root growth angle on rice chromosome 7 (Uga *et al*., 2015).

While much research is focused on traits associated with deep rooting such as steepen root growth angle, traits enhancing soil penetration ability has to also be emphasized. Under drought, soil water content decreases and soil strength becomes increased and the soils begin to compact. The presence of strong or compacted soil layers, often limit the exploitation of deep water and nutrients. Thus, the ability of roots to penetrate hard soils is important for rice productivity and can help plants to survive under drought by drought avoidance strategy (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995).

Among root traits, stele has been shown to be an important predictor for root penetration ability (Chimungu *et al*., 2015). According to a multiple regression model of root penetration, stele diameter showed the highest coefficient (r= 1.1170) and also had the highest significant correlation (r=0.82) with root penetration in correlation analysis of 24 maize genotypes. Root penetration is an important trait for rupturing of the hardpan in the rainfed rice field. Mechanical rupturing can increase yield by approximately 0.5 t/ha in rainfed lowland rice in Bangladesh (Ahmed *et al*., 1996).

Chromosome segments substitution lines (CSSLs) is a novel mapping population which carry a particular chromosome segment from a donor line in the genetic background of the recurrent line. The KDML 105 CSSLs population was derived from a cross between a drought sensitive KDML105 and drought tolerant varieties; DH103 and DH212 (Kanjoo, 2011). The substituted chromosome segments cover a genetic distance of 49, 14.8, 53, 60 and 30 cM on chromosome 1, 3, 4, 8 and 9 respectively (Lanceras *et al*, 2004). These drought tolerant QTL segments had QTLs of grain yield and important agronomic traits related with water stress (Kamoshita *et al*., 2008). This population was evaluated for several agronomic traits including grain yield and yield components under irrigated and stress conditions at the reproductive stage to validate the effects of drought tolerant-QTL (Kanjoo *et al*., 2012).

In this study, we investigated a genotypic variation of stele traits of the KDML105 CSSLs population under well-watered and drought conditions. In addition, we identified QTLs controlling the variation of stele traits.

2. Materials and methods

2.1Plant materials

We used a total set of 135 Chromosome Segment Substitution Lines (CSSLs) obtained from the rice gene discovery unit.

Growth conditions

The 135 CSSLs and KDML105 were evaluated in a natural rain-fed field from September – November, 2016 at Ubon Ratchathani Rice Research Center (URRC) of Ubonratchatani province, Thailand (15°19’55.2”N, 104°41’27.9”E). Plants were grown in sandy soils. The experiment was arranged in a randomized complete block design with three replicates and 2 conditions namely well-watered and drought. Drought was imposed by draining out of the water at panicle initiation stage or at 4 weeks after transplanting. Soil moisture level was maintained at Field Capacity (FC) in well-watered treatment. The progress of drought stress was monitored by visual symptoms Weather data was obtained from a Meteorological Station next to the experimental fields. An average air temperature ranged from 24.8 to 29.7OC. The highest and lowest relative humidity recorded during experiment was 61 to 92%.

2.2 Phenotypic evaluation of stele traits

At harvest, two plants per plot were randomly selected for root anatomical analysis. We measured root anatomical traits including cross sectional area (mm2), stele area, percentage of stele area in a cross section from three nodal root per plant. The root segment was collected at 5 cm away from the base. Root segments were preserved in microtubes with 70% alcohol prior to being processed. Root sections were obtained by using a vibratome. The root sections were then examined under a light microscope. Three sections were selected as subsamples for image capture. The anatomical traits were quantified using GIMP v. 2.8 (GNU Image Manipulation Program; www.gimp.org) and Image J software (Abràmoff *et al*., 2004).

2.3 QTL and Statistical analysis

The QTLs of stele traits were identified by using R software with QTL package (Broman and Wu, 2010). The genotype data of 135 CSSLs and KDML105 consists of 6140 SNP markers. Single marker analysis was applied performed to data of the whole population. LOD of 3 with 1000 permutations was used as a threshold. Statistical analyses, one- and two-way ANOVA was used to evaluate phenotypic differences within the drought and well-watered conditions and among the population. Correlation analysis was performed to establish the relationship among root traits in each condition.

3. Results

Drought reduced an average leaf dry weight and plant height by 16.63% and 6.51%, respectively. Stele area and percentage of stele area were significantly increased by 20.71 % and 14.5% under drought. Significant genotypic variation was observed for stele traits among CSSLs under drought and well-watered conditions ($p< 0.05$). The stele area ranged from 0.015 to 0.047 mm2 in well-watered condition and from 0.014 to 0.063 mm2 in drought condition. Percentage of stele area ranged from 1.60 to 4.53% mm2 and 1.90 to 6.18 % for CSSLs in irrigated and drought conditions, respectively. The range of stele broad sense heritability is 0.32-0.58.

