RESEARCH ARTICLE

Analysis of Genetic Diversity and Survey of QTLs for Grain Yield under Drought Stress in Drought Tolerant Rice Landraces Using DTY QTL-linked Markers

Alpana Anupam^{1,2}, Sanjay Kumar Sinha², Priyamedha¹, Amrita Banerjee¹, Somnath Roy¹ and Nimai P. Mandal¹*

¹Central Rainfed Upland Rice Research Station, ICAR-National Rice Research Institute, Hazaribag-825301, Jharkhand, India ²Sido Kanhu Murmu University, Dumka-814101, Jharkhand, India

(Received: 18 March, 2022; Revised: 03 June, 2022; Accepted: 04 June, 2022)

Genetic diversity in a set of 60 rice genotypes were analyzed using 27 random and 13 grain yield under drought (DTY) quantitative trait loci (QTLs)-linked simple sequence repeats (SSR) markers. The average gene diversity polymorphism information content were 0.53 and 0.46, respectively. The presence of DTY QTLs for grain yield under drought stress was predicted using peak marker in comparison to the positive checks. The DTY QTL $qDTY_{12.1}$, amplified by the peak marker RM 28048, was found in 43.3% of genotypes, and $qDTY_{2.2}$ was detected in only 6.67 % of genotypes using peak marker RM279. Two germplasm accessions, IC389895 and DT 2, possessed maximum number of DTY QTLs and absent is IC525099. Phylogenetic analysis revealed four distinct clusters within the germplasm. Overall, the evaluated genotypes presented a rich source of diversity and contain valuable DTY QTLs which can be utilized in rice genetic enhancement program for drought tolerance.

Key Words: Drought tolerance, DTY QTL, Genetic diversity, Rice, SSR marker

Introduction

Rice is the staple food for more than half of the global population. Worldwide, around 40% of rice cultivated area comes under rainfed environments (Singh et al., 2017). Rainfed rice production systems can be classified into lowland and upland. In Asia rainfed lowland rice area is about 46 million hectares which is almost 30 % of the total world rice area (Maclean et al., 2002). Large rice areas in Bangladesh, Cambodia, Myanmar, Nepal, and Thailand, India, Indonesia, Laos, and Vietnam fall under rainfed ecology (CGIAR, 1998). Rice cultivation in rainfed areas face severe challenges of drought and flooding. Drought will possibly become more frequent due to adverse effect of climate change (World Bank, 2009). Therefore, drought-prone rice systems in rainfed areas require stress tolerant rice varieties along with improved crop management strategies. Recent efforts to identify major QTLs with a large and consistent effect on grain yield under drought condition have marked a valid strategy (Bernier et al., 2007; Kumar et al., 2007; Venuprasad et al., 2009; Vikram et al., 2011). Coordinated drought breeding programmes have exhibited a significant positive trend genetic gain for grain yield over the years under both drought stress as

well as favorable irrigated control conditions (Kumar *et al.*, 2021).

The rice landraces traditionally cultivated by the farmers, contain high level of genetic diversity, and can serve as potential genetic resources for improvement of yield under biotic and abiotic stresses (Choudhury et al., 2013). As the major element of germplasm, genetic diversity is a natural source for rice breeding to encounter existing food requirements (Reig et al., 2016). Identifying rice cultivars with drought tolerance and high level of genetic diversity will aid in rice improvement programme for rainfed drought-prone ecologies. The present study was conducted with a set of 57 rice landraces, selected earlier from a larger set of germplasm based on tolerance to drought, an objective to analyze genetic diversity and predict the presence of DTY QTLs using SSR markers. The knowledge thus generated will help in utilization of these cultivars for rainfed rice improvement.

Materials and Methods

Plant materials and DNA isolation

A set of 60 rice genotypes (Table 1) that include 57drought tolerant rice cultivars and three check varieties

^{*}Author for Correspondence: Email: NP.Mandal@icar.gov.in

viz. Sadabahar, Vandana and Sahbhagi Dhan were used in the study (Table 1). The germplasm set was selected based on tolerance to vegetative stage drought tolerance from 258 rice cultivars mostly from the eastern Indian region (data not shown). During genotyping drought tolerant checks such as N22, IR64Drt1, Vandana, Apo and Way Rarem were used. Total genomic DNA was collected from five-day old seedlings were isolated using a modified cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1987). The concentration of genomic DNA was checked in Nanodrop system 2000c (Thermo, USA), and the actual concentration was adjusted to 50 ng μ L⁻¹.

