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RESEARCH ARTICLE

Exploring the effects of qDTY2.1 genomic regions on yield related traits in rice (*Oryza sativa* L.) under well watered conditions

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Abstract

The quantitative trait locus qDTY2.1 regulates yield under drought stress in rice. This study investigates the role of qDTY2.1 on yield-related traits in rice under well-watered conditions. For comparison, a large qDTY2.1 region (Chr02: 10.7–17.68 Mbp) and its fine-mapped segment (Chr02:10.7–11.4 Mbp) were compared to understand the linkage drag effects. Out of 432 F2 population derived from a cross between Swarna Sub1 and CR Dhan 801 (qDTY2.1 and qDTY3.1), 132 lines possessing only the qDTY2.1 were evaluated for the yield-related traits in well-watered conditions. This analysis showed similar effects for the large (Chr02: 10.7–17.68 Mbp) and fine-mapped (Chr02:10.7–11.4 Mbp) qDTY2.1 region. Besides, the effect of the six haplotypes (two parental and four recombinants) significantly differed ($p < 0.05$) for productive tillers, unfilled grains, and 100-seed weight. Based on the expression in the panicle stage, two candidate genes, namely, a meristem regulator gene (*Os02g0293300; OsFDML1*; Chr02:11.15 Mb) annotated as Arabidopsis homolog of factor of DNA methylation 1 and calcineurin B-like (*Os02g0291000; OsCBL7*; Chr02:11.01 Mb), were identified, which may regulate the yield-related traits in well-watered conditions. Thus, the present findings indicated the statistical effect of large and fine-mapped qDTY2.1 and their haplotypes in affecting the yield-related traits in well-watered conditions.

Keywords: Quantitative trait locus, qDTY2.1, rice, haplotype, yield, well-watered conditions

Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop in the world, providing more than 50% of the world population with essential nutrients (Reddy et al. 2023). Yet, currently, climate change is likely to cause a 51% decline in rice cultivation and production in the twenty-first century (Hussain et al. 2020). Drought is a frequent phenomenon during the crop growing season in South and Southeast Asia and is most encountered in rainfed areas. Drought stress may damage different developmental phases of rice growth, but the most vulnerable is the reproductive stage, leading to a substantial reduction in grain yield. The International Rice Research Institute (IRRI) estimates that more than 23 Mha of rainfed rice, most of which are located in South and Southeast Asia, are affected by drought, causing average yield losses ranging from 15 to over 60%, depending on the severity and timing of the drought (IRRI 2013; Serraj et al. 2011). Due to greater uncertainty in climate and the frequency and severity of drought are the most significant yield-limiting factors (Boyer and Westgate 2004); hence, the development of drought-tolerant rice cultivars is a priority in breeding programs (Vikram et al. 2015).

Conventional breeding for drought tolerance has faced challenges due to the complex quantitative inheritance of the trait, low heritability, and strong environmental interactions (Kumar et al. 2014). Identification of QTLs for

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drought tolerant has made it possible to develop varieties that have both high yield potential and resistant to stress. Of these, some QTLs with large effect, including: q*DTY1.1*, q*DTY2.1*, q*DTY3.1*, and q*DTY12.1,* have been repeatedly confirmed across diverse genetic backgrounds and field conditions (Swamy et al. 2011; Bernier et al. 2009). In particular, q*DTY2*. One has been observed to contribute towards higher grain yield under reproductive-stage drought stress and used extensively in marker-assisted backcross breeding (Venuprasad et al. 2009; Yadav et al. 2013). The success of q*DTY2.1* is well-proven by breeding programs in the rainfed lowlands, particularly in South Asia. For instance, Swarna-Sub1 + q*DTY2.1* introgression lines have shown 30–60% yield advantage over Swarna under reproductive-stage drought stress (Anantha et al. 2016). The positive/negative interactions of alleles within QTLs and with the genetic background (Dixit et al. 2012), as well as by the pleiotropic effect of genes and linkage drag (Xu and Crouch 2008; Vikram et al. 2015; Vikram et al. 2016; Venuprasad et al. 2009; Vikram et al. 2011; Venuprasad et al. 2012), played an important role in determining the overall effect of introgressed loci. The observed linkage drag of the *qDTY* QTLs has been successfully minimized and individual QTLs have been introgressed into improved genetic backgrounds (Vikram et al. 2015).

While identification and introgression of q*DTY2.1* into elite varieties have been successful, there is limited understanding of the allelic variation within this QTL region. Haplotype analysis is now an important method in plant breeding to explore natural genetic variation, understand allelic structure of quantitative traits, and assist in the identification of superior allele combinations with the increasing availability of high-throughput genotyping tools and whole-genome sequencing data (Tian et al. 2009; Zhao et al. 2011). Haplotype analysis, which considers combinations of alleles across multiple loci, is an effective method for analysing this variation and determining favourable allele combinations linked to trait performance (Badoni et al. 2023). It makes the grouping of individuals according to their multi-locus genotypic profiles, enabling the discovery of superior haplotypes for use in breeding programs through haplo-pheno analysis (Muduli et al. 2025). Recent studies using haplotype-based approaches in rice have found superior haplotypes for drought-responsive genes across diverse germplasm panels, highlighting the potential of this method to improve QTL-based selection (Badoni et al. 2023; Anantha et al. 2016). By combining genotypic and phenotypic data, haplotype analysis enhances our ability to detect genotype-phenotype associations, particularly in areas with recombination and structural variation.

