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Research Article

Marker assisted pyramiding of *qDTY* QTLs governing yield under drought stress into rice variety ADT(R)45

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Abstract
Drought occurrence during reproductive stage of rice leads to significant yield loss. Rice production has to be doubled to feed the ever increasing human population. As the yield levels are stagnated under favourable production system, improvement of yield under unfavourable environment is key to attain the projected target. Therefore, the present study was undertaken to improve the drought tolerance of popular rice variety ADT(R)45 by introgressing major effect quantitative trait loci (QTLs) conferring yield under drought ($qDTY_{1.1}$, $qDTY_{3.1}$ and $qDTY_{12.1}$) through marker assisted backcross breeding (MABB). Through MABB, 117 BILs in the background of ADT(R)45 were developed with different combinations of *qDTY* QTLs. The BILs were evaluated under rainfed target production environment and observations on nine biometrical and yield traits were recorded. Variability analysis revealed presence of additive gene action for the traits *viz*., total number of tillers per plant, number of productive tillers per plant, hundred grain weight, yield per hectare and single plant yield. Hence, selection based on these traits will be effective to improve yield under drought stress. Association analysis showed total number of tillers per plant, number of productive tillers per plant, panicle length, hundred grain weight and spikelet fertility percentage were highly correlated with yield per plant. Comparison of BILs with different QTL combinations showed that BILs with single QTL ($qDTY_{12.1}$) was better for most of the traits studied. BILs with single or two QTL combinations were found to perform better under rainfed condition, when compared to BILs with all the three QTLs.

Keywords: Reproductive stage drought, Rainfed, *qDTYs*, rice, BILs, MABB

INTRODUCTION

Rice is the most loved cereal of Asia and the staple food for about 3 billion people in the world. By 2030, it is predicted that production of rice must increase by close to 38% from its current level (Bin Rahman and Zhang, 2022). Drought is one of the major abiotic stresses, which negatively impacts and significantly lowers the yield and productivity of food crops globally up to 70%. (Akte *et al*., 2016). Drought is projected to have a more significant impact on rice yield as a result of decline in water availability due to recent climate change scenarios. Though drought affects rice crop at all the growth stages, reproductive stage is more vulnerable and impacts severely on yield.

Development of rice varieties with tolerance to reproductive stage drought stress is the best strategy to ensure rice production under drought. Since, limited progress has been made in generating drought-tolerant cultivars through conventional breeding, modern approaches like marker assisted breeding can greatly assist in the development of superior drought-tolerant rice cultivars (Khush, 2001). Drought is a heterogeneous stress and its type, occurrence, frequency and severity must be known for formulation of better breeding program. Several QTLs (*qDTY*) governing yield under drought stress have been identified, reported, and successfully employed in breeding programmes [Priyadarshini *et al*. (2014), Shamsudin *et al*. (2016), Sandhu *et al*. (2019), Valarmathi *et al*. (2019), Yadav *et al*. (2019) and Muthu *et al*. (2020)]. Generally, the major effect *qDTY*s have a genetic gain of 10 to 30 %, with a yield advantage of 150 to 500 kg ha^{-1} under reproductive stage drought (Swamy *et al*., 2013). Attempts have been undertaken at IRRI, Philippines and several national programmes to introgress drought resistance in high yielding rice varieties *viz*., IR64, Swarna, and Vandana. Marker assisted breeding using well-defined QTLs will allow to precisely combine high yield potential and reproductive stage drought tolerance as there is low positive correlation between high yield potential and grain yield under reproductive stage drought. Therefore, the present study was undertaken to improve the drought tolerance of popular rice variety, ADT(R)45 through marker assisted backcross breeding (MABB).

MATERIALS AND METHODS

This study was aimed to enhance the drought tolerance of ADT(R)45, a popular rice variety of Tamil Nadu through marker assisted backcross breeding. Hybridization, genotyping, selection and generation advancement were carried out at Tamil Nadu Rice Research Institute, Aduthurai.

The BILs were evaluated under target production environment at Agricultural Research Station, Paramakudi where rainfed rice production is a common method of rice cultivation.

ADT(R)45 is a short duration (115 days) rice variety, widely cultivated in the states of Tamil Nadu, Andhra Pradesh and Telangana due to its high yielding potential under transplanted, irrigated ecologies. Way Rarem an Indonesian upland rice cultivar and Apo (IR55423-01) an upland cultivar developed by IRRI, were used as donors for improving yield under drought. Apo has high-yield potential under aerobic soil conditions and moderate reproductive-stage drought tolerance (Dixit *et al.,* 2016). It possesses drought yield QTLs *viz*., $qDTY_{1.1}$ and $qDTY_{3.1}$ on chromosome 1 and 3 respectively (Venuprasad *et al*., 2009 and Venuprasad *et al*., 2012), while, Way Rarem possess mega drought yield QTL $qDTY_{12.1}$. The QTL was flanked between microsatellite markers RM 28048 and RM 511 on chromosome 12, which has the ability to increase mean yield by 38.8 % to 47.0 % under drought (Bernier *et al*.,2007)).

