

QTL for grain yield under water stress and non-stress conditions over years in rice (*Oryza sativa* L.)**Sunil Kumar Verma^{1*}, Ritu Ravi Saxena¹, Ravi Ratna Saxena², Mary Suchita Xalxo¹, Satish Balkrishna Verulkar³**¹Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492 012 Chhattisgarh, India²Department of Agriculture Statistics and Social Science, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492 012 Chhattisgarh, India³Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492 012 Chhattisgarh, India

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Abstract

A RIL population (F₁₂ and F₁₃ generation) developed from a cross between the two *indica* cultivars, Danteshwari (high yielding variety) and Dagad deshi (drought tolerant local land race). The population was phenotyped under combination of absolute rainfed (RF), terminal stage drought (TSD) and irrigated (I) conditions with direct seeding and transplanting during 2011 and 2012 to identify QTL for grain yield. Genotyping of all 122 RIL lines were done using 162 polymorphic SSR and HvSSR markers. The phenotypic and genotypic data was analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2. The recombinant inbred lines exhibited significant interaction with conditions. In general, the lines performing better under stress were poor under non-stress condition; however, two lines (#17 and 57) performed well under all sets of conditions. These lines had high tillering, ~10 and 27 and spikelet fertility, ~80 and 95% under stress and non-stress conditions, respectively. A total of 20 QTL were detected for grain yield under different conditions by employing QTL cartographer 2.5 and 7 QTL by employing QTL IciMapping 3.2; out of which 5 QTL were common under both the software. Among the major QTL, QTL on chromosome 1 (*qDTY1.1*) under rainfed transplanted condition between RM 3825 (143.7cM) to RM 302 (147.8cM) had LOD score of 5.09, QTL on chromosome 3 (*qDTY3.3*) under irrigated transplanted condition between RM 7 (64.0cM) to RM 232 (76.7cM) had LOD score of 6.59 and QTL on chromosome 11 (*qDTY11.1*) under TSD transplanted condition between RM 21 (85.7cM) to RM 26334 (90.0cM) had LOD score of 7.39. This study has resulted in identification of new major QTL, one on chromosome 11 (*qDTY11.1*) under TSD condition, which is different than most of other QTL reported by earlier workers. These QTL except *qDTY 3.3*, had positive additive effects, indicating that alleles at these loci increase grain yield under different conditions and come from tolerant parent Dagad deshi. Two regions of chromosome #1 and 3 had QTL for grain yield under stress as well as non-stress conditions. These genomic regions associated with grain yield under different conditions will be useful in marker assisted breeding for drought tolerance in rice.

Keywords: Drought tolerance; quantitative trait loci; grain yield; microsatellite markers; rice.**Abbreviations:** I_irrigated; RF_rainfed; TSD_terminal stage drought; Ds_direct seeded; T_ transplanted.**Introduction**

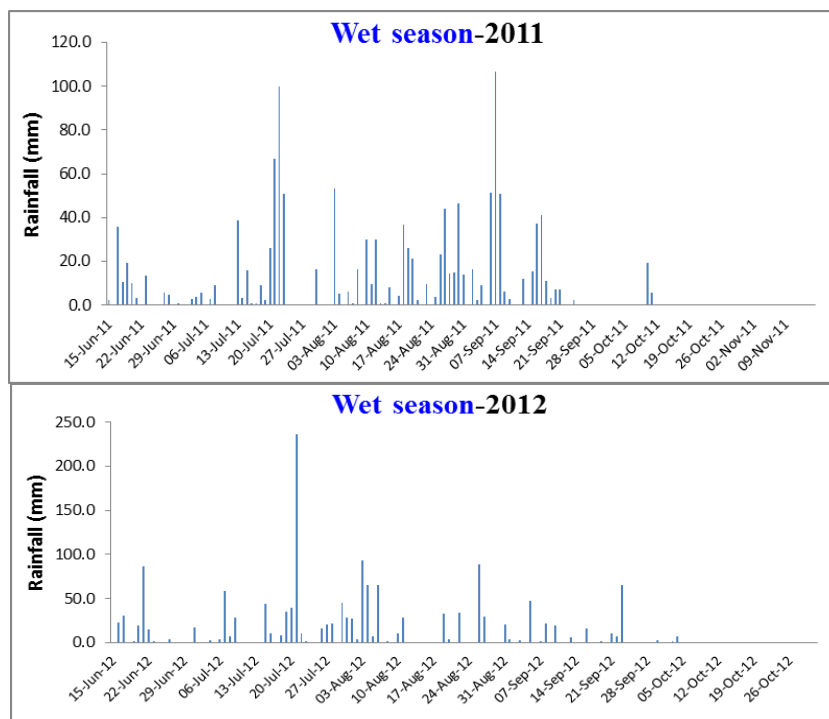
Rice (*Oryza sativa* L.) is a "Global Grain" cultivated widely across the world feeding millions of mankind. Grain yield is major important component for breeder and farmers. It is affected by many biotic and abiotic factors. About 50% of the rice area is grown under intensive irrigated systems, which account for 75% of global rice production, while the other half of the global rice area is rainfed (Zeigler and Barclay, 2008). Of all the abiotic stresses that curtail crop productivity, drought, which often occurs due to unpredictable, insufficient and uneven distribution of rainfall during rice growing seasons, is the main constraint to high yield in rainfed rice production systems in both the lowlands and the uplands (Pandey et al., 2005 and Verulkar et al., 2010) and the most recalcitrant to breeders' efforts (Tuberosa and Salvi, 2006). Recent climate change estimates predict the water deficit to further deteriorate in the years to come (Wassmann et al., 2009) and the intensity and frequency of drought are predicted to become worse (Bate et al., 2008). To

offset the reduction in yield of rice crop in rainfed areas, and to increase overall rice production, new rice varieties with greater adaptability to varied situations is highly essential. It is therefore important to define the target population of environment. Most of the fields in eastern India particularly in Chhattisgarh are banded. The long term rainfall pattern in this region indicates that terminal stage drought is most common. Lot of area is under direct seeding, therefore screening conditions included absolute rainfed conditions (direct seeded and transplanted), terminal stage drought (direct seeded and transplanted) and irrigated condition which simulate the realistic farmers' situation. The term 'drought tolerance' in this study relates to final grain yield rather than to the capacity of the plant to survive in water-limited conditions (Tuberosa and Salvi, 2006). The grain yield under stress conditions has been reported to less heritable being a complex quantitative character reducing its selection efficiency (Rosielle and Hamblin, 1981; Blum,

Table 1. Analysis of variance for grain yield (g/m^2) under split plot design.