The goal of this study is to conduct a haplotype analysis in the q*DTY2.1* region (10.7-20.8 Mbp) using SSR markers for a population derived from the cross between Swarna-Sub1 and Swarna-Sub1+q*DTY2.1*+q*DTY3.1*. The objectives of the present study were to determine the haplotypes within the q*DTY2.1* region; to assess their correlation with phenotypic expression under control conditions, and to discover the most favorable haplotypes for selection using the MAS approach with the intention of developing high-yielding cultivars in control conditions.

Materials and methods

Plant materials

A total of 432 F_2 individuals were derived from a cross between Swarna Sub1 (recurrent parent) and Swarna-Sub1 + q*DTY2.1* + q*DTY3.1* (CR Dhan 802-donor parent). These seeds of F_2s were grown in the research plot of ICAR-CRRI during July-December 2023 in control conditions following the standard agronomic practices. The young leaf samples of F_2s were collected from 430 lines and stored at -20°C for further analysis. Besides, the yield-related data were collected from the matured F_2s individual lines and seeds were harvested for single plant yield data measurements.

DNA extractions and genotyping

Genomic DNA was extracted following a modified CTAB protocol (Doyle 1991). The concentration and purity of DNA were evaluated using a Nano Drop Spectrophotometer and 0.8% agarose gel electrophoresis. For foreground selection, five SSR markers (RM5791, RM521, RM324, RM6374, and VG) linked to the q*DTY2.1* (10.7-20.8 Mbp) region and three SSR markers (RM5791, RM521, RM324) linked to the q*DTY2.1* (10.7-11.4 Mbp) on chromosome 2 were utilized (Table 1). The PCR amplification was performed in 10 µL reaction volumes, and the amplified products were resolved using 3% agarose gel electrophoresis. SSR marker alleles were scored based on banding patterns as homozygous for Swarna-Sub1 (B), homozygous for the donor parent P1 (A), or heterozygous (H). Out of 430 lines, 220 lines that were recombinants within the q*DTY2.1* were identified. Since CR Dhan 802 also carries the q*DTY3.1* QTL, two additional SSR markers (RM520 and RM16030), associated with the *DTY3.1* region, were employed to eliminate lines containing q*DTY3.1*. Thus, a total of 139 lines were selected for yield-related traits. Besides, POWER analysis was performed to identify the minimum sample size required for the trait difference analysis (Supplementary Table S1).

Measurement of agro-morphological traits

A total of 430 genotypes were evaluated under an augmented randomized complete block design (RCBD) comprising four blocks during the *kharif* 2023. The experiment was conducted at the experimental field of ICAR-CRRI, Cuttack. The two parents, CR Dhan 802 and Swarna Sub1, were included as tolerant and susceptible checks, respectively. Checks were replicated five times in each block and each block accommodated 86 genotypes.

Table 1. SSR markers used in the experiment

Marker name	Chromosome	Physical Location	Primer Forward	Primer Reverse
RM 5791	2	10.7 Mb	ACGACGTCACAAAGGGTTCTTGC	GAATACGCTTTCGCCTGCTACG
RM521	2	10.8 Mb	ATGACCCAATTTCTGACTCTAGCC	CATGGGTGGTGCTGTAGATGG
RM 324	2	11.38 Mb	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
RM 6374	2	15.28 Mb	TCACCAGACTCAACAAAGGATCG	TTCACCTTTCTTCTCCCTCATTCC
VG	2	17.68 Mb	GCTAGCCGGTTTAGTTATCAAAAC	TTCAGACGCCTTGAACCTGA
RM520	3	30.7 Mb	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG
RM16030	3	32.5 Mb	GCGAACTATGAGCATGCCAACC	GGATTACCTGGTGTGTGCAGTGTCC

Standard agronomic practices were followed to ensure healthy crop growth and harvested in December 2023. Various agro-morphological traits, including panicle length in cm (PL), single plant yield in g (SPY), number of filled grains (FG), number of unfilled grains (UG), hundred-seed weight (HSW), and number of productive tillers (PT), were recorded from each individual line of F_2s. To assess panicle-related traits, the total number of panicles per plant was first counted and recorded as the number of productive tillers. All panicles were then harvested and stored in brown paper covers for subsequent yield-related trait analysis. The collected panicles were oven-dried at 50 °C for three days. Following drying, the three uppermost panicles from each plant were selected for measuring panicle length. Additionally, the number of filled and unfilled grains per panicle was counted manually. The weight of 100 well-filled grains was measured in grams using a precision balance. For calculating single plant grain yield (g), the total weight of all harvested panicles was recorded using an analytical balance (Mettler Toledo JE303G) (Nayak et al. 2024).

Haplo-pheno analysis

Haplo-pheno analysis within the q*DTY2.1* region was conducted to identify the effect of the haplotypes for yield-related traits under control conditions. Haplotypes occurring in fewer than 10 lines were excluded from the analysis, following the criteria outlined by Singh et al. (2024). Genotypes were grouped based on their haplotype profiles, and the corresponding phenotypic data were analysed to assess trait performance within each group. To determine superior haplotypes, the mean values of each trait were calculated for every haplotype group. Comparative analysis of trait means was performed to identify statistically significant differences among haplotypes. Box plots were generated using SR plot to visualize phenotypic variation across haplotypes (Tang et al. 2023). Statistical significance of trait differences between haplotype groups was assessed using the Wilcox rank-sum test (Lehmann 2006). Two candidate genes have been identified using rap-db within the fine-mapped QTL region (Chr02: 10.7-11.4 Mbp) (https://rapdb.dna.affrc.go.jp/). The expression of the putative candidate genes was further analysed Rice RNA Seq database.