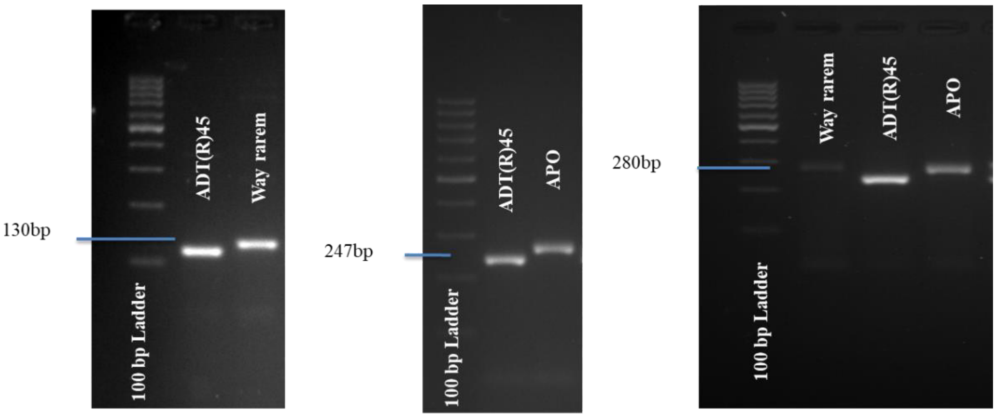
Initially independent crosses between ADT(R)45 and Apo and ADT(R)45 and Way Rarem were effected at Tamil Nadu Rice Research Institute (TRRI), Aduthurai during 2018-19. Parental polymorphism survey for the target QTLs *viz*., $qDTY_{1.1}$, $qDTY_{3.1}$ and $qDTY_{12.1}$ were done with respective SSR markers *viz*., RM11928, RM520 and RM511 reported by Bernier *et al*. (2007) and Venuprasad *et al.* (2009) (**Table 1. and Fig. 1**.). All the three SSR markers exhibited polymorphism between the parents *viz*., ADT(R)45, Apo, and Way Rarem. True F_1s were fixed through selected peak SSR primers and the F_1s were then backcrossed with recipient parent, ADT(R)45. The BC_1F_1s were genotyped with QTL specific peak markers RM11928 for $qDTY_{1.1}$, RM520 for $qDTY_{3.1}$ and RM511 for $qDTY_{12.1}$. The backcross cycle was repeated to advance BC_1F_1 to BC_2F_1 and confirmed genotypically with respective markers. QTL positive BC_2F_1 of both crosses were again backcrossed with recurrent parent ADT(R)45 as well as intermated among them to produce intermated F_1s. Foreground selection of BC_3F_1 of both crosses and intermated F_1s were performed with respective QTL specific SSR markers. A panel of 36 polymorphic SSR markers were used for background selection in QTL positive BC_3F_1s plants as well as intermated F_1s. Based on Recurrent Genome Recovery (RGR) and phenotype of the plant, one plant each from BC_3F_1 of ADT45/Apo, BC_3F_1 of ADT45/Way Rarem and intermated F_1 were selected and forwarded as BC_3F_2 and intermated F_2 populations (**Fig. 2.**).

Marker assisted selection (MAS) was followed in two BC_3F_2 and one intermated F_2 populations for drought yield QTLs. A total of 148 plants with different combinations of QTLs were identified using QTL linked SSR markers from three populations. These selected 148 BC_3F_2 and intermated F_2s were advanced as BC_3F_3 and intermated F_3, which were raised in progeny rows at TRRI, Aduthurai. From the plant phenotypic observations, 31 BC_3F_3 progenies out of 148 progeny rows were rejected as they were found to be further segregating for morphological traits. The remaining 117 progenies were found to be phenotypically uniform and homozygous. Five plants were selected from these 117 progenies and genotyped for drought yield QTLs. Seeds from the tagged plants were harvested and used for evaluation under drought as well as irrigated environments as Backcrossed Inbred Lines (BILs).

Fresh leaf samples were collected from progenies and parents and used for extracting DNA by modified CTAB protocol (Doyle and Doyle, 1990). Isolated genomic DNA was used for SSR genotyping. SSR genotyping was done by PCR (50 ng of template DNA, 2X PCR Mater-mix and 10µM primers) by using following thermal cycler program: one cycle of 95ºC for 5 min, 35 cycles of 95ºC for 30sec, 55ºC for 30 sec and 72ºC for 30 sec followed by final extension of one cycle at 72ºC for 10 min. PCR products

Table 1. Details of *qDTY* QTLs and their linked markers utilized in foreground selection

QTL	Donor	Chromo-some No	Marker interval (QTL region)	Linked marker	Position	Reference
$\mathit{qDTY}_{1.1}$	Apo	1	RM486–RM472 (34.95-37.88 Mb)	RM11928	37.58	Venuprasad *et al.* (2012)
$\mathit{qDTY}_{3.1}$	Apo	3	RM520-RM16030 (30.91-32.50)	RM520	30.91	Venuprasad *et al.* (2009)
$\mathit{qDTY}_{12.1}$	Way Rarem	12	RM28048-RM511 (14.11-17.40)	RM511	17.40	Bernier *et al.* (2007)