Source of variation	Degree of freedom	Mean sum of square	
		Wet season -2011	Wet season -2012
Replication	1	160835.20	56705.80
Environments (a)	4	4567631.99*	5615989.50**
Error (a)	4	444623.50	218679.00
genotypes (b)	123	105695.04**	73793.90**
a \times b	492	38590.03**	16936.47**
Error (b)	615	13844.19	7276.39

* = Significant at 0.05 probability level; ** = Significant at 0.01 probability level

**Fig 1.** Daily rainfall pattern during wet season-2011 and 2012.

1988; Edmeades et al., 1998 and Fukai and Cooper, 1995). However, the secondary traits have not been successfully used in breeding program. In the recent years, broad sense heritability for yield under stress conditions has been reported to be similar to that of non-stress conditions (Blum et al., 1999; Lafitte and Courtois, 2000; Atlin and Lafitte, 2002; Babu et al., 2003; Lanceras et al., 2004 and Venuprasad et al., 2007). Recently the effective selection for grain yield under stress condition has been reported (Venuprasad et al., 2007; Kumar et al., 2008 and Verulkar et al., 2010). Conventional breeding for drought resistance is slow in attaining progress due to poor understanding of genetic control of drought resistance. Molecular markers help in identification of quantitative trait loci (QTL) associated with drought resistance traits and their indirect selection using marker assisted selection. Several quantitative trait loci (QTL) for the grain yield have been identified and mapped on different rice chromosomes using molecular markers (Moncada et al., 2001; Bernier et al., 2007; Fu et al., 2010; Vikram et al., 2011 and Yadaw et al., 2013), but their refinement and genetic dissection are yet underway to truly understand the quantitative variation and genes contributing to the trait, which limits their effective utilization in breeding programs. This exhaustive study was undertaken to identify QTL under ten well-defined conditions mainly representing the major target population of environment over two years

using the mapping population developed by locally adapted highly drought tolerant donor.

Results and Discussion

Analysis of variance

Analysis of variance was performed for grain yield in both the year following split plot design (Table 1). The mean sum of squares for environments were significant in both the year, showed that the condition created in this experiment was different to each other. The genotype \times environment interaction was also significant at 0.01 probabilities, indicating differential response of genotypes to environment. Significant G \times E interaction under water stress conditions have also been reported by Mall et al., 2012. An important significant interaction was also observed under direct seeded and transplanted condition with genotypes, which indicates differential performance of genotypes under these conditions. A significant area of rice in eastern India is under direct seeding; therefore, its evaluation under direct seeded conditions is essentially required. Since G \times E was significant, each environment was analyzed separately according to a randomized complete block design (Gomez and Gomez, 1984). In individual environments, genotypes were also significant (Table 2).

Table 2. Analysis of variance for grain yield (g/m^2) under RCBD design.

Source of variation	Degree of freedom	Mean sum of square, wet season -2011				
		I T	RF Ds	RF T	TSD Ds	TSD T
Replications	1	1841383.24**	32386.66*	25365.99*	1791.26	24266.85**
Genotypes	123	128569.19**	27219.68**	19526.74**	43698.63**	14987.48**
Error	123	44414.01	7760.60	4628.41	6313.25	2335.13

Source of variation	Degree of freedom	Mean sum of square, wet season -2012				
		I Ds	I T	RF Ds	RF T	TSD T
Replications	1	16393.44**	578947.25**	133328.84**	2339.61	165545.44**
Genotypes	123	12579.52**	56175.51**	30179.12**	33605.29**	10369.36**
Error	123	1434.42	16480.43	8371.07	7209.86	2470.92

Where, I= Irrigated, RF= Rainfed, TSD= Terminal Stage Drought, Ds= Direct seeded, T= Transplanted,

* = Significant at 0.05 probability level and ** = Significant at 0.01 probability level

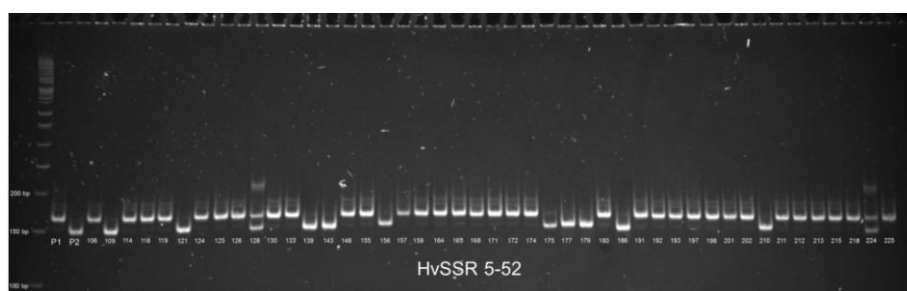


Fig 2. Banding pattern of HvSSR 5-52 for genotyping of population.

Heritability

Heritability is an important genetic parameter. Broad-sense heritability of grain yield *per se* under different conditions is presented in Table 3. Grain yield showed medium heritability 0.49, 0.56 and 0.62 under irrigated transplanted, rainfed direct seeded and rainfed transplanted condition, respectively during wet season 2011; and 0.54, 0.57, 0.65 and 0.61 under irrigated transplanted, rainfed direct seeded, rainfed transplanted and TSD transplanted conditions, respectively during wet season 2012. It also showed high heritability under TSD direct seeded (0.75) and TSD transplanted condition (0.73) during wet season 2011. It is noted that during both the years heritability under stress conditions were not lower than that of the irrigated control. Recent literature also reports high heritability of grain yield under stress conditions (Atlin et al., 2004; Bernier et al., 2007; Venuprasad et al., 2007 and Kumar et al., 2008) and direct selection for yield under a managed upland drought environment.