SSR markers used

A total of 40 SSR markers, 27 random and 13 DTY QTL-linked were used in the study (Supplementary Table 1). The random markers were sampled from GCP panel of 50 SSRs (http://gramene.org/markers/microsat/50_ssr.html). The primer sequences and annealing temperatures are available at Gramene Database (http:// www.gramene.org).

Polymerase chain reaction and scoring of amplicons

Polymerase chain reaction (PCR) was carried out in a total volume of 10 μ L reaction having 50 ng of template DNA, 0.5 μ M of each forward and reverse primers, 0.2 mM of dNTPs, 1X PCR buffer with 20 mM MgCl₂, and

Table 1. List	of rice	genotypes	used in	1 the	study
---------------	---------	-----------	---------	-------	-------

1 U of *Taq* DNA polymerase (Thermo Fisher Scientific, USA). PCR was done in a thermocycler (VeritiTM, Applied Biosystems) using following conditions: 5 min at 94°C; 35 cycles of 45 s at 94°C 45 s at annealing temperature and 45 s at 72°C; followed by 5 min at 72°C. PCR amplicons were visualized in 3-4% agarose gels stained with SYBR Safe DNA gel stains (Invitrogen) using a UV-transilluminator. During scoring, the band with the lowest molecular weight was assigned allele number 1 and the progressively heavier bands were scored incrementally. DNA molecular weight standards were used in agarose gels to determine amplicon sizes.

Screening of DTY QTLs

Out of 14 DTY QTLs linked SSR markers, nine peak markers were used to screen for the presence of drought tolerant yield QTLs (Supplementary Table 1). The size of amplified fragment of each genotype under study was compared with the amplicon size of the respective DTY QTL positive check to score for the presence of DTY QTLs.

SSR Data analysis

The summary statistics of the SSR markers matrices such as number of alleles, major allele frequency, observed heterozygosity, expected heterozygosity gene diversity and polymorphism information content were estimated. The phylogenetic tree was constructed using Roger's

	IC/ collector No.		IC/ collector No.		IC/ collector No.
1	RSR2/JLM-9	21	IC-389895	41	IC568239
2	RSR2/JLM-12	22	IC-419206	42	IC568303
3	RSR2/JLM-34	23	IC-454009 sathi	43	IC568288
4	RSR2/JLM-40	24	IC-454256	44	IC568236
5	RSR/SKY-22	25	IC-454498X BALA	45	IC568294
6	KP-2036	26	IC-454599 KRISHNA	46	IC568223
7	SKSS-05	27	IC-454372X	47	IC568237
8	SKSS-06	28	IC-454628 PTB 28	48	IC568262
9	SKSS-09	29	IC-454634 PTB 30	49	RO-46
10	SKSS-12	30	IC-459347 AC-45	50	JCR-1875
11	SKSS-15	31	IC515116	51	SKY-67
12	PTP/DC-40	32	IC515117	52	SKY-68
13	PTP/DC-43	33	IC525099	53	DT 2
14	PTP/DC-61	34	IC538351	54	DT 10
15	DPS/OPD-179	35	IC538653	55	DT 13
16	DPS/OPD-187	36	IC75844	56	DT 14
17	NR-25 JADAN	37	IC548644	57	DT 35
18	NR-26 CHHATRI DHAN	38	IC568278	58	Vandana
19	NR-31 LAL DHAN	39	IC568250	59	Sahbhagi Dhan
20	IC-264006	40	IC568228	60	Sadabahar

Indian J. Plant Genet. Resour. 35(2): 250–256 (2022)

1972 genetic distance and UPGMA method using Power Marker V3.25 (Liu and Muse, 2005). The phylogenetic tree was constructed using Tree View Software.

Results and Discussion

Screening for DTY QTLs

The molecular weight of each of the peak SSR markers associated with each DTY QTLs: $qDTY_{1,l}$, $qDTY_{1,2}$, $qDTY_{2,2}$, $qDTY_{2,3}$, $qDTY_{3,l}$, $qDTY_{3,2}$, $qDTY_{4,l}$, $qDTY_{6,l}$ and $qDTY_{12,1}$) was compared with the amplicon size of the respective DTY QTL-positive check (Supplementary Fig. 1). The positive checks are mentioned in Table 2. The genotypes under study were found to possess more than one DTY QTLs. Two germplasm, IC389895 and DT 2, possessed maximum number of DTY QTLs, while 11 genotypes such as Sadabahar ($qDTY_{3,1}$), NR-31LALDHAN ($qDTY_{3,2}$), IC568288 ($qDTY_{3,2}$), IC538653 ($qDTY_{3,2}$), DT10 ($qDTY_{6,1}$), DT13 ($qDTY_{2,3}$), RSR2/