RM511 130bp qDTY12.1 RM520 247bp qDTY3.1 RM11928 280bp qDTY12.1

Fig. 1. Polymorphism between parents for peak SSR marker

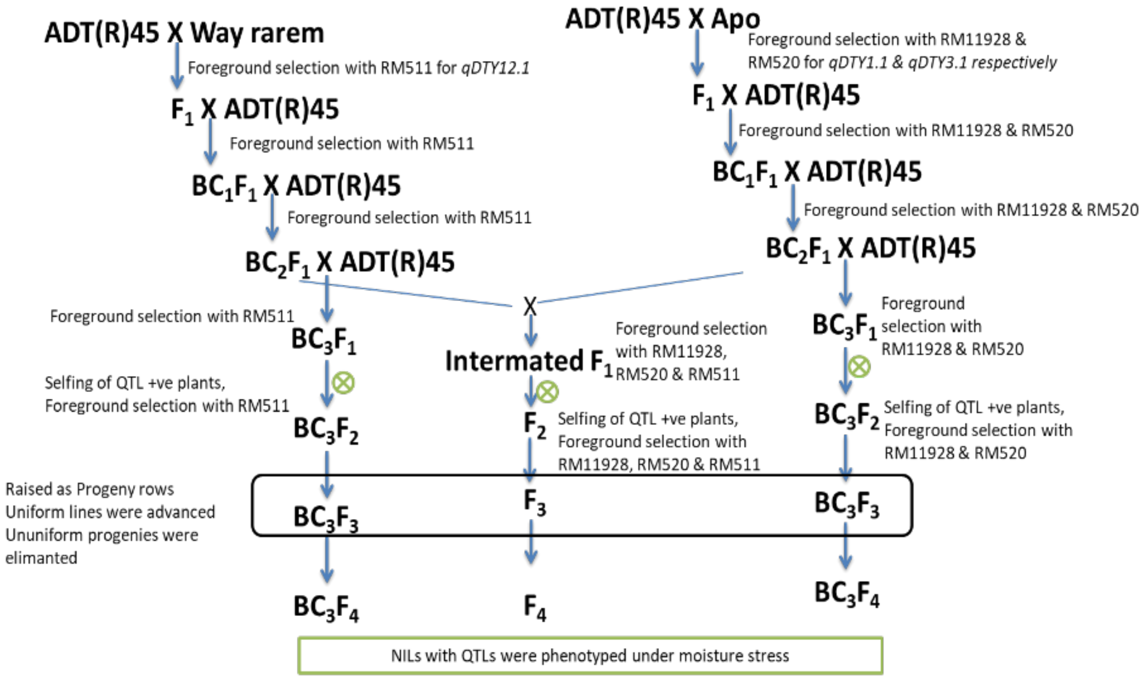


Fig. 2. Schematic representation of introgression of *qDTYs* into the background of ADT(R)45

were resolved by 3 % agarose gel electrophoresis, stained with Ethidium Bromide and visualized with UV trans-illuminator. Allelic pattern of each SSRs among the progenies was scored in comparison with the parents.

The 117 BILs along with parents were evaluated during *Rabi* (*Samba*), 2020-2021 at Agricultural Research Station, Paramakudi which is the target production environment, where rice is grown under rainfed ecosystem. The BILs were direct seeded in randomized block design with two replications. Seeds of each genotype were sown in three lines of 3-meter length with a row spacing of 20 cm and between the genotypes/plots 30 cm spacing was provided. First soaking rain was received on second day after sowing and germination was observed on 5-7th day after sowing. Two weeks after germination, thinning was carried out to provide a plant to plant spacing of 15 cm.The crop was grown completely under rainfed condition without any supplementary irrigation.

Eight biometrical traits *viz*., days to fifty per cent flowering, plant height (cm), panicle length (cm), number of productive tillers per plant, total number of tillers per plant, spikelet fertility percentage, hundred grain weight (g) and single plant yield (g) were recorded in five plants per entry per replication. Yield per hectare (t/ha) was worked out using the plot yield and plot size of entries. The variability parameters *viz*., mean, variance, standard deviation and standard error were worked out by adopting the standard methods by Panse and Sukhatme (1961) and analysis of variance was carried out using TNAUSTAT Statistical package (Manivannan, 2014). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were worked out by method suggested by Burton (1952), Heritability (h^2) in the broad sense was calculated according to Lush (1940) and Genetic advance as percentage of mean was carried out according to Johnson *et al*. (1955). Genotypic and phenotypic correlation coefficients were worked out using TNAUSTAT Statistical package as per the method suggested by Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

Analysis of variance for the nine traits indicated that variance due to genotypes were highly significant for all the traits studied (**Table 2**), which suggests that significant amount of variability is present in the study material. The mean values of the traits were used for analyzing genetic variability parameters like mean, range, standard deviation, phenotypic coefficient of variation (PCV), Genotypic coefficient of variation (GCV), heritability in broad sense (h^2) and genetic advance as percentage of mean (**Table 3**). PCV was found to be higher than that of GCV for all the traits studied. Based on Sivasubramanian and Menon (1973) classification, PCV and GCV was high only for total number of tillers per plant and moderate for number of productive tillers per plant, hundred grain weight, single plant yield and yield per hectare. However, the difference between PCV and GCV for the traits studied were negligible. Hence, it could be concluded that genotype played a strong role for the expression of these traits under rainfed condition, as influence of environment was minimum for the traits expression.

Heritability plays major role in the improvement of any trait (Dubey *et al*., 2019). Broad sense heritability was observed to be high for all the traits. The traits *viz.,* total number of tillers per plant, number of productive tillers per plant, hundred grain weight, yield per hectare and single plant yield recorded high genetic advance as percentage of mean as well as high heritability, which indicates the prevalence of additive gene action for these traits. Hence, selection of genotypes based on these traits may be effective (Hema *et al.,* (2019), Prasannakumari *et al.* (2020), Bhargava *et al.* (2021), Manivelan *et al.* (2022) and Devi *et al.* (2022).).