Intensity of stress

Rainfall pattern is presented in Fig. 1. During wet season 2011 total 1328 mm water received in crop period where maximum 15 continuous rainless day at flowering and 6 continuous rainless days at tillering stage. During 2012 total 1640 mm rainfall was received where maximum 6 continuous rainless days at flowering and 4 continuous rainless days twice at growth stage. Mean grain yield and percent reduction in grain yield under both sets of water-stress conditions compared with an irrigated control over years is presented in Table 3. The drought intensity index (DII) values for yield varied from 0.47 (TSD Ds) to 0.62 (TSD T) and 0.53 and 0.54 under RF T and RF Ds, respectively during wet season 2011; and from 0.28 (RF T) to 0.52 (TSD T) and 0.33 under RF Ds during wet season 2012. Except during 2012 under rainfed conditions, quite severe level of water stress was

imposed. Soil moisture content at 30 cm depth ranged from min 17.83% to max 22.74% with an average of 20.32% and under TSD transplanted condition min 15.76% to max 21.07% with an average of 18.95%. Tensiometer reading at maximum water stress ranged from 35-46 Kp at 30 cm depth under TSD transplanted 2011. RWC ranged from 70.49% to 99.83%. This level of stress is required to clearly discriminate between drought-tolerant and susceptible genotypes. Most breeding programs screening for drought tolerance fail to impose a sufficiently severe stress in their trials and thus are not able to accurately select drought-tolerant lines (Kumar et al., 2008).

Correlation of grain yield under different conditions

Correlation between different conditions of grain yield was evaluated at $P \leq 0.05$ and $P \leq 0.01$. A total of 45 pair wise combinations were formed among 10 conditions of grain yield of which 32 coefficients of correlation estimates were found to be significant at 1% level of significance and four combinations were significant at 5% level of significance (Table 4). Highly significant positive correlation 0.58 between RF Ds and TSD Ds 2011, 0.42 between IT 2011, 2012 and 0.43 between TSD T 2011, 2012 was found for grain yield. Grain yield of rainfed condition exhibited positive significant correlation with grain yield of TSD condition indicating that both conditions had similar water stress. Correlation of grain yield between irrigated transplanted conditions exhibited significant negative correlation with rainfed direct sown condition, which to a great extent indicate that genotypes performance cannot be predicted based on its performance under one set of condition.

Performance of RIL lines under different condition

Within 122 RILs, top 15 high yielding lines under different conditions during 2011 and 2012 are presented in Table 5.

Table 3. Mean, range and other parameters of grain yield under irrigated (I), rainfed (RF), terminal stage drought (TSD), direct seeded (Ds) and transplanted (T) conditions.

Parameters/ Conditions	Wet season -2011				Wet season -2012					
	I	RF	RF	TSD	TSD	I	I	RF	RF	TSD
	T	Ds	T	Ds	T	Ds	T	Ds	T	T
Sowing	15 June	30 June	10 July	26 June	18 July	15 June	29 June	29 June	15 June	29 June
Transplanting	14 July	-	29 July	-	5 Aug	-	14 July	-	9 July	9 Aug
Rainfall (15 Jun- 15 Dec)	1328 mm				1640 mm					
No. of rainless days	81	77	73	78	70	67	63	63	67	63
Range	139- 1417	67-578	42-561	19-778	14-435	37-555	291-994	140-807	125-748	140-600
Mean grain yield (g/m ²)	601	278	285	318	229	236	625	417	451	298
% reduction over irrigated	-	53.74	52.58	47.09	61.90	-	-	33.28	27.84	52.32
DII	-	0.54	0.53	0.47	0.62	-	-	0.33	0.28	0.52
Heritability h ² _(bs)	0.49	0.56	0.62	0.75	0.73	0.79	0.55	0.57	0.65	0.62