Fig. 1. UPGMA clustering of 60 rice genotypes using 40 SSR markers

Indian J. Plant Genet. Resour: 35(2): 250–256 (2022)

JLM-9 $(qDTY_{12.1})$, RSR2/JLM-40 $(qDTY_{12.1})$, RSR/ SKY-22 $(qDTY_{12.1})$ and RSR2/JLM-12 $(qDTY_{12.1})$ were found to possess only one DTY QTL (Supplementary Fig. 2). IC-454599 and DPS/OPD-187 showed specific amplicon for $qDTY_{1.1}$ using both the linked markers RM431 and RM 11943. Only four genotypes: SKSS-12, SKSS-15, IC568250 and DT-2 showed $qDTY_{2.2}$ specific amplicon using RM 279. Specific amplicon for $qDTY_{12.1}$ was noted in 43.3% of the total genotypes using RM28048. A total of 21 genotypes were found to possess $qDTY_{6.1}$ based on the banding pattern of linked marker RM589. DTY QTLs such as $qDTY_{2.3} qDTY_{3.1}$, $qDTY_{3.2}$ and $qDTY_{4.1}$ were predicted to be present in 26.66%, 21.67%, 28.33% and 18.33% of the total genotypes, respectively. The DTY QTLs such as $qDTY_{2.2}$ and $qDTY_{3.1}$ had a consistent effect across seasons under lowland drought stress conditions (Dixit *et al.*, 2012; 2014). Under severe drought stress, a grain yield advantage of 0.8-1.0 t ha⁻¹ was reported the variety IR64 through the introgression of two QTLs ($qDTY_{2.2}$ and $qDTY_{4.1}$) (Swamy *et al.*, 2013). The qDTY12.1 was also reported to show a consistent effect across environments for high grain yield under drought stress at reproductive-stage in the background of popular high-yielding but drought-susceptible variety (Mishra *et al.*, 2013). Therefore, screening of the genotypes for the possible presence of DTY QTLs will further generate scope for validation and utilization of the promising rice germplasm in drought breeding.

DTY QTLs (peak marker)	Positive check	Promising genotypes	Number (%)
qDTY _{1.1} (RM431)	N22	RSR2/JLM-34, DPS/OPD-187, IC-264006, IC-454009, IC-454256, IC- 454498X BALA, IC-454599 KRISHNA, IC-454372X, IC568239, IC568303, IC568288, RO-46, DT 35	13 (21.67%)
qDTY _{1.1} (RM11943)	N22	SKSS-12, PTP/DC-43, DPS/OPD-187, NR-25 JADAN, NR-26 CHHATRI DHAN, IC-389895, IC-454599, IC-459347 AC-45, IC515116, IC538351, IC75844, IC568278, IC568236, IC568294, JCR-1875, SKY-68, DT 2	17 (28.33%)
qDTY _{1.2} (RM3825)	N22	SKSS-05, SKSS-06, SKSS-15, PTP/DC-43, DPS/OPD-187, IC-454256, IC-454498X BALA, IC-454599, IC-454628 PTB 28, IC-454634 PTB 30, IC- 459347 AC-45, RO-46, SKY-67, DT 2	14 (23.33%)
qDTY _{2.2} (RM279)	IR64 Drt-1	SKSS-12, SKSS-15, IC568250, DT 2	4 (6.67%)
qDTY _{2.3} (RM 3212)	Vandana	RSR2/JLM-9, SKSS-12, PTP/DC-40, DPS/OPD-179, IC-389895, IC-419206, IC515117, IC568239, IC568303, IC568236, IC568294, IC568223, SKY-68, DT 2, DT 13, DT 14, Vandana	16 (26.66%)
qDTY _{3.1} (RM520)	Аро	KP-2036, SKSS-09, SKSS-12, PTP/DC-61, DPS/OPD-179, IC-389895, IC515117, IC568239, IC568294, IC568223, IC568237, DT 14, Sadabahar	13 (21.67%)
qDTY _{3.2} (RM22)	Vandana	KP-2036, SKSS-06, SKSS-09, PTP/DC-40, PTP/DC-61, DPS/OPD-179, NR- 25 JADAN, NR-31 LAL DHAN, IC-264006, IC-389895, IC-419206, IC-459347 AC-45, IC538351, IC538653, IC548644, IC568228, IC568239, IC568288, SKY-67, SKY-68, DT 2, DT 35, Vandana	23 (38.33%)
qDTY _{4.1} (RM518)	IR64 Drt-1	PTP/DC-61, IC-389895, IC-454256, IC-454628 PTB 28, IC568278, IC568250, IC568303, IC568237, SKY-67, DT 2, DT 35	11 (18.33%)
qDTY6.1 (RM589)	Vandana	SKSS-05, SKSS-09, PTP/DC-40, DPS/OPD-179, DPS/OPD-187, NR- 26CHHATRI DHAN, IC-389895, IC-454498XBALA, IC-45KRISHNA, IC- 454372X, IC-454634 PTB 30, IC515116, IC515117, IC75844, IC568288, IC568223, IC568262, SKY-68, DT 10, DT 35, Vandana	21 (35%)
<i>qDTY</i> _{12.1} (RM28048)	Way Rarem	RSR2/JLM-9, RSR2/JLM-12, RSR2/JLM-34, RSR2/JLM-40, RSR/SKY-22, SKSS-05, SKSS-09, PTP/DC-40, PTP/DC-43, PTP/DC-61, IC-389895, IC-419206, IC-454009, IC-454498X BALA, IC568239, IC568288, IC568236, IC568294, IC568223, IC568237, IC568262, RO-46, JCR-1875, SKY-67, SKY-68, DT 2, Shabhagi Dhan	26 (43.33%)