Genotypic and phenotypic correlation coefficients for all nine traits under moisture stress are given in **Table 4**. Single plant yield had high significant positive association with total number of tillers per plant, number of productive tillers per plant, panicle length and hundred grain weight. The result was in line with the report of Prisca Seeli *et al.* (2021), while, spikelet fertility was correlated significantly and positively with single plant yield. Hence, selection for these traits will indirectly increase yield under moisture stress. Due importance was given to these traits while comparing the performance of BILs in relation to QTL combination.

Table 2. Analysis of variance of RBD for various morphological traits under moisture stress

Source	Degrees of freedom	Mean sum of squares								
		Days to fifty per cent flowering	Plant height	Total number of tillers per plant	Number of productive tillers per plant	Panicle length	Spikelet fertility percentage	Hundred grain weight	Single plant yield	Yield per Hectares
Replication	1	0.5854	0.763	0.086	1.4985	0.0683	2.5815	0.0116	0.0018	0.2149
Genotypes	119	24.7493**	91.21**	18.9336**	7.9871**	5.5962**	91.499**	0.1739**	36.4747**	1.1971**
Error	122	3.028	0.6996	0.6654	0.5677	0.4348	2.1567	0.004	0.9108	0.2224

**significant at 1% level

Table 3. Variability parameters for various morphological traits under moisture stress

Parameters /Characters		Days to fifty per cent flowering	Plant height	Total number of tillers per plant	Number of productive tillers per plant	Panicle length	Spikelet fertility percentage	Hundred grain weight	Single plant yield	Yield per Hectares
Parents Mean	ADT(R)45	85.5	79.8	9.4	7.9	18.4	73.3	14.23	1.614	3.900
	Apo	94.0	100.5	12.3	10.4	23.3	83.4	21.54	2.515	4.767
	Way Rarem	90.5	87.1	12.2	7.8	26.7	82.5	26.03	2.301	5.867
BILs	Mean	90±1.23	81.2±0.59	14.5±0.58	11.0±0.53	20.9±0.47	77.5±1.04	22.6±0.67	2.253±0.04	4.789±0.35
	Range	81.0-100.0	68.5-101.9	8.0-24.8	5.8-18.0	16.9-25.3	55.5-91.0	13.46-33.88	1.547-2.935	3.066-7.033
Standard Deviation		13.65	6.56	6.40	5.91	5.17	11.52	0.50	7.48	3.70
Critical Difference (5%)		3.41	1.64	1.60	1.48	1.29	2.88	0.12	1.87	0.92
Coefficient of Variation (%)		1.93	1.03	5.65	6.90	3.14	1.89	2.82	4.23	9.84
Phenotypic Coefficient of Variation (PCV) %		4.14	8.33	21.67	18.93	8.28	8.83	13.25	19.17	17.60
Genotypic Coefficient of Variation (GCV)%		3.66	8.26	20.92	17.63	7.66	8.62	12.95	18.70	14.57
Heritability in broad sense (h^2) %		78.20	98.48	93.21	86.73	85.58	95.39	95.48	95.13	68.67
Genetic advance as percentage of mean (GAM) %		6.67	16.89	41.60	33.82	14.60	17.35	26.06	37.57	24.88

Table 4. Genotypic and phenotypic correlation coefficients for nine biometrical traits in BIL population under moisture stress

Traits	DFF	PH	TNT	NPT	PL	SF	HGW	YPH	SPY
DFF	**1.00**	0.18*	-0.12	-0.06	0.32**	0.03	0.10	0.19*	0.07
PH	0.20*	**1.00**	-0.32**	-0.26**	0.32**	0.13	0.15	0.35**	0.13
TNT	-0.13	-0.33**	**1.00**	0.84**	-0.08	-0.10	0.06	-0.24**	0.33**
NPT	-0.09	-0.28**	0.87**	**1.00**	-0.09	-0.08	0.09	-0.22*	0.35**
PL	0.41**	0.34**	-0.10	-0.13	**1.00**	0.13	0.19*	0.28**	0.30**
SF	0.05	0.13	-0.09	-0.06	0.15	**1.00**	-0.07	0.18*	0.17*
HGW	0.12	0.15	0.07	0.10	0.21*	-0.07	**1.00**	0.21*	0.36**
YPH	0.27**	0.43**	-0.30**	-0.33**	0.39**	0.23*	0.25**	**1.00**	0.15
SPY	0.10	0.14	0.35**	0.38**	0.32**	0.19*	0.37**	0.16	**1.00**

*Significant at 5% level, **significant at 1% level, Upper diagonal- Phenotypic correlation coefficients, Lower diagonal - Genotypic correlation coefficients

DFF- Days to 50% flowering, PH- Plant height, TNT- Total number of tillers per plant, NPT- Number of productive tillers per plant, PL- Panicle length, SF- Spikelet fertility percentage, HGW- Hundred grain weight, YPH- Yield per hectare and SPY- Single plant yield.

Trait wise mean values of BILs with different *qDTY* QTL combinations are tabulated in **Table 5**. Slight increase in duration for about 5 to 10 days was observed in BILs as compared to that of recurrent parent ADT(R)45. However, the BILs with all the three QLTs ($qDTY_{1.1}$+ $qDTY_{3.1}$+ $qDTY_{12.1}$) were statistically on par with ADT(R)45. BILs with $qDTY_{12.1}$ among single QTL BILs and BILs with $qDTY_{1.1}$ and $qDTY_{12.1}$ among double QTL combinations were on par with grand mean.