Statistical analysis

The mean values of three plants used for recording the observations were used for analysis. The data analysis feature of R-Studio was used to perform Analysis of variance (ANOVA), initial descriptive statistics and genetic parameters (variances, genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance) were analysed using "augmented RCBD" package (Federer 1961) whereas correlation between different traits was analysed using the "corrplot" package to understand the relationship between the traits. The PCA is prepared using the 'Statistics Kingdom' website (https://www.statskingdom.com/). The statistical significance among the means of haplotype groups was determined by a non-parametric test (Kruskal-Wallis test) using R-studio (Nayak et al. 2025). A power analysis using ClinCalc to evaluate the adequacy of haplotype group size(Supplementary Table S1).

Results

Phenotypic, genotypic variation, and descriptive statistics of recombinant genotypes

Under well-watered conditions, the selected 139 F_2 lines possessing qDTY2.1 and not qDTY3.1 showed substantial phenotypic variation for the key agro-morphological traits. The panicle length (PL) ranged between 17.47 cm and 33.92 cm. The single plant yield (SPY) ranged from 10.07 g to 30.23

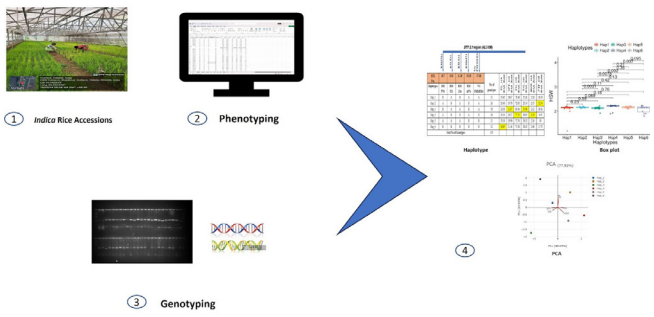


Fig 1. Graphical representation: 1) A diverse Indica rice of genotypes was cultivated, 2) Phenotyping involved the collection of agro-morphological traits data, 3) Genotype of collected leaf sample and 4) Haplotype analysis association of significance traits in box plot and principal component analysis (PCA)

Table 2. Descriptive statistics of agro-morphological traits

Trait	Mean	SE	SD	Min	Max	Skewness	Kurtosis
PL	29.21	0.17	1.97	17.47	33.92	-3.32	8.97
SPY	20.61	0.37	4.22	10.07	30.23	0.02	2.24
FG	72.04	0.82	9.48	41.50	96.50	-0.38	3.55
UG	26.02	0.98	11.29	10.17	88.67	1.73	8.78
HSW	2.14	0.01	0.11	1.19	2.28	-5.28	2.23
PT	10.92	0.23	2.64	4.00	16.00	-0.14	2.16

PL = Panicle length (cm), SPY = Single plant yield (g), FG = Filled grains (nos), UG = Unfilled grains (nos), HSW = Hundred seed weight (g), PT = Productive tillers (nos), SE = Standard error, SD = Standard deviation. Min = Minimum, Max = Maximum

Table 3. Genetic parameters of agro-morphological traits

Trait	Mean	PV	GV	EV	GCV	PCV	ECV	h2 (bs)	GA	GAM
PL	29.21	3.90	3.69	0.21	6.58	6.76	1.58	94.58	3.85	13.20
SPY	20.61	17.93	17.50	0.44	20.30	20.55	3.21	97.56	8.52	41.35
FG	72.04	90.07	88.57	1.50	13.06	13.17	1.70	98.33	19.25	26.72
UG	26.02	128.09	124.93	3.17	42.95	43.49	6.84	97.53	22.77	87.50
HSW	2.14	0.01	0.01	0.00	4.92	4.93	0.33	99.55	0.22	10.12
PT	10.92	6.95	6.28	0.67	22.94	24.13	7.47	90.41	4.92	45.00

PL = Panicle length (cm), SPY = Single plant yield (g), FG = Filled grains (nos), UG = Unfilled grains (nos), HSW = Hundred seed weight (g), PT = Productive tillers (nos), PV = Phenotypic variance, GV = Genotypic variance, EV = Environmental variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation, h^2 (bs) = Broad sense heritability, GA = Genetic advance, GAM = Genetic advance as per mean

Table 4. Comparison of average agronomic trait with parent

Trait	RP (Swarna Sub1)	DP(CR Dhan 802)	Genotypes	
			Mean	Range
PL	30.3	28.9	29.3	28.58-30.06
SPY	19.82	21.61	20.62	19.71-21.67
FG	79	74	72.25	69.47-77.35
UG	44	22	25.76	20.65-35.97
HSW	1.9	2.14	2.14	2.09-2.20
PT	14	13	10.96	10.00-12.35