Where, DII= Drought Intensity Index

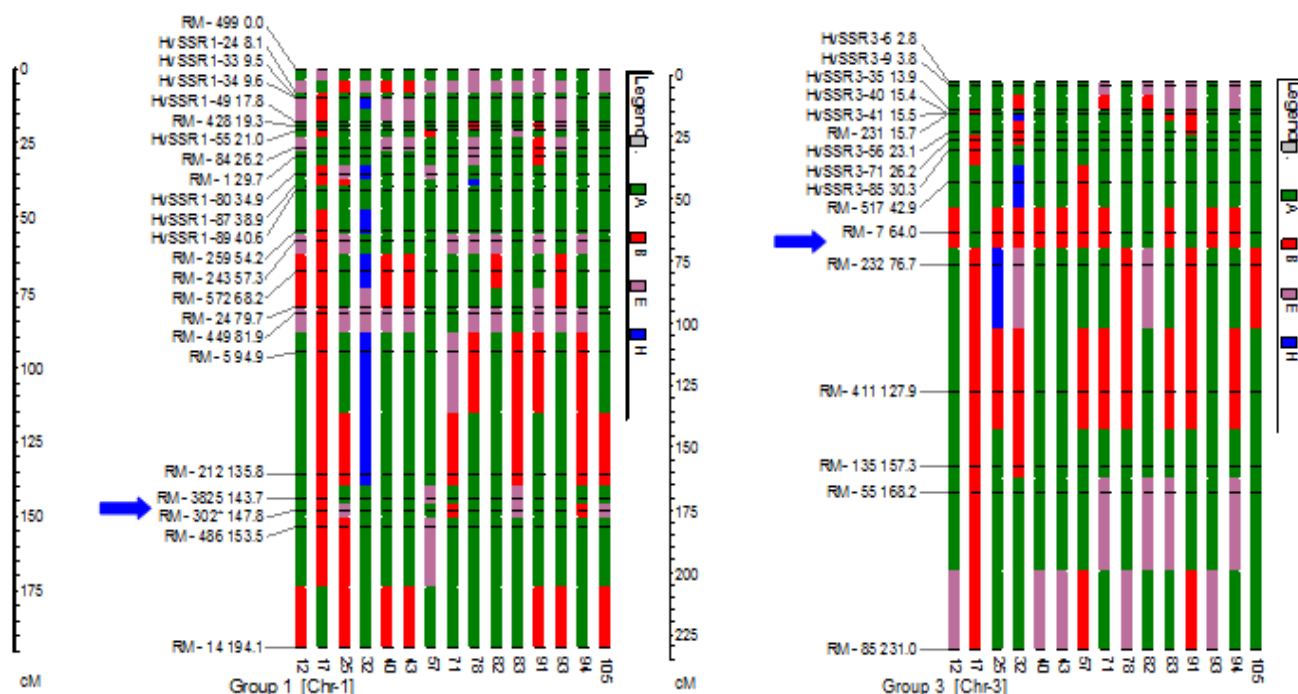


Fig 3. Graphical genotype of chromosome 1 and 3 of Danteshwari × Dagad deshi showing expected proportion of introgression.

During both the years line number 25, 71, 82 and 105 had highest grain yield under irrigated condition, line number 43, 78 and 83 had highest grain yield under rainfed condition, while line number 12, 40 and 93 had highest grain yield under terminal stage drought (TSD) condition. Line number 13, 17, 64 and 78 had highest grain yield under direct seeded condition and line number 17, 40, 43, 83 and 93 had highest grain yield under transplanted condition. Line number 25, 64 and 105 had highest grain yield under early sown condition and line number 17, 40, 57 and 78 had highest grain yield under late sown condition. On overall basis only two lines # 17 and 57 had highest yield under all conditions in both the years, over the parents and mean of particular condition. These lines had high tillering, ~10 and 27 and spikelet fertility, ~80 and 95% under stress and non-stress conditions, respectively. So these can be use for further breeding programme and also useful for farmers.

Genotyping of population

For the purpose of genotyping 830 microsatellite primers were screened, out of which 162 were polymorphic, exhibiting 19.52% polymorphism. Differences among polymorphic markers were observed for allele segregation in RILs (Fig. 2). Out of 162 markers, 73 (45.06%) exhibited normal 1:1 segregation at 1% level of significant in χ^2 test, rest exhibited skewed distribution towards either parents. The marker RM 171 produces more female type alleles (86.1%) with less male type alleles (12.3%). On the other hand RM 277 produces more male type allele (83.6%) with 11.5% female. RM 171 marker had high A: B ratio (7.0) such skewed distribution for markers has also been reported by Cai et al., 2011. Expected proportion of integration of parents into lines was analyzed with the help of GGT2.0 based on genotypic data (Fig. 3). GGT of high yielding lines showing that major QTL region of chromosome 1 contributed from

Table 4. Correlation between grain yield under different conditions.

Correlation		GY wet season -2011					GY wet season -2012				
Coefficient		I T	RF Ds	RF T	TSD Ds	TSD T	I D/s	I T	RF Ds	RF T	TSD T
GY	I T	1									
wet	RF Ds	-0.21*	1								
season	RF T	-0.04	0.46**	1							
-2011	TSD Ds	0.21*	0.58**	0.43**	1						
	TSD T	0.00	0.31**	0.61**	0.24**	1					
GY	I Ds	0.35**	0.02	-0.02	0.42**	-0.06	1				
wet	I T	0.42**	-0.02	0.28**	0.23**	0.47**	0.34**	1			
season	RF Ds	0.06	0.35**	0.32**	0.23**	0.30**	0.30**	0.40**	1		
-2012	RF T	0.51**	0.06	0.22**	0.37**	0.36**	0.39**	0.71**	0.35**	1	
	TSD T	0.19*	0.05	0.29**	0.19*	0.43**	0.28**	0.59**	0.42**	0.49**	1

Where, GY=grain yield, I= Irrigated, RF= Rainfed, TSD= Terminal Stage Drought, Ds= Direct seeded, T= Transplanted, * = Significant at 0.05 probability level and ** = Significant at 0.01 probability level