With a range of 81 to 100 days, skewness (0.37) and kurtosis (-0.19) the trait mean days to fifty per cent flowering of BILs showed normal distribution, which indicates that BILs might majorly falls around mean as parents ADT(R)45 (86) and Apo (94) falls on extreme ends of the distribution curve respectively. Bernier *et al.* (2007) reported the co-location of QTLs for days to flowering in the region of $qDTY_{12.1,}$ which indicates that the interaction of those QTLs may result in increased days taken for

Table 5. Trait wise mean values of BILs with different *qDTY* QTL combinations

Genotype	DFF	PH	TNT	NPT	PL	SF	HGW	SPY	YPH
ADT(R)45	86	79.8	9.4	7.9	18.4	73.3	1.614	14.23	3.900
Apo	94	100.5	12.3	10.4	23.3	83.4	2.515	21.54	4.767
Way Rarem	91	87.1	12.2	7.8	26.7	82.5	2.301	26.03	5.867
$qDTY_{1.1}$	93	86.4	13.9	10.8	21.9	77.0	2.227	21.31	5.560
$qDTY_{3.1}$	90	82.1	11.3	9.2	21.3	76.6	2.236	20.76	5.159
$qDTY_{12.1}$	89	79.4	15.7	11.8	20.5	77.0	2.239	23.44	4.515
$qDTY_{1.1}$+ $qDTY_{3.1}$	92	85.4	14.0	10.6	20.9	78.6	2.331	22.49	5.159
$qDTY_{1.1}$+ $qDTY_{12.1}$	91	76.6	13.4	9.6	21.4	82.5	2.448	21.94	4.600
$qDTY_{3.1}$+ $qDTY_{12.1}$	94	82.7	11.7	9.0	21.7	79.6	2.328	21.60	5.193
$qDTY_{1.1}$+ $qDTY_{3.1}$+ $qDTY_{12.1}$	88	86.1	12.1	8.8	22.4	79.6	2.267	20.38	5.100
C.D.	3.4	1.6	1.6	1.5	1.3	2.9	0.12	1.87	0.92
Overall mean	90.0	81.4	14.4	10.9	21.0	77.5	2.251	22.55	4.79

DFF- Days to 50% flowering, PH- Plant height, TNT- Total number of tillers per plant, NPT- Number of productive tillers per plant, PL- Panicle length, SF- Spikelet fertility percentage, HGW- Hundred grain weight, YPH- Yield per hectare and SPY- Single plant yield.

flowering in BILs with $qDTY_{12.1}$. Increased days for fifty per cent flowering in this study was in contradiction to earlier reports of reduce in days to flowering under drought stress in introgressed lines with QTLs from Apo under controlled stress (Valarmathi **et al**. (2019), Priyadarshini **et al.** (2018) and Venuprasad **et al.** (2011)). It indicates, differential interaction of drought QTLs with flowering QTLs under managed stress and rainfed environments.

Mean plant height of BILs ranged from 68.6 to 101.0 cm. BILs with $qDTY_{12.1}$ was shortest single QTL BIL with mean of 79.4 cm. The result may be due to the QTL $qDTY_{12.1}$ being co located with QTLs for plant height in its region (Vikram **et al**., 2016 and Bernier **et al**., 2007). Among the two QTL combinations, BILs possessing $qDTY_{1.1}$ and $qDTY_{12.1}$ were significantly shorter in plant height than ADT(R)45. The reduction in height may be due to the tight linkage between the QTL $qDTY_{1.1}$ and *sd1* gene (Lafitte **et al**., 2006 and Vikram **et al**., 2015).

Total number of tillers per plant was significantly high over parent ADT(R)45 in all the 7 QTL classes. Profuse tillering may also be the result of pleotropic effect of *sd1* gene as reported by Vikram **et al**. (2015). But none of the classes showed significant increase over grand mean. BILs with $qDTY_{12.1}$ recorded high number of tillers per plant than other QTL combinations. Among single QTL BILs, $qDTY_{1.1}$ and $qDTY_{12.1}$ BILs were on par with overall mean and possessed significantly high number of productive tillers than ADT(R)45. Among two QTL combinations, BILs with $qDTY_{1.1}$ and $qDTY_{3.1}$ and BILs with $qDTY_{1.1}$ and $qDTY_{12.1}$ registered increased number of productive tillers than recurrent parent. BILs with all the three QTLs ($qDTY_{1.1}$+ $qDTY_{3.1}$+ $qDTY_{12.1}$) were found to be on par with ADT(R)45 for this trait.

Mean panicle length of BILs were higher than ADT(R)45 and lesser than the donor parents. Similar intermediate mean values of panicle length for drought introgressed progenies were reported by Prisca Seeli **et al.** (2021). It may be due to the incomplete dominance of the alleles for this trait. BILs possess all the three $qDTY_{1.1}$, $qDTY_{3.1}$ and $qDTY_{12.1}$ registered significantly high mean value for panicle length than grand mean. All other BIL classes showed statistically on par with overall mean.

Mean spikelet fertility percentage of the BILs were higher than ADT(R)45 and lesser than that of donors. However, BILs possessing $qDTY_{1.1}$ and $qDTY_{12.1}$ were significantly higher than overall mean spikelet fertility percentage. The results revealed that both mega effect QTLs were maintained high spikelet fertility in rainfed condition. It is in concurrent with the earlier reports by Bernier **et al**. (2007) and Vikram **et al**. (2011).