SSR polymorphisms

The summary statistics of the SSR markers is presented in Supplementary Table 2. A total of 133 alleles were detected in 60 rice genotypes using 40 SSR markers. The number of alleles per locus ranged from 2 (RM495, RM507, RM514, RM338, RM455, and RM44) to 5 (RM518, RM6368, and RM118) with an average of 3.325 alleles/locus. The average numbers of alleles per locus observed in this study correspond to other previous studies (Cho et al., 2000; Anand et al., 2012; Nachimuthu et al., 2015). The level of polymorphism among the present genotypes was assessed by calculating PIC values of the 40 SSR loci. The PIC value ranged from 0.138 (RM 161) to 0.677 (RM 16030), with an average 0.457. These values are also consistent with PIC value (0.475) obtained in Anupam et al. (2017) while studying genetic diversity of rice germplasm from the state of Tripura, India. Altogether, 17 SSR markers including 8 tightly linked DTY QTL markers (RM 279, RM 22, RM 520, RM 3212, RM 518, RM 11943, RM 3825 and RM16030) recorded PIC values >0.50. A PIC value higher than 0.5 indicates higher polymorphism and are extremely useful in distinguishing the polymorphism rate of markers at specific locus (Botstein et al., 1980; Dewoody et al., 1995). Present study indicated that the DTY QTLs linked SSR markers were more informative than that of random SSR marker. Yadav et al. (2013) also indicated that average genetic diversity at genomic loci assessed by QTL-linked markers is more than that revealed by random markers and reported that study of diversity based on drought QTLs revealed the existence of greater variability at the functional regions of the genome. Gene diversity of expected heterozygosity value was highest (0.726) for DTY QTL linked SSR marker (RM16030) which generated the maximum 5 bands while, the lowest value (0.142) was noted with random SSR marker (RM161). The average gene diversity in the present germplasm was 0.525. The gene diversity value is a fundamental measure of genetic variation in a population and describes the proportion of heterozygous genotypes expected under Hardy-Weinberg equilibrium (Nei, 1973). Gene diversity obtained in the present study is comparable to those reported in previous studies such as 0.52 in Nachimuthu et al. (2015) and 0.54 in Choudhary et al. (2013). Major allele frequency is maximum with marker RM161 (0.925), minimum with 0.350(RM 16030, RM 3825). The most common allele at each locus ranged from 92% (RM161) to 35% (RM16030 and

RM 3825). The average major allele frequency in the present study was higher as compared to the previous studies on Indian rice varieties (Upadhyay *et al.*, 2012) and Korean landraces (Li *et al.*, 2014).