PL = Panicle length (cm), SPY = Single plant yield (g), FG = Filled grains (nos), UG = Unfilled grains (nos), HSW = Hundred seed weight (g), PT = Productive tillers (nos), RP = Recurrent parent, DP = Donor parent

g, with a mean of 20.61 g. The productive tillers ranged from 4 to 16, with a mean of 10.92 tillers. There was considerable variation in the filled grain (FG), ranging from 41.50 to 96.50 grains per plant, with a mean of 72.04. Similarly, unfilled grains (UG) also varied from 10.17 to 88.67 per panicle. The 100-seed weight (HSW) ranged from 1.19 to 2.28 g, with a population mean of 2.14 g (Table 2). In the present study, the phenotypic coefficient of variation (PCV) ranged from 4.93 - 43.49%. Genotypic coefficient of variation (GCV) between 4.92 to 42.95% (Fig. 2A). Further, broad-sense heritability estimates were high (>60%) for all the traits evaluated. In comparison, the genetic advance as a percentage of the mean was also high (>20%) for nearly all traits, except for PL (13.20) and HSW (10.12) (Fig. 2B, 2C).

Correlation among the agro-morphological traits

Correlation analysis of the agronomic traits showed a highly significant positive correlation between the number of filled grains and productive tillers (r = 0.41***). Similarly, panicle length also exhibited a significant positive correlation with both productive tillers (r = 0.24**) and filled grains (r = 0.18*). Additionally, a significant positive correlation was observed between unfilled grains and filled grains (r = 0.25**). The single plant yield demonstrated a significant positive correlation with filled grains and unfilled grains (r = 0.19*), suggesting a relation between yield-related traits. However, the unfilled grains showed a significant negative correlation with panicle length (r = -0.17*) and hundred seed weight (r = -0.30***) (Fig. 3).

Trait performance of F_2 lines in comparison to parental lines

The panicle length in the F_2 population was intermediate between Swarna Sub1 (30.3 cm) and CR Dhan 802 (28.9

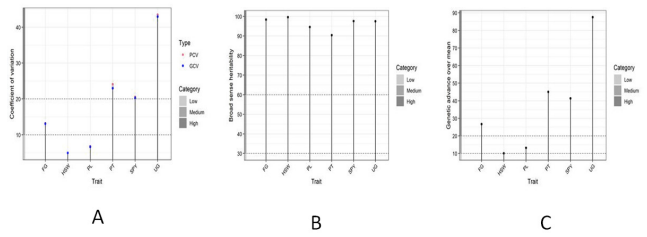


Fig. 2. A) Distribution of traits showing GCV and PCV, B) Distribution of traits showing Broad sense heritability, and C) Distribution of traits showing Genetic advance

cm), with a narrow range of 28.58–30.06 cm (Table 4). The single plant yield (SPY) was higher in the F_2 population (20.62 g) than in Swarna Sub1 (19.82 g), but slightly lower than CR Dhan 802 (21.61 g). Filled Grains (FG) per panicle in F_2 population averaged 72.25, lower than both Swarna Sub1 (79) and CR Dhan 802 (74), with a wider range (69.47–77.35). Unfilled Grains (UG) were significantly reduced in F_2 population compared to Swarna Sub1 (44), aligning closer to the CR Dhan 802 (22). Hundred Seed Weight (HSW) was higher in F_2 population (2.14 g), matching the CR Dhan 802 and surpassing the Swarna Sub1 (1.9 g), with minimal variation (2.09–2.20 g). Productive Tillers (PT) were lower in F_2 population (10.96) than both Swarna Sub1-14, CR Dhan 802-13), with a moderate range (10–12.35).

Haplotype identification using SSR markers

Five polymorphic markers (RM5791, RM521, RM324, RM6374, and VG) linked to the q*DTY2.1* QTL region (Chr02: 10.7-20.8 Mbp) were used for the haplotype analysis in the mapping population to understand the difference in the haplotypes of q*DTY2.1* for the yield-related traits. Among the 8 haplotypes identified, two haplotypes were considered minor haplotypes due to their frequency of less than 5 per cent (One includes 5 genotypes containing parent alleles and another one includes two genotypes containing missing alleles) and only the remaining six haplotypes were taken for further analysis. The haplotype 1 (Hap_1) has 23 genotypes, haplotype 2 (Hap_2) comprises 26 genotypes, haplotype 3 (Hap_3) comprises 30 genotypes, haplotype 4 (Hap_4) comprises 20 genotypes, haplotype 5 (Hap_5) comprises 21 genotypes, and haplotype 6 (Hap_6) comprises 12 genotypes (Fig. 4A).

Similarly, three polymorphic markers (RM5791, RM521, RM324) were used by shortening the QTL region (Chr02: 10.7-11.4 Mbp), and there is a slight difference in no of genotypes in the three haplotype groups, i.e., Hap_1 (23), Hap_3 (32), Hap_6 (15) (Fig. 4B).