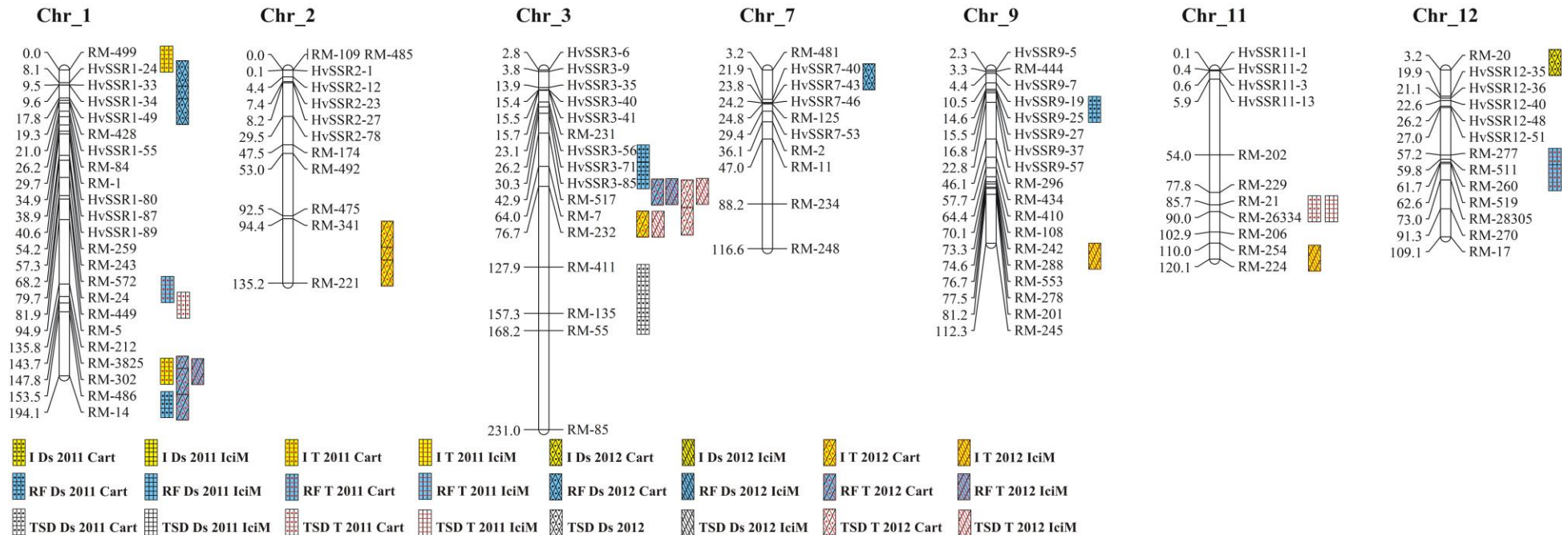


Fig 4. Genetic map locating all QTL for grain yield under different conditions by QTL Cartographer 2.5 and QTL IciMapping 3.2.

female parent and chromosome 3 from both the parents, which can be used in selection of desirable lines.

Identification of QTL

The genotypic data and field based phenotypic data of grain yield was analyzed using QTL cartographer 2.5 and QTL IciMapping 3.2. Results from the QTL analysis are presented in Table 6-8 and Fig. 4-6. A total of 20 QTL were identified for grain yield under different conditions using cartographer, while 7 QTL were identified using QTL IciMapping, out of which 5 QTL were common with both the software. The LOD score for QTL ranged from 3.0 to 7.4 for mapping with cartographer and 3.43 to 5.99 using QTL IciMapping. These QTL were found to be present on chromosome #1, 2, 3, 7, 9, 11 and 12. Two major QTL were identified; one on chromosome #11 between RM 21 (85.7cM) to RM 26334 (90.0cM), LOD score of 7.39 with 27 % phenotypic variance in cartographer and LOD score of 4.28 with 17 % phenotypic variance in QTL IciMapping under terminal stage drought (transplanted) condition. This QTL had positive additive effects, indicating that alleles at this loci increase grain yield under different condition come from tolerant parent Dagad deshi. A perusal of published literature from Moncada et al., 2001; Bernier et al., 2007; Kumar et al., 2007; Fu et al., 2010; Gomez et al., 2010; Vikram et al., 2011; Marathi et al., 2012 and Yadaw et al., 2013 reveals that this is a new QTL with major effect and can be tentatively named as *qDTY 11.1*. Another major effect QTL for grain yield under irrigated transplanted condition was identified on chromosome #3 between RM 7 to RM 232 with LOD score of 6.59. This QTL had positive additive effects (74.96) with 13 % phenotypic variance. A region encompassing RM 7 and RM 517 had QTL (*qDTY3.3*) for grain yield under irrigated, terminal stage drought as well as rainfed conditions, which indicates that this region is important for grain yield under all sets of conditions and therefore could be a major target region for increasing grain yield. Bernier et al., 2007 and Marathi et al., 2012 also reported QTL on chromosome 3 for grain yield under different condition but the region on chromosome is different than the already reported *DTY 3.1* and *DTY 3.2*. QTL Region of chromosome 1 between RM 572 to RM 449 (~4 LOD, negative additive effect and 19 % phenotypic variance under QTL cartographer) and chromosome 3 between HvSSR 3-85 to RM 517 (~3.5 LOD, positive additive effect and 11% phenotypic variance) was associated with grain yield under both TSD and rainfed transplanted condition 2011 and 2012, respectively. The only stable region that had significant effects in both years under rainfed condition was located on chromosome 1 between RM 486 to RM 14 (~3.70 LOD, positive additive effect and ~10% phenotypic variance) and chromosome 3 between HvSSR 3-85 and RM 232 (~3.40 LOD, positive additive effect and ~12% phenotypic variance). QTL present between RM 517 to RM 7 on chromosome 3 and between RM 24 to RM 449 on chromosome 1 is associated with all the three conditions irrigated, rainfed and TSD condition, which can be used for increasing the grain yield under all the sets of conditions. One major QTL *DTY 1.1* has been reported by Moncada et al., 2001; Kumar et al., 2007; Fu et al., 2010; Gomez et al., 2010; Vikram et al., 2011 and Yadaw et al., 2013, which contribute significantly for grain yield under water stress. The other QTL identified on chromosome #1 under rainfed condition between HvSSR 1-24 to HvSSR 1-49 (3.50 LOD, negative additive effect and 6 % phenotypic variance) seems to be a new QTL on this chromosome, and can be designated as *qDTY 1.2*. Our QTL *qDTY 1.1* is similar to earlier reported *qDTY 1.1* by Vikram et al., 2011; Dixit et al., 2012; Ghimire

et al., 2012 and Venuprasad et al., 2012. QTL *qDTY 2.3* identified on chromosome 2 is similar to earlier reported QTL *qYLD 2.3* by Vikram et al., 2011; Dixit et al., 2012 and Mishra et al., 2013; and *qtl 2.1* by Bernier et al., 2007. QTL *qDTY 3.2* identified on chromosome 3 is similar to earlier reported *qDTY 3.2* by Vikram et al., 2011; Dixit et al., 2012; Mishra et al., 2013 and Yadaw et al., 2013 and; *qtl 3.1* by Bernier et al., 2007. QTL *qDTY 12.1* identified on chromosome 12 is present on similar region of earlier reported *qDTY 12.1* by Dixit et al., 2012 and Mishra et al., 2013; and *qtl12.1* by Bernier et al., 2007. Of the 14 QTL (*qDTY*) identified in the study, 10 QTL were found to be novel. QTL *qDTY 1.2* and *qDTY 1.3* on chromosome 1; *qDTY 3.3* and *qDTY 3.4* on chromosome 3; *qDTY 7.1* on chromosome 7; *qDTY 9.2* and *qDTY 9.3* on chromosome 9; *qDTY 11.1* and *qDTY 11.2* on chromosome 11; *qDTY 12.2* on chromosome 12 identified is present on different region than earlier reported QTL. A perusal of QTL identified under direct sown condition versus transplanted conditions revealed that grain yield under both the conditions is contributed by different QTL regions. The genotypes exhibited significant interaction with the method of establishment, the growth pattern under both the conditions varies, the primary yield contributing traits are different thus it is expected that QTL for grain yield under both the conditions should differ. Fig. 5 indicates the different important regions of rainfed under both the conditions. Only one epistasis QTL affecting grain yield were identified under irrigated transplanted condition (Fig. 6). QTL at marker interval RM 5 - RM 212 on chromosome 1 showed significant interaction with QTL on chromosome 7 i.e. RM 2 - RM 11 explaining 5.57 LOD with 52.47% phenotypic variation (Table 8).