Correlation analyses were performed for stele area, percentage of stele area, leaf dry weight and plant height under well-watered and drought conditions (Fig.2). Only percentage of stele area was negatively correlated with leaf dry weight (r= -0.14, r= -0.12 at $p<0.05$) in well-watered and drought conditions while stele area had no correlation with leaf dry

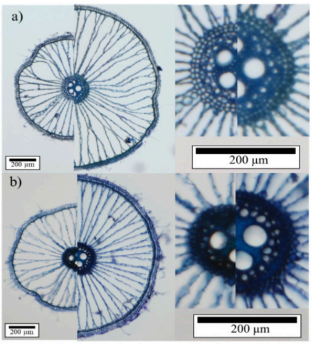

Figure 1. Cross-section images showing genotypic differences in root stele area in rice: (a) CSSL7 and CSSL117 under well-watered condition and (b) CSSL103 and CSSL137 drought. The plants were harvested at 79 days after sowing from field-grown plants at URRC. Scale bars depict 200 µm. and gray presents well-watered condition. Y-axis: Number of individuals.

weight or plant height in both conditions. The CSSLs responded to drought by increasing the stele area and percentage of stele area by approximately 20.21% and 0.52% (Fig. 3).

Using QTL analysis, we identified three significant SNPs markers (Table 3). In well-water condition, 1 SNPs markers for stele area located on chromosome 4 at 1.233 Mbp with total phenotypic variance explained by 11.36%. In case of drought, 2 SNPs markers were found on chromosome 1 and 9 with total phenotypic variance explained by 20.51%. For all SNPs contribute by DH except for M4_1233306 on chromosome 4 under well-watered condition.

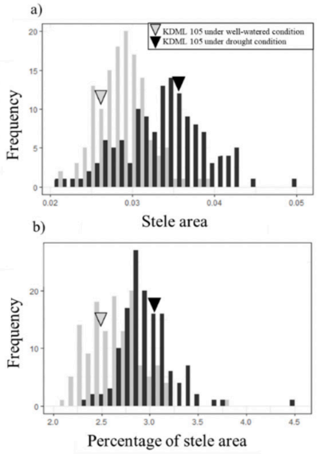

Figure 2. The distribution of (a) stele area and (b) percentage of stele area in the CSSL population under drought and well-watered conditions. Black presents drought condition.

Table 1. Summary of stele trait mean value for 135 CSSLs and KDML105 that were quantified in imaging analysis of well-watered and drought conditions.

Trait	Mean ± SD	Range X	KDML ± SD	Genotype effect	Broad-sense (H^2)
Well-watered condition					
Stele area (mm^2)	0.0286 ± 0.0046	3.13	0.0287 ± 0.0046	***	0.52
Percentage of stele area	2.62 ± 0.47	0.22	2.52 ± 0.42	**	0.32
Plant height (cm)	102.2±7.14	1.5	95.47±3.41	**	0.92
Leaf dry weight (g)	23.52±3.56	2.59	19.93±2.12	**	0.42
Drought condition					
Stele area (mm^2)	0.034 ± 0.0076	4.50	0.036 ± 0.069	***	0.58
Percentage of stele area	3.00 ± 0.46	3.26	3.22 ± 0.62	***	0.44
Plant height (cm)	95.58±8.20	1.44	88.23±4.25	**	0.85
Leaf dry weight (g)	19.61±3.26	1.99	17.15±1.68	ns	0.32

Drought effect on stele traits and shoot traits.

Table 2. Phenotypic correlation coefficients among stele area (SA), percentage of stele area (%SA), plant height (PH), root thickness (RT) and leaf dry weight (LDW) of a) well-watered and b) drought conditions. ***, **, * indicated significant level at p<0.001, p<0.01, p<0.05 as Spearman's principle correlation.

a)

	RT	SA	%SA	LDW
SA	0.53***			
%SA	-0.63***	0.29***		
LDW	0.1	-0.019	-0.14*	
PH	0.06	0.084	0.07	0.32***

b)

	RT	SA	%SA	LDW
SA	0.77***			
%SA	-0.41***	0.23***		
LDW	0.09	0.06	-0.12*	
PH	0	-0.0083	-0.03	0.42***

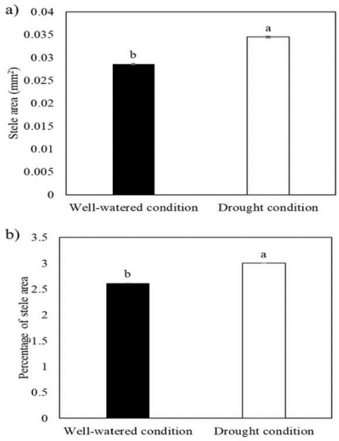

Figure 3. Stele area of CSSLs under well-watered and drought conditions. Black: well water and white: drought) ($p < 0.05$).