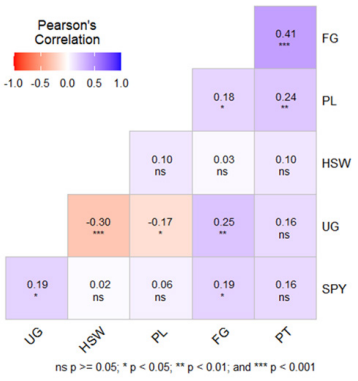
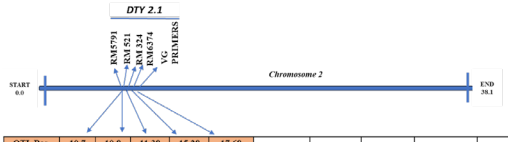


Fig. 3. Correlation between different agro-morphological traits

Haplotype–trait associations

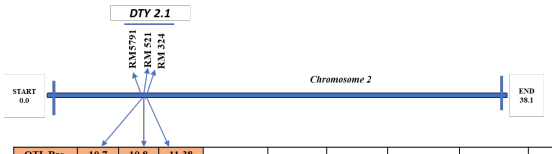
Analysis of phenotypic data across haplotype groups, including 6 primers (Chr02: 10.7-20.8 Mbp), showed statistically significant differences for unfilled grains (p=0.00**), hundred seed weight (p=0.02*), and productive tillers (p=0.01*) were significantly different. Except for these three traits, the mean value of other traits was statistically nonsignificant. Further, Hap_4 had the lowest unfilled grains (20.65 grains), while Hap_3 had the highest (35.95 grains) per panicle (Fig. 4A). Besides, Hap_2 and Hap_6 recorded higher numbers of productive tillers (12.34 and 11.75, respectively), whereas Hap_5 (10) and Hap_3 (10.36) had lower tiller numbers. The haplotype, Hap_4, had the highest HSW (2.19 g) and Hap_6 had the lowest HSW (2.08g). Similarly, Hap_6 and Hap_1 showed relatively longer panicle lengths (>29.5 cm). But Hap_3 had the shortest average panicle length (28.58 cm). For the single plant yield, Hap_3 exhibited the highest average SPY of 21.67 g, followed closely by Hap_6 (21.48 g). However, Hap_2 showed the lowest SPY (19.70 g). Further, Hap_4 showed the highest average filled grains (77.35 grains), followed by Hap_5 (72.76). These results collectively indicate that certain haplotypes, particularly Hap_3andHap_4 are associated with superior agronomic performance under controlled conditions.

Analysis of phenotypic data across haplotype groups,



QTL Pos.	10.7	10.8	11.38	15.28	17.68							
Haplotypes	RM 5791	RM 521	RM 324	RM 6374	VG PRIMER	No. of genotypes	PL_ Rabi_2024	SPY_ Rabi_2024	FG_ Rabi_2024	UG_ Rabi_2024	HSW_ Rabi_2024	PT Rabi_2024
Hap_1	B	A	B	A	A	23	29.43	20.67	70.30	21.26	2.10	10.39
Hap_2	A	B	A	B	A	26	29.40	19.70	72.03	23.34	2.15	12.34
Hap_3	B	A	A	B	A	30	28.58	21.67	69.46	35.96	2.12	10.36
Hap_4	A	B	B	A	B	20	29.16	20.27	77.35	20.65	2.19	10.9
Hap_5	A	A	A	B	B	21	29.18	19.90	72.76	24.52	2.16	10
Hap_6	B	B	B	B	B	12	30.05	21.48	71.58	28.83	2.08	11.75
Total No of Genotypes						132	p = 0.32	p = 0.51	p = 0.09	p = 0.00**	p = 0.02	p = 0.01

Fig. 4A. Selected haplotypes with the representation of DTY2.1 QTL region (Chr02: 10.7-20.8 Mbp). Hap_1 = Haplotype 1, Hap_2 = Haplotype 2, Hap_3 = Haplotype 3, Hap_4 = Haplotype 4, Hap_5 = Haplotype 5, Hap_6 = Haplotype 6, A = CR Dhan 802 allele, B = Swarna Sub1 allele. Statistical significance test was performed using non-parametric Kruskal–Wallis, Wilcoxon test with 5% level of significance.



QTL Pos.		10.7	10.8	11.38							
Haplotypes		RM 5791	RM 521	RM 324	No. of genotypes	PL_ Rabi_2024	SPY_ Rabi_2024	FG_ Rabi_2024	UG_ Rabi_2024	HSW_ Rabi_2024	PT_ Rabi_2024
Hap_1		B	A	B	25	29.38	20.59	70.16	20.88	2.11	10.36
Hap_2		A	B	A	26	29.40	19.71	72.04	23.35	2.16	12.35
Hap_3		B	A	A	32	28.60	21.57	69.53	34.88	2.13	10.41
Hap_4		A	B	B	20	29.16	20.28	77.35	20.65	2.20	10.90
Hap_5		A	A	A	21	29.19	19.91	72.76	24.52	2.17	10.00
Hap_6		B	B	B	15	29.82	21.41	71.87	27.20	2.09	11.87
Total No of Genotypes					139	p = 0.39	p = 0.51	p = 0.06	p = 00**	p = 0.02	p = 0.008

Fig. 4B. Selected haplotypes with the representation of DTY2.1 QTL region(Chr02: 10.7-11.4 Mbp). Hap_1 = Haplotype 1, Hap_2 = Haplotype 2, Hap_3 = Haplotype 3, Hap_4 = Haplotype 4, Hap_5 = Haplotype 5, Hap_6 = Haplotype 6, A = CR Dhan 802 allele, B = Swarna Sub1 allele. Statistical significance test was performed using non-parametric Kruskal–Wallis, Wilcoxon test with 5% level of significance.

including 3 primers (Chr02: 10.7-11.4 Mbp), also showed statistically significant differences for unfilled grains (p=0.00**), hundred seed weight (p = 0.02*), and productive tillers (p=0.008**) were significantly different (Fig. 4B). There is a minor difference in the mean value. These results also indicate that Hap_3andHap_4 are associated with better agronomic performance.