Materials and Methods

Planting materials

A recombinant inbred line (RIL) population of 271 lines in F₁₁ generation was developed from a cross between Danteshwari and Dagad deshi; and maintained by single seed descent method. Dagad deshi is a local land races having deep root, strong culm, high spikelet fertility, exhibits late leaf rolling under water stress while the other parent, Danteshwari is a high yielding but susceptible to drought. A preliminary experiment was conducted during 2010 in which whole RIL population of 271 was evaluated under water stress (absolute rainfed condition) and irrigated condition and based on performance under these two conditions and important traits like grain yield, leaf rolling, plant height and root pulling resistance; highest and lowest genotypes were selected. Selected 122 RILs in F₁₂ and F₁₃ generations were subsequently used for this study.

Experimental design and environments

The trials were conducted during wet season-2011 and 2012. In each year the whole RIL population was evaluated under five different conditions of different water management and crop establishment as described in Table 3. Under each condition the trial was conducted in RCBD with two replications with each genotype having 3 rows of 1.5m length. The seed rate was maintained at 2.5 g/m² for transplanted conditions and 6.0 g/m² under direct seeding. The field trial was conducted under combination of irrigated, rainfed, TSD, direct seeded, transplanted, early and late condition at research cum instructional farm of IGKV, Raipur (C.G.), (21° 16' N and 81° 36' E at altitude of 289.6 meter above sea level). The experiments were conducted in sandy

Table 5. Top 15 high yielding lines under different conditions.

Conditions		Line numbers with its grain yield (g/m ²) in bracket															P ₁	P ₂	Mean
Wet season-2011	I	88	115	18	70	82	67	55	105	71	<u>57</u>	6	25	83	120	56			
	T	(1417)	(1278)	(1194)	(1194)	(1194)	(1139)	(1125)	(1111)	(1083)	(1055)	(1028)	(1028)	(944)	(944)	(889)	(725)	(500)	(601)
	RF	17	110	49	13	61	33	87	19	44	1	109	32	78	97	45			
	Ds	(578)	(578)	(567)	(522)	(600)	(489)	(489)	(478)	(478)	(478)	(478)	(456)	(456)	(456)	(411)	(300)	(375)	(279)
	RF	<u>57</u>	17	93	40	43	54	69	39	87	44	85	106	78	83	32			
	T	(561)	(513)	(513)	(497)	(487)	(455)	(439)	(423)	(402)	(402)	(402)	(402)	(397)	(397)	(392)	(325)	(400)	(286)
	TSD	17	64	44	105	13	110	1	78	7	56	67	63	83	61	73			
	Ds	(778)	(656)	(622)	(622)	(622)	(611)	(589)	(578)	(544)	(544)	(522)	(511)	(500)	(500)	(489)	(310)	(325)	(318)
	TSD	12	<u>57</u>	93	43	87	119	86	89	6	22	8	17	116	37	40			
Wet season -2012	T	(435)	(421)	(412)	(407)	(398)	(394)	(389)	(380)	(356)	(347)	(343)	(338)	(338)	(338)	(338)	(325)	(330)	(231)
	I	64	17	9	18	105	113	60	13	76	25	36	67	1	15	16			
	Ds	(555)	(392)	(370)	(363)	(363)	(363)	(356)	(348)	(348)	(341)	(341)	(341)	(333)	(333)	(333)	(325)	(178)	(237)
	I	93	25	105	43	71	78	12	99	64	109	28	17	82	16	40			
	T	(995)	(990)	(958)	(948)	(943)	(927)	(927)	(880)	(875)	(870)	(859)	(854)	(844)	(833)	(833)	(807)	(599)	(625)
	RF	17	89	<u>57</u>	64	60	69	51	111	22	105	78	117	16	33	47			
	Ds	(807)	(711)	(711)	(689)	(689)	(652)	(637)	(930)	(622)	(615)	(585)	(578)	(570)	(563)	(556)	(444)	(518)	(417)
	RF	110	83	93	105	25	43	64	1	40	89	78	109	115	12	121			
	T	(748)	(733)	(711)	(704)	(704)	(659)	(652)	(652)	(644)	(637)	(637)	(637)	(637)	(630)	(630)	(415)	(467)	(451)
TSD	25	12	17	62	40	10	83	93	60	100	82	50	<u>57</u>	22	16				
T	(600)	(444)	(430)	(422)	(422)	(415)	(407)	(407)	(393)	(393)	(393)	(393)	(385)	(385)	(378)	(318)	(311)	(298)	

Where, I= Irrigated, RF= Rainfed, TSD= Terminal Stage Drought, Ds= Direct seeded and T= Transplanted