Genetic Structure Analysis

The assessment of genetic diversity of germplasms is one of the potential approaches which lead to identification of diverse parents for designing effective breeding strategy for hybridization (Sajib et al., 2012; Nachimuthu et al., 2015). SSR markers have remarkable potential to discriminate between rice genotypes compared to other molecular markers (Xiao et al., 1996). The genetic distance based UPGMA clustering divided the genotypes into four major clusters (Fig. 1). The Cluster I consisted of five genotypes with all the nine DTY QTLs except $qDTY_{3,l}$. Eight genotypes including 3 check varieties were grouped in Cluster II. The Cluster III was comprised of 24 genotypes. In this cluster, RSR/SKY-22 and RSR2/ JLM-40 had the lowest genetic distance of 0.05 followed by RSR2/JLM12 - RSR2/JLM40 (0.09). All the nine DTY OTLs were detected within the members of Cluster III. Altogether 23 genotypes grouped in Cluster IV, and eight DTY QTLs, excluding qDTY₂, were found within this cluster. In Cluster IV, the highest genetic distance was recorded between PTP/DC-61 and Vandana (0.80) followed by PTP/DC 40 and DT 2 (0.71). Importance of clustering of these genotypes using both DTY QTL linked markers and random SSR markers can be easily visualized as the released varieties could be improved for drought tolerance using distantly related germplasm without narrowing the genetic base, for instance, rice variety Sadabhar was found to distantly related to IC568250 (genetic distance = 0.65). IC568250 was found to have specific banding for multiple QTLs $(qDTY_{2})$ and $qDTY_{4,1}$) and thus can be crossed with Sadabahar to generate superior drought tolerant progenies without narrowing the genetic base.

Conclusion

In the present study, screening of sixty drought tolerant rice genotypes for the possible presence of drought QTLs and the level of genetic diversity provided the scope of utilizing of these genotypes in rice varietal improvement programme for drought stress. The study of genetic diversity among the genotypes enhanced the possibility of using a more diverse donor for drought stress, minimizing the effect of genetic erosion through widening of genetic base. Use of DTY QTL-linked markers in both screening of the genotypes as well as studying of genetic diversity among these genotypes gave valuable information about the amplification profile of these markers that can be useful for monitoring introgression of DTY QTLs in drought susceptible varieties.

*Supplementary Table or Figure mentioned in the article are available in the online version.

References

- Anand D, KV Prabhu, AK Singh (2012) Analysis of molecular diversity and fingerprinting of commercially grown Indian rice hybrids. J. Plant Biochem. Biotechnol. 21(2):173-79.
- Anupam A, J Imam, SM Quatadah, A Siddaiah, SP Das, M Variar, NP Mandal (2017) Genetic diversity analysis of rice germplasm in Tripura State of Northeast India using drought and blast linked markers. *Rice Sci.* 24: 10-20.
- Bernier J, A Kumar, V Ramaiah, D Spaner, G Atlin (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci.* 47(2): 507-516.
- Botstein D, RLWhite, M Skolnick, RW Davis (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* **32:** 314-331.
- CGIAR (1998) Press Release 'CGIAR Urges Halt to Granting of Intellectual Property Rights for Designated Plant Germplasm', February 11.
- Cho YG, T Ishii, S Temnykh, X Chen, L Lipovich, WD Park, N Ayres, S Cartinhour, SR McCouch (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theor Appl. Genet.* **100**: 713-722.
- Choudhury B, LM Khan, S Dayanandan (2013). Genetic structure and diversity of indigenous rice varieties (*Oryza sativa* L.) in eastern Himalayan region of northeast India. *Springer Plus* 2: 228–237.
- DeWoody JA, RL Honeycutt, LC Skow (1995) Microsatellite markers in white-tailed deer. J. Hered. 86(4): 317–319.
- Dixit S, A Singh, MTS Cruz, PT Maturan, M Amante, A Kumar (2014) Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet.* 15: 16.
- Dixit S, BPM Swamy, P Vikram, HU Ahmed, MTS Cruz, M Amante, D Atri, H Leung, A Kumar (2012) Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. *Theor. Appl. Genet.* **125(1):** 155-169.
- Doyle JJ, JL Doyle (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.
- Kumar A, A Raman, S Yadav, SB Verulkar, NP Mandal, ON Singh, P Swain, T Ram, *et al.* (2021) Genetic gain for rice yield in rainfed environments in India. *Field Crops Res.* 260:107977.