BILs with $qDTY_{1.1}$ and $qDTY_{12.1}$ recorded significantly high hundred grain weight over grand mean. BILs with QTL combinations other than $qDTY_{1.1}$ and $qDTY_{12.1}$ were on par with overall mean and significantly high over recurrent parent ADT(R)45. It may be due to the boldness of the Apo donor parent. The result was in line with earlier reports by Priyadarshini **et al.** (2018).

The results showed that all the BILs classes registered significantly more yield than ADT(R)45 and on par with overall mean. Among single QTL BILs, those possessing $qDTY_{1.1}$ were high yielders. Among two QTL combinations, BILs with $qDTY_{1.1}$ and $qDTY_{3.1}$ and BILs with $qDTY_{3.1}$ and $qDTY_{12.1}$ registered high yield. All the BILs with different QTL combinations recorded significantly more single plant yield than ADT(R)45 and on par with overall mean.

Among single QTL BILs, BILs with *qDTY*$_{12.1}$ recorded more yield. BILs with *qDTY*$_{1.1}$ and *qDTY*$_{3.1}$ showed more grain yield per plant than other two QTL combinations. Surprisingly BILs with three QTLs (*qDTY*$_{1.1}$+ *qDTY*$_{3.1}$+ *qDTY*$_{12.1}$) recorded less single plant yield than other combinations in target production environment. This might be due to negative interaction between QTLs (Shamsudin *et al*., 2016, Vikram *et al*., 2016, Priyadarshini *et al.,* 2018, Sahebi *et al.,* 2018, Ćalić *et al.,* 2022). Contrastingly positive interaction of three QTLs was reported by Dixit *et al*. (2012).

Considering mean values of all the traits, among the single QTL BILs, those with *qDTY*$_{12.1}$ were relatively better than the ones possessing *qDTY*$_{1.1}$ and *qDTY*$_{3.1}$. However, BILs possessing *qDTY*$_{1.1}$ had higher yield per hectare than other two group of BILs. BILs with *qDTY*$_{1.1}$ and *qDTY*$_{12.1}$ performed comparatively better for most of the traits than that of BILs with other two *qDTY* QTL combinations. All the two *qDTY* QTL combinations had registered significantly high single plant yield than recurrent parent and on par with overall mean. Positive digenic interaction between two different *qDTY* QTLs was found to be beneficial in yield enhancement under moisture stress (Shamsudin *et al*. (2016), Vikram *et al*. (2016), Priyadarshini *et al*. (2018), Yadav *et al.* (2019), Sandhu *et al.* (2019), Anyaoha *et al.* (2019), Muthu *et al.* (2020), Ndikuryayo *et al.* (2023) and Raj and Nadarajah (2023). BILs with all the three QTLs surprisingly recorded less single plant yield than all other BILs.

Superior performance of BILs over susceptible parent ADT(R)45 can be explained by better values recorded by them for yield attributing characters like total number of tillers per plant, number of productive tillers per plant, panicle length, hundred grain weight and spikelet fertility which were highly significant associates with single plant yield under rainfed condition.

Days to fifty per cent flowering was affected by moisture stress and days to flowering was increased in BILs. The BILs having QTLs from donor, Apo, were found to be late in flowering than other BILs and recurrent parent, which might be due to physiological imbalance caused by drought stress. Delayed flowering due to moisture stress and panicle emergence under resurgence of favourable condition was earlier reported by Lilley and Fukai (1994) and Lafitte *et al*. (2006). The traits total number of tillers per plant and number of productive tillers per plant were found to be highly associated with grain yield under stress. Profuse tillering was observed in BILs which may be due to association of *sd1* gene with *qDTY*$_{1.1}$ (Vikram *et al*., 2015). The pleotropic effect of *sd1* on panicle number was reported by Murai *et al*. (2002). From the study, we can conclude that the traits total number of tillers per plant, number of productive tillers per plant, panicle length, hundred grain weight and spikelet fertility could be used for selection in moisture stress environment for enhancing yield under stress. The single QTL BILs with *qDTY*$_{12.1}$ were found to perform better under rainfed moisture stress condition. Similarly, Priyadarshini *et al.* (2018) found out that single QTL lines with *qDTY*$_{2.2}$ performed better than lines with two or three *DTY* QTLs. Venuprasad *et al*. (2009) also found that single QTL lines with *qDTY*$_{3.1}$ performed better under water deficit condition than combination of *qDTY* QTLs. All the BILs with combination of *qDTY* QTLs were on par with overall mean for yield, whereas showed varied performance for other traits. This indicates that addition of QTLs have not directly influence yield under stress as expected; this may be due to negative interaction between different *qDTY* QTLs and also due to linkage between other undesirable genes/QTLs present in the same region. Based on this study, it is concluded that *qDTY* QTLs with single or combination of two QTLs were most effective to ensure the yield under drought stress in the target production environment when compared to more than two QTL combinations.

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REFERENCES

Akte, J., Yasmin, S., Bhuiyan, M. J. H., Khatun, F., Roy, J. and Goswami, K. 2016. In vitro screening of rice genotypes using polyethylene glycol under drought stress. *Progressive agriculture*, **27**(2): 128-135. [Cross Ref]

Anyaoha, C.O., Fofana, M., Gracen, V., Tongoona, P. and Mande, S. 2019. Introgression of Two Drought QTLs into FUNAABOR-2 Early Generation Backcross Progenies Under Drought Stress at Reproductive Stage. *Rice Sci.,* **26**: 32–41. [Cross Ref]

Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. and Atlin, G. 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Science*, **47**(2): 507-516. [Cross Ref]

Bhargava, K., Shivani, D., Pushpavalli, S. N. C. V. L., Sundaram, R. M., Beulah, P. and Senguttuvel, P. 2021. Genetic variability, correlation and path coefficient analysis in segregating population of rice. *Electronic Journal of Plant Breeding*, **12**(**2**): 549-555. [Cross Ref]

Bin Rahman, A. N. M. and Zhang, J. 2022. Trends in rice research: 2030 and beyond. *Food and Energy Security*, p. e390. [Cross Ref]

Burton, G.W. 1952. Quantitative inheritance in grasses. *Pro VI Int Grassl Cong, 1952*, pp.277-283.