Significant differences between the haplotypes

By using 6 primers in the QTL region (Chr02: 10.7-20.8 Mbp) among the six traits studied, three traits showed statistically significant differences between the haplotypes, i.e., unfilled grains, hundred seed weight, and productive tillers. Further, Hap_1 was found to be statistically different from Hap_2 (0.038), Hap_3 (4.9e-05), Hap_5 (0.045) and Hap_6 (0.029) for unfilled grains (Fig. 6). Similarly, Hap_2 was significantly different from Hap_3 (0.00036) for unfilled grains. Hap_3 was significantly different from Hap_4 (3.2e-05) and Hap_5 (0.0015) and Hap_4 with Hap_6 (0.014) for unfilled grains also. For 100 seed weight, Hap_1 shows a significant difference with Hap_3 (0.02) and Hap_4 (1.1e-05), Hap_2 with Hap_3 (0.0002) and Hap_4 (9.2e-06), Hap_3 with Hap_4 (2.9e-05) and Hap_5 (0.0036), Hap_4 with Hap_5 (0.0001) and Hap_6 (0.0064). Similarly, the significant difference in productive tillers (PT) was observed between Hap_1 with Hap_2 (0.013), Hap_2 with Hap_3 (0.0038) and Hap_5 (0.0083) and Hap_3 with Hap_6 (0.041)(Fig. 5A). These results indicate Hap_2 and Hap_3 are significantly associated with the yield related traits i.e. hundred seed weight and productive tillers with better agronomic performance.

Similarly, by shortening the QTL region (Chr02: 10.7-11.4 Mbp), three traits (UG, HSW, PT) also showed statistically significant differences between the haplotypes. For unfilled grains, Hap_1 was significantly different from Hap_2 (0.018), Hap_3 (3.6e-05), Hap_5 (0.03), and Hap_6 (0.021). Similarly, there was a significantly different in Hap_2 with Hap_3 (0.0014), Hap_3 with Hap_4 (9.6e-05) and Hap_5 (0.0049), Hap_5 with Hap_6 (0.026). For hundred seed weight, there was a significant different in Hap_1 with Hap_4 (7.1e-06), Hap_2 with Hap_3 (0.00012) and Hap_4 (9.2e-06), Hap_3 with Hap_5 (0.0026), Hap_4 with Hap_5 (0.0001) and Hap_6 (0.0017). Further, for productive tillers Hap_1 show significant difference with Hap_2 (0.0089) and Hap_6 (0.042), Hap_2 with Hap_3 (0.0035) and Hap_5 (0.0083), Hap_3 with Hap_6 (0.017), Hap_5 with Hap_6 (0.046)(Fig 5B). These results indicate Hap_2 and Hap_3 are also significantly associated with the yield-related traits, i.e. hundred seed weight and productive tillers with better agronomic performance.

Principal component analysis of selected haplotypes

In the larger QTL region (Chr02: 10.7-20.8 Mbp), the PCA (Principal Component Analysis) explained 77.93% of the total phenotypic variance across two principal components (PC1 = 48.09%, PC2 = 29.84%), highlighting major phenotypic contributions from traits like SPY, FG, and HSW. The Hap_3 is located on the extreme negative side of both PC1 and PC2, strongly associated with high SPY and UG (Fig. 6A). Further, Hap_5 lies on the negative PC2 axis with positive PC1, suggesting good performance for FG and HSW, but slightly lower for SPY. The Hap_2 clusters on the positive PC1/PC2 quadrant, linked more to PL and PT, but less with yield traits. The two haplotypes, Hap_4 and Hap_6, on the far positive side of PC1 or PC2, seem to contribute less to SPY and HSW. The PCA clearly distinguished the trait-based grouping of haplotypes, with Hap_3and Hap_5 aligning with yield-related vectors (SPY, FG, HSW).

In the shorter QTL region (Chr02: 10.7–11.4 Mbp), there was a slight difference in PCA, 77.26% of the total phenotypic variance across two principal components (PC1 = 30.49%, PC2 = 46.76%), than the larger QTL region. Similarly, the result showed Hap_3and Hap_5 also aligned with the yield-related traits(Fig. 6B).

Candidate gene identification

Two candidate genes, namely calcineurin b-like 7 (*OsCBL7*:*Os02g0291000* - chr02:11015384..11018203) and factor of DNA methylation like 1 (*OsFDML1*:*Os02g0293300* - chr02:11151750..11156506), were identified in the QTL region (Chr02:10.7-11.4 Mbp) responsible for positive modulation of drought stress and regulation of floral organ specification and meristem determination, respectively. *OsCBL7* was found to be highly expressed in reproductive tissues (flower bud and flower), and *OsFDML1* exhibits the highest expression in the panicle before flowering and the panicle after flowering. From the expression analysis, it was found that the *OsCBL7* was specific to drought stress responsiveness, with data points of 483. Similarly, OsFDML1 expression was specific to reproductive tissues, comprising 121 data points (Supplementary Fig. 1).