Table 3. QTL analysis output of stele area of well-watered condition by single marker analysis via R in a chromosomal substitution population of 135 lines.

Condition	Peak marker	Chr.	Position (Mbp)	LOD	PVE	Total PVE	Allele
Well water	M4_1233306	4	1.233	3.534	11.36	11.36	KDML 105
Drought	1_41229421	1	41.229	3.46	11.13	20.51	DHL
	M9_10172938	9	10.173	3.1	10.03		DHL

Discussion

Previous studies have been reported that stele traits are important for root penetration into hard soil layers under drought (Chimungu *et al.*, 2015). This adaptive strategy associates with drought avoidance in plant under water stress by increasing water uptake from soil. In this study, we found large genotypic variations for stele traits in the KDML 105 CSSL population ($p<0.01$). Drought significantly increased stele area and the percentage of stele area. Compared to the study by Uga *et al.* (2008) in which the researchers studied 117 lines of F3 population dereived from a cross between IR64 and Kinangang Patong, an average value of the stele area of the CSSL population fell into the same range (Uga *et al.*, 2008) with slightly smaller steal area.

The size of stele has been shown to be positively correlated with root thickness and xylem, which influence water uptake and water use efficiency (Uga *et al.*, 2008). Among monocot species, stele size can be largely different being 0.035-0.04 mm2 in barley (Luxova, 1989) to 0.08-1.17 mm2 in maize (Burton *et al.*, 2013). Compared to maize, rice in our study had approximately 2.75-fold smaller stele area. This information could imply that rice had lower water conductance according to the Poiseuille's law, which describes a positive relationship between the rate of fluid flux and the size of a transporting apparatus (Frensch and Steudle, 1989).

Although increasing the size of stele and root thickness under drought could enhance soil penetration, it could be detrimental to above-ground development. This is evidenced by the negative relationship found between the percentage of steal area and leaf dry weight. To increase mechanical strength for soil penetration, plants allocate more photosynthates to develop thicker roots at the expense of shoot growth (Kato *et al.*, 2006). However, the formation of roots in deep soil strata required additional oxygen supply and prevention of oxygen loss because oxygen availability is decreased with increasing soil depth.In this case, small stele tends to prevent oxygen deficiency by reducing radial oxygen loss (Gibbs *et al*., 1998). Thus, the beneficial stele size in rice should not be too small to allow for deep soil penetration but not too large to preserve oxygen in the root tissues.

Kadam *et al*. (2015) reported that stele diameter was not affected by water deficit stress but stele diameter in proportion to root diameter (SD: RD) was strongly affected by water deficit stress. In this study, we found that rice responded to drought by increasing 20.21% of stele area and 0.52% of the percentage of stele area. The increasing stele size may help maintain water in vascular tissue (Henry *et al*., 2012b). The increase in the percentage of stele area under drought also indicate that rice reduced radial distance by decreasing cortical width, which resulted in improved radial hydraulic conductance (Kadam *et al*., 2015)

We identified 3 QTLs associated with stele traits. The QTLs are located on chromosome 1, 4, and 9. Among CSSLs, the largest stele area in drought is CSSL135 and the smallest is 43. CSSL135 and CSSL43 contained chromosomal segment in chromosome 9 and 3, respectively. Uga *et al*. (2008) reported two significant QTLs for stele transversal area, qSTA-2 (STELE TRANSVERSAL AREA) and qSTA-9 on chromosome 2 and 9 (Uga *et al*., 2008). Our QTLs did not colocalize with these reported QTLs. However, SNP marker, 1_4122942 located at 41,229,421 bp on chromosome 1 was foud to be co-localized with a QTL for penetrated root thickness identified from a DHL population (IR64 x Azucena) (Zheng *et al*., 2000).

Our findings suggest that a large phenotyoic variation for stele traits exist among the CSSL population. Rice increased stele size under drought possibly to facilitate soil penetration. The identified QTL was found to be colocalized with a previously reported QTL for root thickness. The validation of the QTLs and identification of candidate genes controlling in the variation of stele traits will be necessary further steps toward molecular plant breeding targeting stele traits.

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