- Kumar R, R Venuprasad, GN Atlin (2007) Genetic analysis of rainfed lowland rice drought tolerance under naturally occurring stress in eastern India: Heritability and QTL effects. *Field Crops Res.* **103(1):** 42-52.
- Li FP, YS Lee, SW Kwon, G Li, YJ Park (2014) Analysis of genetic diversity and trait correlations among Korean landrace rice (*Oryza sativa* L.). *Genet. Mol. Res.* 13(3): 6316-31.
- Liu K, SV Muse (2005) Power Marker: an integrated analysis environment for genetic marker data. *Bioinformatics* 21: 2128-2129.
- Maclean JL, D Dawe, B Hardy, GP Hettel (eds) (2002) *Rice Almanac.* IRRI, WARDA, CIAT, FAO, Los Baños (Philippines), Bouaké (Côted'Ivoire), Cali (Colombia) and Rome (Italy), 253 p.
- Mishra KK, P Vikram, RB Yadaw, BPM Swamy, S Dixit, MT Sta Cruz, P Maturan, S Marker, A Kumar (2013) *qDTY12.1*: A locus with a consistent effect on grain yield under drought in rice. *BMC Genet.* 14: 12.
- Nachimuthu VV, R Muthurajan, S Duraialaguraja, R Sivakami, BA Pandian, G Ponniah, K Gunasekaran, MKSK Swaminathan, R Sabariappan (2015) Analysis of population structure and genetic diversity in rice germplasm using SSR markers: An initiative towards association mapping of agronomic traits in *Oryza sativa* L. *Rice.* 8: 30.
- Nei M (1973) Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321-3323.
- Palanog AD, BPM Swamy, NAA Shamsudin, S Dixit, JE Hernandez, TH Boromeo, PCS Cruz, A Kumar (2014) Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. *Field Crops Res.* 161: 46-54.
- Reig-Valiente JL, J Viruel, E Sales, L Marqués, J Terol, M Gut, S Derdak, M Talon, C Domingo (2016) Genetic diversity and population structure of rice varieties cultivated in temperate regions. *Rice.* 9: 58.
- Sajib AM, MM Hossain, ATMJ Mosnaz, H Hossain, MM Islam, MS Ali and SH Prodhan (2012) SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). J. Biosci. Biotechnol. 1: 107-116.
- Sandhu N, A Singh, S Dixit, MTS Cruz, PC Maturan, RK Jain, A Kumar (2014) Identification and mapping of stable QTL with main and epistasis effect on rice grain yield under upland drought stress. *BMC Genet.* 15: 63.
- Singh K, CJ McClean, P Büker, SE Hartley, JK Hill (2017) Mapping regional risks from climate change for rainfed rice cultivation in India. *Agric. Syst.***156**: 76-84.
- Swamy BPM, HU Ahmed, A Henry, R Mauleon, S Dixit, P Vikram, R Tilatto, SB Verulkar, P Perraju, NP Mandal, M Variar, S Robin, R Chandrababu, ON Singh, JL Dwivedi, SP Das, KK Mishra, RB Yadaw, TL Aditya, B Karmakar, K Satoh, A Moumeni, S Kikuchi, H Leung, A Kumar (2013) Genetic, physiological, and gene expression analyses reveal that multiple QTL enhance yield of rice mega-variety IR64 under drought. *PLoS One* 8(5): e62795.

- Upadhyay P, CN Neeraja, C Kole, VK Singh (2012) Population structure and genetic diversity in popular rice varieties of India as evidenced from SSR analysis. *Biochem. Genet.* 50: 770-83.
- Venuprasad R, CO Dalid, M Del Valle, D Zhao, M Espiritu, MTS Cruz, M Amante, A Kumar, G Atlin (2009) Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor. Appl Genet.* 120(1): 177–190.
- Venuprasad R, ME Bool, L Quiatchon, MTS Cruz, M Amante, GN Atlin (2012) A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. *Mol. Breed.* 30(1):535-547.
- Vikram P, BPM Swamy, S Dixit, HU Ahmed, MT Sta Cruz, AK Singh, A Kumar (2011) *qDTY1.1*, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genet.* 12: 89.
- Xiao JH, SN Grandillo, SN Ahn, SR McCouch, SD Tanksley, JM Li, LP Yuan (1996) Genes from wild rice improve rice yield. *Nature*. 384: 223-224.
- Yadav S, A Singh, MR Singh, N Goel, KK Vinod, T Mohapatra, AK Singh (2013) Assessment of genetic diversity in Indian rice germplasm (*Oryza sativa* L.). Use of random versus trait-linked microsatellite markers. J. Genet. 92(3): 545-557.