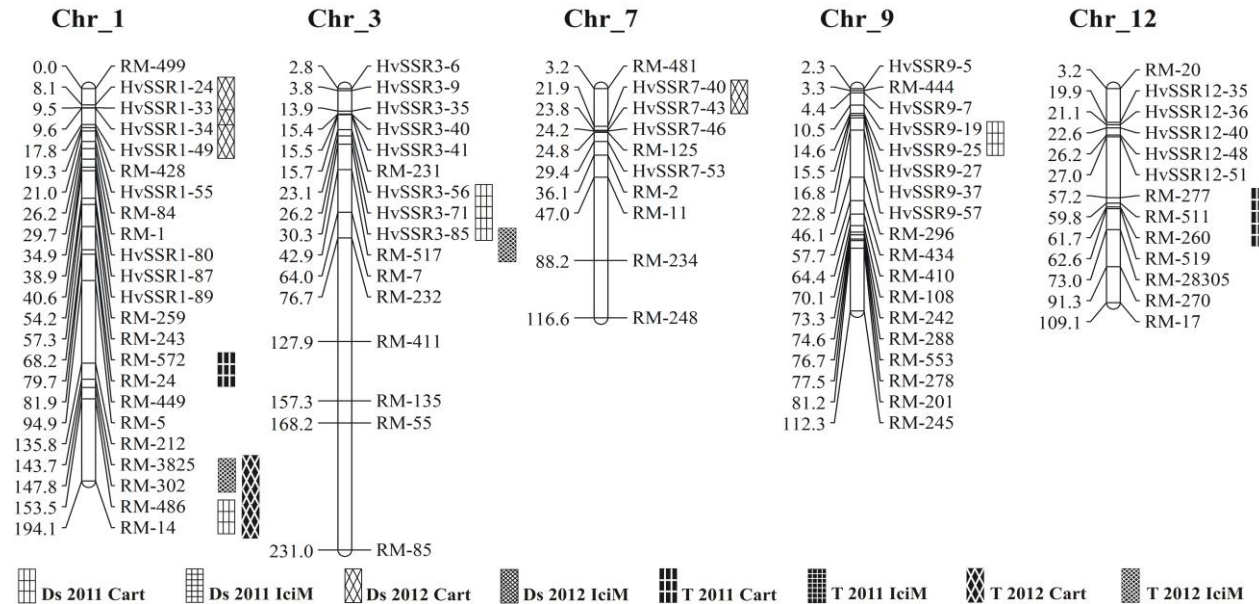
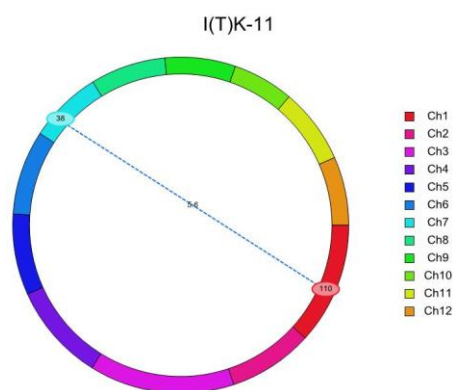


Fig 5. Genetic map locating all QTL for grain yield under rainfed conditions by QTL Cartographer 2.5 and QTL IciMapping 3.2.

Table 6. QTL identified for grain yield under different conditions by QTL cartographer 2.5.

Conditions	QTL	Chr	Marker interval	LOD	R ² (%)	Add
I T 2011	<i>qDTY 1.2</i>	1	RM 499 to HvSSR 1-24	3.41	11.10	90.93
	<i>qDTY 1.1</i>	1	RM 3825 to RM 302	3.06	8.98	77.50
I Ds 2012	<i>qDTY 12.2</i>	12	RM 20 to HvSSR 12-35	3.46	8.68	19.20
I T 2012	<i>qDTY 2.3</i>	2	RM 341 to RM 221	3.61	8.93	41.85
	<i>qDTY 3.3</i>	3	RM 7 to RM 232	6.59	13.38	74.90
RF Ds 2011	<i>qDTY 1.1</i>	1	RM 486 to RM 14	3.38	8.52	40.21
	<i>qDTY 3.2</i>	3	HvSSR 3-56 to HvSSR 3-85	3.06	7.51	-34.40
	<i>qDTY 9.2</i>	9	HvSSR 9-19 to HvSSR 9-25	3.35	8.41	-38.29
RF T 2011	<i>qDTY 1.3</i>	1	RM 572 to RM 24	4.64	19.96	-45.15
	<i>qDTY 12.1</i>	12	RM 277 to RM 260	3.00	7.29	28.24
RF Ds 2012	<i>qDTY 1.2</i>	1	HvSSR 1-24 to HvSSR 1-49	3.50	6.31	-42.78
	<i>qDTY 7.1</i>	7	HvSSR 7-40 to HvSSR 7-43	3.44	11.77	59.06
RF T 2012	<i>qDTY 1.1</i>	1	RM 3825 to RM 302	5.09	15.08	51.74
	<i>qDTY 1.1</i>	1	RM 302 to RM 14	4.02	13.51	50.55
	<i>qDTY 3.2</i>	3	HvSSR 3-85 to RM 517	3.22	11.28	47.53
TSD Ds 2011	<i>qDTY 3.4</i>	3	RM 411 to RM 55	4.29	15.19	-60.86
TSD T 2011	<i>qDTY 1.3</i>	1	RM 24 to RM 449	3.23	8.98	-28.83
	<i>qDTY 11.1</i>	11	RM 21 to RM 26334	7.39	27.34	58.13
TSD T 2012	<i>qDTY 3.2</i>	3	HvSSR 3-85 to RM 517	3.24	8.84	26.38
	<i>qDTY 3.3</i>	3	RM 7 to RM 232	4.38	10.74	-25.56

I=Irrigated, RF= Rainfed, TSD= Terminal Stage Drought Ds= Direct sown, T= Transplanted

**Fig 6.** Epistasis effect of QTL for grain yield on chromosome #1 and 7 by QTL IciMapping 3.2.

or clay loam inceptisols, with soil pH ranging from 6.8 to 7.4 and organic carbon of 0.32-0.34%. Each field was selected for different condition so that could be managed in different ways. All normal agronomic practices were followed in all the conditions except irrigation and drainage. For irrigated condition continuous water was maintained in these experiments from sowing / transplanting to 10 days before maturity. For rainfed experiments fields were never irrigated and rainwater was drained just after rain so as to allow quick appearance of drought, thus keeping the fields free from standing water throughout the season. Sowing and transplanting for TSD experiments was delayed by approximately 20-25 days so as to coincide the reproductive-stage stress of the crop with the dry spell after the withdrawal of monsoon. The crop was irrigated till 4 weeks after transplanting and thereafter the paddy was drained. Precaution was taken to select a field on higher topology with light soils, which loses its soil moisture rapidly to allow the development of severe drought stress in the field. This situation led to exposure of the breeding material to terminal stage drought only.