Ćalić, I., Groen, S. C., Choi, J. Y., Joly-Lopez, Z., Hamann, E., Natividad, M. A., Dorph, K., Cabral, C. L., Torres, R. O., Vergara, G. V., Henry, A., Purugganan, M. D. and Franks, S. J. 2022. The influence of genetic architecture on responses to selection under drought in rice. *Evolutionary Applications*, **15**: 1670–1690. [Cross Ref]

Devi, R, K., Hari, Y, Sathish Chandra, B. and Rajendra Prasad, K. 2022. Genetic association, variability and path studies for yield components and quality traits of high yielding rice genotypes (*Oryza sativa L.*). *International Journal of Bio-resource and Stress Management,* **13**(**1**): 81-92. [Cross Ref]

Dixit, S., Kumar, A. and Woldring, H. 2016. Paper 1 Water scarcity in rice cultivation: current scenario, possible solutions, and likely impact. *Regional: Development and Dissemination of Climate-Resilient Rice Varieties for Water-Short Areas of South Asia and Southeast Asia*, p.3.

Dixit, S., Swamy, B.M., Vikram, P., Ahmed, H.U., Sta Cruz, M.T., Amante, M., Atri, D., Leung, H. and Kumar, A. 2012. Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. *Theoretical and Applied Genetics*, **125**:155-169. [Cross Ref]

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation from Small Quantity of tissue. *Phytochem bul*, **19**:1-15.

Dubey, S. and Rangaiah, S. 2019. Broad sense and narrow sense heritability in F4 and F5 generations of finger millet, *Eleusine coracana* (L.) gaertn. *Electronic Journal of Plant Breeding*, **10**(1): 66-75. [Cross Ref]

Hema, T., Saravanan, S., Kannan, R., Shoba, D. and Pillai, M. A. 2019. Studies on genetic variability, association and path coefficient analysis in F_2 derivatives of CR1009× WP 22-2 for earliness and semi-dwarfism in rice (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, **10**(**2**): 585-591. [Cross Ref]

Johnson, H.W. Robinson, H.F and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soybean. *Agronomy Journal*. **47**:413-418. [Cross Ref]

Khush G.S. 2007. Rice breeding for 21st century. *Book on Science, Technology and Trade for Peace & Prosperity*. pp. 64-65.

Lafitte, H.R., Li, Z.K., Vijayakumar, C.H.M., Gao, Y.M., Shi, Y., Xu, J.L., Fu, B.Y., Yu, S.B., Ali, A.J., Domingo, J. and Maghirang, R. 2006. Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. *Field Crops Research*, **97**(1):77-86. [Cross Ref]

Lilley, J.M. and Fukai, S. 1994. Effect of timing and severity of water deficit on four diverse rice cultivars I. Rooting pattern and soil water extraction. *Field Crops Research*, **37**(3):205-213. [Cross Ref]

Lush, J.L. 1940. Intra-sire correlations or regressions of offspring on dam as a method of estimating heritability of characteristics. *Journal of animal science*, **33**(1):293-301.

Manivannan,N. 2014. TNAUSTAT-Statistical package. Retrived from https://sites.google.com/site/tnaustat

Manivelan, K., Juliet Hepziba, S., Suresh, R., Theradimani, M., Renuka, R. and Gnanamalar, R.P. 2022. Inherent variability, correlation and path analysis in lowland rice (*Oryza sativa* L.). *Biological Forum – An International Journal,* **14**(**2**): 771-778.

Murai, M., Takamure, I., Sato, S., Tokutome, T. and Sato, Y. 2002. Effects of the dwarfing gene originating from 'Dee-geo-woo-gen'on yield and its related traits in rice. *Breeding science*, **52**(2):95-100. [Cross Ref]

Muthu, V., Abbai, R., Nallathambi, J., Rahman, H., Sasikala, R., Kambale, R., Thulasinathan, T., Bharathi, A. and Raveendran, M. 2020. Pyramiding QTLs controlling tolerance against drought, salinity, and submergence in rice through marker assisted breeding. *PLoS ONE,* **15**(1): e0227421. [Cross Ref]

Ndikuryayo, C., Ndayiragije, A., Kilasi, N.L. and Kusolwa, P. 2023. Identification of drought tolerant rice (*Oryza Sativa* L.) genotypes with asian and african backgrounds. *Plants*. **12**(**4**):922. [Cross Ref]

Panse, V.G. and Sukhatme, P.V. 1961. *Statistical methods for agricultural workers, 2nd ed*. ICAR. New Delhi. 12-13.