Analysis of Genetic Diversity and Survey of QTLs for Grain Yield under Drought Stress in Drought Tolerant Rice Landraces

Marker	Chromosome	Random/ DTY QTL-linked	Reference/Source
RM 237	1	Random	See http://gramene.org/markers/microsat/50_ssr.html for details of random SSR markers
RM 283	1	Random	
RM 495	1	Random	
RM 154	2	Random	
RM 408	2	Random	
RM 452	2	Random	
OSR 13	3	Random	
RM 514	3	Random	
RM 338	3	Random	
RM124	4	Random	
RM 161	5	Random	
RM 507	5	Random	
RM413	5	Random	
RM 162	6	Random	
RM133	6	Random	
RM118	7	Random	
RM125	7	Random	
RM 455	7	Random	
RM 152	8	Random	
RM 447	8	Random	
RM284	8	Random	
RM 433	8	Random	
RM 44	8	Random	
RM 316	9	Random	
RM215	9	Random	
RM 484	10	Random	
RM 536	11	Random	
RM431	1	qDTY _{1.1}	Vikram <i>et al.</i> , 2011
RM 11943	1	qDTY _{1.1}	Vikram <i>et al.</i> , 2011
RM 3825	1	qDTY _{1.2}	Sandhu et al., 2014
RM 279	2	qDTY _{2.2}	Swamy et al., 2013, Palanog et al., 2014
RM1367	2	qDTY _{2.3}	Palanog et al., 2014, Sandhu et al., 2014
RM 3212	3	qDTY _{2.3}	Palanog et al., 2014, Sandhu et al., 2014
RM 520	3	qDTY _{3.1}	Dixit et al., 2014
RM16030	3	qDTY _{3.1}	Dixit et al., 2014
RM 22	3	qDTY _{3.2}	Vikram <i>et al.</i> , 2011
RM 518	4	qDTY _{4.1}	Swamy et al., 2013
RM16368	4	qDTY _{4.1}	Swamy et al., 2013
RM 589	6	qDTY _{6.1}	Venuprasad et al., 2012
RM28048	12	qDTY _{12.1}	Bernier et al., 2007
RM28130	12	qDTY _{12.1}	Bernier et al., 2007

Alpana Anupam et al.

Marker	Major allele frequency	Allele No	Gene Diversity	Observed heterozygosity	Polymorphism information content
RM 152	0.74	3.00	0.41	0.30	0.37
RM 154	0.53	3.00	0.61	0.02	0.54
RM 162	0.63	3.00	0.50	0.02	0.42
OSR13	0.54	4.00	0.55	0.12	0.45
RM 161	0.93	4.00	0.14	0.02	0.14
RM 237	0.81	3.00	0.33	0.03	0.30
RM 283	0.48	3.00	0.61	0.00	0.53
RM 316	0.48	3.00	0.59	0.03	0.51
RM 408	0.72	3.00	0.42	0.00	0.34
RM 447	0.38	4.00	0.66	0.10	0.59
RM 452	0.52	3.00	0.52	0.03	0.40
RM 495	0.58	2.00	0.49	0.00	0.37
RM 507	0.92	2.00	0.15	0.00	0.14
RM 514	0.85	2.00	0.26	0.00	0.22
RM 536	0.53	4.00	0.62	0.08	0.55
RM 3825	0.35	4.00	0.71	0.00	0.65
RM 11943	0.41	4.00	0.69	0.02	0.64
RM 279	0.57	4.00	0.61	0.00	0.56
RM 520	0.46	4.00	0.65	0.08	0.58
RM 518	0.47	5.00	0.67	0.03	0.62
RM 22	0.45	3.00	0.64	0.00	0.57
RM28130	0.73	3.00	0.42	0.00	0.37
RM28048	0.68	4.00	0.49	0.17	0.44
RM 3212	0.44	4.00	0.67	0.05	0.61
RM1367	0.50	4.00	0.55	0.00	0.44
RM16368	0.50	3.00	0.57	0.00	0.48
RM16030	0.35	5.00	0.73	0.05	0.68
RM431	0.65	3.00	0.51	0.00	0.44
RM118	0.48	5.00	0.68	0.00	0.63
RM124	0.72	4.00	0.45	0.00	0.41
RM125	0.63	3.00	0.50	0.00	0.41
RM133	0.58	3.00	0.56	0.00	0.49
RM284	0.52	3.00	0.59	0.00	0.50
RM 338	0.75	2.00	0.38	0.00	0.31
RM 433	0.45	3.00	0.64	0.00	0.57
RM 484	0.50	3.00	0.53	0.00	0.42
RM215	0.57	3.00	0.52	0.00	0.41
RM413	0.57	4.00	0.59	0.00	0.52
RM 455	0.75	2.00	0.38	0.00	0.31
RM 44	0.57	2.00	0.49	0.00	0.37
Mean	0.58	3.33	0.53	0.03	0.46