Rainfall and hydrology

The total rainfall during the experimental period and number of rainless days during the RF and TSD crop period are

presented in Table 3 and Fig. 1. The total rainfall was 1328 mm in 2011 and 1640 mm in 2012, 85% of which occurred from mid-June to September 30. The numbers of rainless days in rainfed trial were 63 in 2012 and 73 in 2011. Similarly, the number of rainless days during the stress period in the TSD trial ranged from 63 days in 2012 and 78 days in 2011. All the rainwater was very effectively drained on the same day, thus leading to the imposition of stress even if we had a gap of more than 9 days.

Phenotypic observations

Observation recorded for grain yield on g/m² under all the sets of conditions. Daily rainfall data for the cropping season (June-December), along with parching water level, tensiometer reading and soil moisture content (gravimetric method) at 30 cm, were recorded at regular intervals throughout the season.

Genomic DNA isolation and PCR

For developing genotypic data based on SSR and HvSSR markers, DNA was extracted from fresh leaf with the help of MiniPrep method (Doyle and Doyle, 1987). Polymerase

Table 7. QTL identified for grain yield under different conditions by QTL IciMapping 3.2.

Conditions	QTL	Chr	Position (cM)	Left marker	Right markers	LOD	R ² /PVE (%)	Add
TSD T 2011	<i>qDTY 11.1</i>	11	89.10	RM-21	RM-26334	4.28	17.12	36.69
I T 2012	<i>qDTY 9.3</i>	9	74.30	RM-242	RM-288	3.53	9.80	54.20
	<i>qDTY 11.2</i>	11	116.10	RM-254	RM-224	4.76	17.92	70.97
RF T 2012	<i>qDTY 1.1</i>	1	144.00	RM-3825	RM-302	5.99	20.26	58.77
	<i>qDTY 3.2</i>	3	39.80	HvSSR3-85	RM-517	3.43	16.97	56.37
TSD T 2012	<i>qDTY 3.2</i>	3	42.80	HvSSR3-85	RM-517	3.81	11.34	26.34
	<i>qDTY 3.3</i>	3	64.80	RM-7	RM-232	4.34	13.28	-26.17

Where, I= Irrigated, RF= Rainfed, TSD= Terminal Stage Drought, Ds= Direct seeded and T= Transplanted

Table 8. Epistasis QTL identified in Danteshwari × Dagad deshi RIL population for grain yield by QTL IciMapping 3.2.

Trait name	Irrigated transplanted 2011
Chromosome1	1
Position1	110.00
Left marker1	RM 5
Right marker1	RM 212
Chromosome2	7
Position2	38.20
Left marker2	RM 2
Right marker2	RM 11
LOD	5.57
PVE (%)	52.48
Add1	49.68
Add2	80.06
Add by add	182.93

chain reaction (PCR) amplification was performed in a total volume of 20 µl and the reaction mixture contained 10 X assay buffer, 1 mM dNTP mix, 5 pM forward and reverse primers, 40 ng of template DNA and 1 unit *Taq* polymerase in Applied Biosystems thermal cycler. After an initial denaturation step of 95°C for 5 minutes, the amplification was carried out for 34 cycles comprising 1 minutes each of 94°C, 55°C and 72°C. The final elongation step was extended to 7 minutes at 72°C followed by 4°C. After the PCR reaction was completed, 5 µl of 6 X loading dye was added to PCR amplicons and 7 µl (PCR product with dye) was loaded on 5% PAGE in a minivertical electrophoresis system (CBS scientific, model MGV-202-33). DNA fragments were then stained with ethidium bromide and visualized with a UV transilluminator Bio-rad XR⁺ (Fig. 2). Genotyping of 122 lines was done using 162 polymorphic (out of 830 tested) SSR markers in molecular markers laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur. The banding pattern was scored as A, B and H for female, male and heterozygous banding pattern, respectively. These markers were quite evenly distributed across the entire twelve chromosomes. (Supplementary Table 1).

Statistical analysis

Mean and range were calculated based on observation of grain yield. Mean was calculated by dividing sum of all value to number of individuals. Smallest and largest value present in observation was denoted as range, which providing the information about the extent of variability present in the genotypes. The replicated data of each line were used for analysis of variance under split plot design and RCBD with the help of SPSS 16.0. When G × E was significant, each environment was analyzed separately according to a randomized complete block design. The split plot design and RCBD experimental design as per the method of Fisher (1935). Heritability was calculated as the ratio of genotypic variance to phenotypic variance (total variance) through the

formula suggested by Hanson et al. (1956). A correlation coefficient (r) was calculated for grain yield under different conditions by using the standard procedure of Searle (1961). A Chi square test was performed for testing segregation of markers (goodness of fit) by using standard procedure suggested by Steel and Torrie 1960.

Drought intensity index

The drought intensity index was calculated for each condition using the following formula given by Ramirez-Vallejo and Kelly (1998):

$$DII = 1 - (Y_S/Y_I)$$

Where, Y_S= Stress condition overall mean yield
Y_I= Irrigated condition overall mean yield

QTL analysis

The linkage map was constructed with QTL Cartographer 2.5 (Fig. 4 and 5). Graphical genotyping of these molecular data (Fig. 3) was done using GGT 2.0 (Van Berloo, 1999). The phenotypic and genotypic data was analyzed using QTL cartographer 2.5 (Wang et al., 2005) and QTL IciMapping 3.2 (Li et al., 2007) (Composite Interval Mapping) with a threshold value of 3.0 LOD, was used for declaring the presence of a suggestive QTL.

Conclusion

A stable and major QTL had been identified on chromosome #1, 3 and 11 for stress and non-stress condition. This study has resulted in identification of new QTL which can be useful in marker assisted breeding for drought tolerance in rice, QTL pyramiding and further dissected or fine mapped by developing isogenic lines. These are much useful for local area by using locally adapted highly drought tolerant donor.

Acknowledgements

We thank to Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) for providing funds and facility.

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