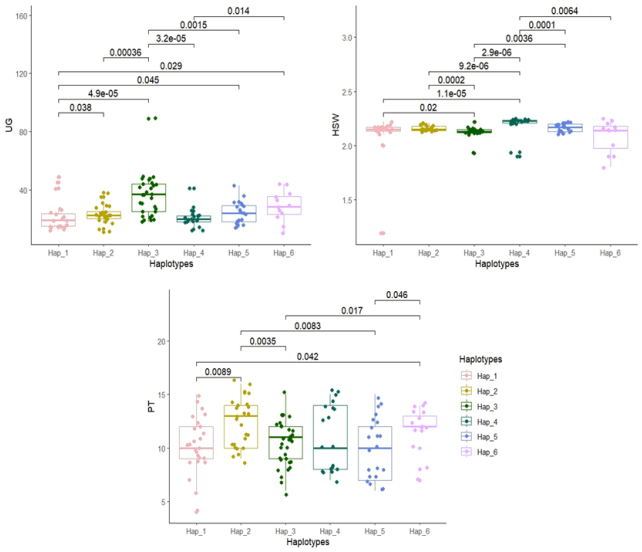


Fig. 5A. Box plot showing the distribution six haplotypes (Chr02: 10.7-20.8 Mbp) for the three significant traits i.e., HSW, PT, and UG. Statistical significance test was performed using the non-parametric Kruskal–Wallis, Wilcoxon test with 5% level of significance.

Discussion

This current study, conducted under well-watered conditions, provided valuable insights into the genomic regions of qDTY2.1 affecting the yield-related traits in rice. Initially, qDTY2.1 (Chr02: 10.7–20.8 Mbp; Venuprasad et al., 2009) was mapped in a larger genomic region and further narrowed through fine mapping (Chr02: 10.7-11.4 Mbp; Singh et al., 2016). Additionally, most of the introgressed lines of different qDTYs released as varieties showed large QTL region introgression (0.8–5.6 Mbp) (Kumar et al, 2020). Therefore, this study was conducted to understand the effects of the qDTY2.1 genomic regions in well-watered

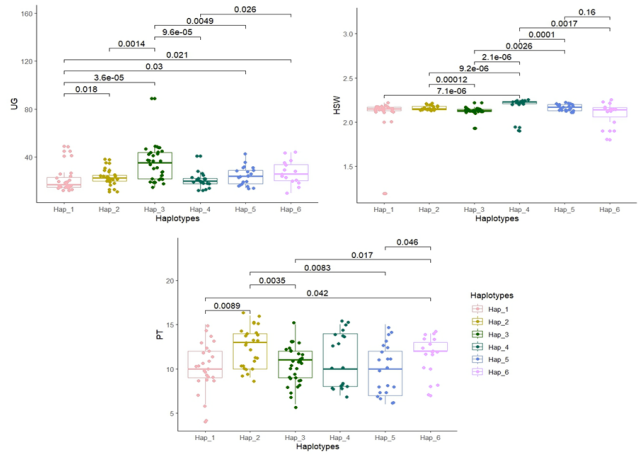


Fig. 5B. Box plot showing the distribution six haplotypes (Chr02: 10.7-11.4 Mbp)for the three significant traits, i.e., HSW, PT and UG. Statistical significance test was performed using non-parametric Kruskal–Wallis, Wilcoxon test with 5% level of significance.

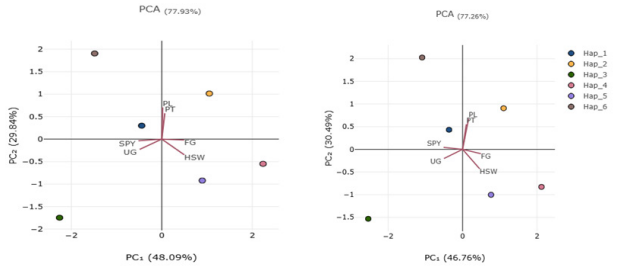


Fig. 6. Principal component analysis (PCA) of 6 haplotypes based on 6 phenotypic traits. A) QTL region (Chr02: 10.7-20.8 Mbp). B) QTL region(Chr02: 10.7-11.4 Mbp)

conditions for yield-related traits. Specifically, large qDTY2.1 (10.7–17.68 Mbp) and fine-mapped region (10.7–11.38 Mbp) were compared to differentiate the linkage drag effects of qDTY2.1 with larger genomic fragments. However, this analysis showed the effect of the larger genomic regions and only the fine-mapped regions of qDTY2.1 were almost the same on yield-related traits under well-watered conditions. For example, both analyses showed significant differences among the haplotypes for productive tillers, unfilled grains, and hundred seed weight in well-watered conditions. Further, the recombinant haplotypes showed 3.8 to 19.02% increase in the no. of productive tillers relative to the parental haplotypes. This indicates that rather than the large genomic regions, fine-mapped genomic regions of qDTY2.1 have a significant effect on the yield-related traits in well-watered conditions. Accordingly, candidate gene analysis also identified a meristem regulator gene (Os02g0293300; *OsFDML1*; Chr02:11.15 Mb) annotated as the Arabidopsis homolog of factor of DNA methylation 1 in rice (Zheng et al. 2023). Besides, a candidate gene for drought tolerance, namely, calcineurin B-like (Os02g0291000; *OsCBL7*; Chr02:11.01 Mb) within the qDTY2.1 was also identified. Thus, the close proximity of these candidate genes might be regulating the yield traits under well-watered and drought stress conditions. The above-mentioned putative candidate genes require further functional validation through gene expression studies, knock-out, or transgenic approaches in future research.