Supplementary Table 2. Genetic diversity information of 40 SSR loci in 60 rice gen	notypes
--	---------

Analysis of Genetic Diversity and Survey of QTLs for Grain Yield under Drought Stress in Drought Tolerant Rice Landraces



Supplementary Fig. 1. Representative agarose gel photographs using SSR markers linked to different DTY QTLs

Alpana Anupam et al.

S.No.	Genotype	qDTY1.1 (RM111943)	qDTY1.1 (RM431)	qDTY1.2 (RM3825)	qDTY2.2 (RM279)	qDTY2.3 (RM3212)	qDTY3.1 (RM520)	qDTY 3.2 (RM22)	qDTY 4.1 (RM518)	qDTY 12.1 (RM28048)	qDTY 6.1 (RM 589)
1	RSR2/JLM-9									N I	
2	RSR2/JLM-12									N I	
3	RSR2/JLM-34		N			×				N I	
4	RSR2/JLM-40									N	
5	RSR/SKY-22					1 1				N	
6	KP-2036						N	Ń			
7	SKSS-05			N						N	×
8	SKSS-06			N				X		_	
9	SKSS-09	_		-		N	N	N		N.	N.
10	SKSS-12	N I		_	N		V			_	V
11	SKSS-15		-	N	N		-	_	-		
12	PTP/DC-40	_		-		_	_	N	_	N	
13	PTP/DC-43	N	_	N		N	_	-	-	N	
14	PTP/DC-61					N	N	N.	N.	N	
15	DPS/OPD-1/9					-	N	N	-	-	N
10	NP 26 IADAN		× 1	N.		-	-	-	-	-	V
10	NR-25 JADAN	-	-	-			-	× 1	-	-	
10	NR-20 CHHATRI	×	-	-		-	-	1	-	-	
20	IC-264006	-	-	-	-	-	-		_	-	
20	10-204000	-		-		-	1		1	1	-
22	IC-410206		-	-				1	-		3
23	IC-454009 SATHI	-	1	-		1	-	_	-		
24	IC-454256	-	3	N		-			1	_	
25	IC-454498X BALA	-	ż	1 J		V				~	1
26	IC-454599	V	1	1 J		J.					1 J
27	IC-454372X		V	-		V	-				Ż
28	IC-454628 PTB 28	-		V		Ň	_		×		
29	IC-454634 PTB 30		-	V		Ň	_				×
30	IC-459347 AC-45	N		V		V		N			
31	IC515116	V				V					V
32	IC515117						N				V
33	IC525099										
34	IC538351	N						N			
35	IC538653							Ń			
36	IC75844	N I								_	
37	IC548644					V		N I			
38	IC568278	N				N			N		N
39	IC568250				N				N		
40	IC568228							N			
41	IC568239		N	_		-	N	X		N I	N
42	IC568303		N	_					N	_	
43	IC568288		N.					N		V	
44	IC568236	N	_	-		-			-	N	
45	IC568294	V	_	-			N			N	
46	IC568223	-	-	-			V		-	-Y	
47	10568237	-	-	-		-	N		N	L¥_	
48	10568262	-	-	-				_	-	- V	
49	KO-40	-	N N	V		V	_		-	-	
50	JUK-18/5	V	_				-		-	-	
51	SKT-0/	-	-	-			-	-	- ·		
52	DT 2	1	-		el.	-	-	N. N.	al	1	
53	DT 10	N.	-	N.	N N	Y	-	- X	×		
54	DT 10	-	-	-	-	-	-	-	-		
50	DT 14	-	-	-		1	al			-	
57	DT 35	-	-					1	al .		-
59	VANDANA	-	- ·	-		-		1		-	
59	SAHBHAGI							<u> </u>		1	J
60	SADABAHAR			-			1			-	
~	A REPORT OF A REPORT	-	-	-			_				

Supplementary Fig. 2. DTY QTL survey in 60 rice genotypes