Prasannakumari, M., Akilan, M., Kalaiselvan, S., Subramanian, A., Janaki, P. and Jeyaprakash, P. 2020. Studies on genetic parameters, correlation and path analysis for yield attributes and Iron content in a backcross population of rice [*Oryza sativa* (L.)]. *Electronic Journal of Plant Breeding*, **11**(**03**):881-886. [Cross Ref]

Prisca Seeli, F.D., Manonmani, S., Pushpam, R. and Raveendran, M. 2021. Parent progeny regression analysis in segregating generations of drought QTLs pyramided rice lines (*Oryza sativa* L.). *Electronic Journal of Plant Breeding*, **12**(4):1178-1188. [Cross Ref]

Priyadarshini, S.K., Raveendran, M., Manonmani, S. and

Robin, S. 2014. Effect of QTLs controlling grain yield under drought stress in the genetic background of ADT45 rice variety. *Indian Journal of Genetics and Plant Breeding*, **74**(03):374-377. [Cross Ref]

Priyadarshini, S.K., Raveendran, M., Manonmani, S. and Robin, S. 2018. Studies on early backcross generation inbred lines of rice (*Oryza sativa* L.) for yield under stress evaluated under different moisture regimes. *Journal of Pharmacognosy and Phytochemistry*, **7**(1S):1236-1243.

Raj, S.R.G. and Nadarajah, K. 2023. QTL and Candidate Genes: Techniques and Advancement in Abiotic Stress Resistance Breeding of Major Cereals. *Int. J. Mol. Sci.* **24**: 6. [Cross Ref]

Sahebi, M., Mohamed M., Hanafi, M. Y., Rafii, T. M. M. Mahmud, Parisa Azizi, Mohamad Osman, Rambod Abiri, Sima Taheri, Nahid Kalhori, M. Shabanimofrad, Gous Miah and Narges Atabaki. 2018. Improvement of drought tolerance in rice (*Oryza sativa* L.): genetics, genomic tools, and the WRKY gene family. *BioMed Research International*. [Cross Ref]

Sandhu, N., Dixit, S., Swamy, B.P.M., Raman, A., Kumar, S., Singh, S.P., Yadaw, R.B., Singh, O.N., Reddy, J.N., Anandan, A. and Yadav, S. 2019. Marker assisted breeding to develop multiple stress tolerant varieties for flood and drought prone areas. *Rice*, **12**(1): 1-16. [Cross Ref]

Shamsudin, N.A.A., Swamy, B.M., Ratnam, W., Sta. Cruz, M.T., Sandhu, N., Raman, A.K. and Kumar, A. 2016. Pyramiding of drought yield QTLs into a high quality Malaysian rice cultivar MRQ74 improves yield under reproductive stage drought. *Rice*, **9:**1-13. [Cross Ref]

Singh, A.S. and Chaudhary, B.D. 1977. Biometrical methods in Quaitative Genetic Analysis. *Kalyani Publ*., New Delhi. Pp. 54-63.

Sivasubramanian, S. and Menon, P.M. 1973. Genotypic and phenotypic variability in rice. *Madras Agric. J.*, **60**(9-12): 1093-96.

Swamy, B.P.M., Ahmed, H.U., Henry, A., Mauleon, R., Dixit, S., Vikram, P., Tilatto, R., Verulkar, S.B., Perraju, P., Mandal, N.P., Variar, M. and Robin, S. 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. [Cross Ref]

Valarmathi, M., Muthukumar, M., Rahman, H., Sasikala, R. and Raveendran, M. 2019. Development of early maturing, high yielding, drought tolerant rice variety with superior grain quality through molecular breeding and its performance evaluation. *Journal of pharmacognosy and phytochemistry*, **8**(2):338-346

Venuprasad, R., Bool, M.E., Quiatchon, L. and Atlin, G.N. 2012. A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds. *Theoretical and Applied Genetics*, **124**(2):323-332. [Cross Ref]

Venuprasad, R., Dalid, C.O., Del Valle, M., Zhao, D., Espiritu, M., Sta Cruz, M.T., Amante, M., Kumar, A. and Atlin, G.N. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics,* **120**(1):177-190. [Cross Ref]

Venuprasad, R., Impa, S.M., Gowda, R.V., Atlin, G.N. and Serraj, R. 2011. Rice near-isogenic-lines (NILs) contrasting for grain yield under lowland drought stress. *Field Crops Research*, **123**(1):38-46. [Cross Ref]

Vikram, P., Kadam, S., Singh, B.P., Lee, Y.J., Pal, J.K., Singh, S., Singh, O.N., Mallikarjuna Swamy, B.P., Thiyagarajan, K., Singh, S. and Singh, N.K. 2016. Genetic diversity analysis reveals importance of green revolution gene (*sd1* Locus) for drought tolerance in rice. *Agricultural research*, **5**:1-12. [Cross Ref]

Vikram, P., Swamy, B.M., Dixit, S., Ahmed, H.U., Teresa Sta Cruz, M., Singh, A.K. and Kumar, A. 2011. *qDTY* 1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC genetics*, **12:**1-15. [Cross Ref]

Vikram, P., Swamy, B.M., Dixit, S., Singh, R., Singh, B.P., Miro, B., Kohli, A., Henry, A., Singh, N.K. and Kumar, A. 2015. Drought susceptibility of modern rice varieties: an effect of linkage of drought tolerance with undesirable traits. *Scientific reports*, **5**(1):14799. [Cross Ref]

Yadav, S., Sandhu, N., Majumder, R.R., Dixit, S., Kumar, S., Singh, S.P., Mandal, N.P., Das, S.P., Yadaw, R.B., Singh, V.K. and Sinha, P. 2019. Epistatic interactions of major effect drought QTLs with genetic background loci determine grain yield of rice under drought stress. *Scientific Reports*, **9**(1):1-13. [Cross Ref]