The traits, namely single plant yield, unfilled grains, and productive tillers, showed high genetic advance as a percentage of the mean (>40%) in the lines possessing only the qDTY2.1. The GCV value is close to the PCV value, implying a low environmental coefficient of variance. In support of the present findings, Roy and Shill (2000) reported genetic parameters of traits in rice under well-watered conditions, where PCV and GCV showed close values, implying a low environmental coefficient of variation (< 5%). This suggests that well-irrigated conditions can substantially minimize the environmental homogeneity. The high heritability and high genetic advance observed for single plant yield,

hundred seed weight and filled grains per panicle and better haplotypes of the qDTY2.1 under well-watered conditions could be utilized in breeding programs for trait improvement of rainfed shallow lowland varieties(Roy and Shil 2020; Konate et al. 2016). In support of these findings, qDTY2.1 introgression in Pusa44 reported higher yield than the recurrent parent even in well-watered conditions (Oo et al. 2021). In contrast, qDTY2.1 introgression in TN11 showed yield inferiority in well-watered conditions (Bordeos et al. 2025). Generally, backcross breeding approaches aim to maintain high background recovery with introgression of the specific regions as foreground selection (Kumar et al. 2018; Bordeos et al. 2025). However, the observed low genetic advance of panicle length and hundred seed weight, which exhibited high heritability, points towards non-additive gene action or a greater influence of environmental factors, making these traits less susceptible to direct selection (Nayak et al, 2025). Therefore, phenotypic selection for yield and productive tillers in the qDTY2.1 introgressed line would be a relevant approach not only for enhancing the drought tolerance but also for improving the genetic gain for yield of the popular variety even in well-watered conditions. These findings are further corroborated by the highly positive correlation between filled grains and productive tillers (r = 0.41***), indicating the tiller number association with higher grain-filling capacity and yield improvement (Kumar et al. 2012). Additionally, these findings also align with the increased single plant yield (SPY) of qDTYs introgressed lines in different backgrounds in well-watered conditions (Kumar et al. 2018). qDTY2.1 haplotype difference analysis showed H2 and H3 performed relatively better than other haplotypes. This supports previous findings that q*DTY2.1* confers a 30–60% yield advantage even in non-stressed field trials (Swamy et al. 2011; Bernier et al. 2009; Anantha et al. 2016). Overall, the results highlight the potential of haplotype-based selection in improving complex traits, where interactions between alleles within and across QTL regions play a critical role (Dwivedi et al. 2021). The power analysis indicated showed a minimum of 11 genotypes per haplotype is sufficient to detect the significant difference between the groups.

In conclusion, the fine-mapped region of the qDTY2.1 (Chr02: 10.7–11.4 Mbp) affects the yield-related traits in well-watered conditions. Further, high genetic advance for productive tillers and single plant yield indicates that the selection among the qDTY2.1 introgressed lines would be an effective strategy for enhancing the genetic gain for yield in well-watered conditions. The superior haplotype of qDTY2.1 identified in well-watered conditions for yield-related traits requires evaluation under reproductive stage drought stress conditions and multi-environmental trials remain to be validated to confirm the stability of the superior haplotype. Further, two candidate genes identified (*OsCBL7*; calcineurin B-like and *OsFDML1*: Factor of DNA methylation 1) need further validation.

Supplementary materials

Supplementary Table S1 and Supplementary Fig. 1 are provided and can be accessed at www.isgpb.org.

Author's contribution

Conceptualization of research (PC, JLK, APD); Designing of the experiments (PC, JLK, SP); Contribution of experimental materials (JLK, RV); Execution of field experiments and data collection (SP, MP, MKL, Su S, Sar S, RS); Analysis of data and interpretation (SP, PC, GN); Preparation of the manuscript (SP, PC, APD, SS).

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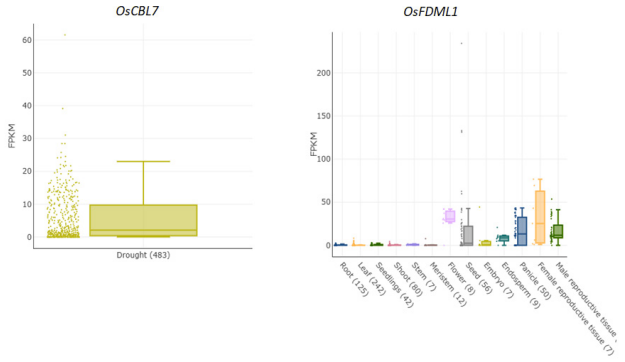
Supplementary Table S1. Power analysis data for sample size detection

Trait Name	Haplotype Data Range	No. of genotypes (Group A)	No. of genotypes (Group B)	Sample Size using Power Analysis (Group A)	Sample Size using Power Analysis (Group A)
PL	28.55 – 30.06	30	12	11	11
SPY	19.71-21.67	26	30	17	17
FG	69.47-77.35	30	20	19	19
UG	20.65-35.97	20	30	3	3
HSW	2.09-2.20	12	20	10	10
PT	10.00-12.35	21	26	21	21



Supplementary Fig. 1. Expression analysis of A. OsCBL7:Os02g0291000B. OsFDML1:Os02g0293300