



Development and evaluation of Pusa 44 backcross derived lines possessing *qDTY2.1*, *qDTY3.1* and their combinations indicate differential yield response under severe reproductive stage drought stress

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(Received: January 2021; Revised: April 2021; Accepted: May 2021)

Abstract

Reproductive stage drought stress (RSDS) tolerance in rice with higher productivity is very important in the context of climate change. With increasingly fluctuating rainfall distribution, maintaining higher productivity in the niche areas of rice production such as northern-western India is a challenge. Pusa 44 has been one of the most popular high yielding cultivars of northern-western India especially in the states of Punjab and Haryana. A medium duration rice variety with long slender grains, Pusa 44 is highly sensitive to drought and can lose up to 65% yield when exposed to the unexpected RSDS. To address this challenge, marker-assisted backcross breeding was adopted to introgress two QTLs governing yield under RSDS namely, *qDTY2.1* and *qDTY3.1* from the donors, IR81896-B-B-195 and IR81896-B-B-142 possessing both the QTLs. Through marker assisted foreground and phenotypic selection several backcross derived inbred lines (BILs; BC₃F₄) were developed, out of which Pusa 1823-12-31-12-12 (*qDTY3.1*), Pusa 1823-12-62 (*qDTY2.1+qDTY3.1*), Pusa 1823-12-82 (*qDTY2.1+qDTY3.1*), Pusa 1823-12-55 (*qDTY2.1+qDTY3.1*), and Pusa 1969-13-21-10-24 (*qDTY2.1*) were found to significantly out-yield Pusa 44 to the tune of 2.0 to 2.5 times under RSDS. Lines possessing *qDTY2.1* were found relatively less stable for drought response, than those possessing both the QTLs. These improved BILs are important genetic resource for further genetic studies as well as donors for improving rice yields under RSDS.

Key words: Drought stress tolerance, *qDTY2.1*, *qDTY3.1*, marker assisted backcross breeding, tolerance indices, climate resilience

Introduction

Among the top rice cultivating ASEAN countries of the world, drought stress is emerging as one of the major challenges for rice cultivation in nine of them namely China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines and Pakistan. Of the current total world rice production of 495.49 million tons, with an average productivity of 4.55 t.ha⁻¹, 65% is constantly under the threat of yield loss due to the unfavourable rainfall distribution during the rice-growing seasons. Several drought-prone rice environments exist in these nations, that are constantly challenged by the vagaries of rainfall fluctuations and high temperature. Populated by high rice consuming people, rice production is vital to food security of these nations. Therefore, to augment targeted rice varietal development programme and to sustain rice production, it is essential to improve the drought endurance of cultivars through systematic breeding using strategies such as marker-assisted backcross breeding (Fisher et al. 2003; Duan and Cai 2012).

Pusa 44 is one of the high yielding rice cultivars of north-western India particularly popular in the states of Punjab and Haryana. Pusa 44 is a high input responsive variety with wide adaptation, having medium to long duration and high yielding potential. Popular for its amenability to mechanical harvesting

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

by the virtue of its sturdy stem, Pusa 44 also possesses long slender grains with a translucent appearance and good cooking quality. Being a cultivar better suited to irrigated and nutrient-rich environments, Pusa 44 suffers greatly under unfavourable conditions such as drought-prone environments. It is highly sensitive to drought stress, particularly during the reproductive stage. Since this cultivar possesses several desirable attributes including high yield, development of Pusa 44 rice with the drought stress-tolerance is a potential solution to combat the unpredictable weather situations that may arise in the event of climate change. Plant type of a futuristic rice cultivar should possess high tillering ability, longer panicle, high number of filled grains per panicle, medium plant height, deep root system, good seedling vigour, higher germination and good emergence rate (Oo et al. 2021). Pusa 44 possess several of these features that make it an ideal candidate for breeding for tolerance to drought. Using an ideal cultivar aids in development of good drought tolerant plant types that can be targeted for both upland and lowland environments (Dixit et al. 2014). Further, it would also help in extending the cultivation of high yielding variety such as Pusa 44 across areas that are drought-prone.

Rice plant possesses several genetic attributes that can articulate drought tolerance in a cultivar. Several quantitative trait loci (QTLs) associated with grain yield under drought stress have been mapped in rice mostly derived from drought tolerant landraces. Two such QTLs are *qDTY2.1* and *qDTY3.1* reported by Venuprasad et al. (2009) on chromosome 2 and chromosome 3, respectively. Several physiological parameters related to drought stress have been recognised to be associated with *qDTY2.1* and *qDTY3.1* such as lower leaf canopy temperature, cell membrane stability, higher relative water content, dark-green leaf colour, higher total chlorophyll content, higher photosynthesis rate, thick leaf, waxy layer and anthocyanin pigmentation. These traits might be linked to drought-responsive proteins and plant growth hormones such as abscisic acid (ABA), gibberellic acid (GA) etc. (Farooq et al. 2010; Gao et al. 2011; Åebela et al. 2018). Several breeding lines have been developed carrying these QTLs, specifically under elite backgrounds. IR81896-B-B-142 and IR81896-B-B-195 possessing the QTLs, *qDTY3.1* and *qDTY2.1* have been developed at International Rice Research Institute (IRRI). In this study, we have used IR81896-B-B-142 and IR81896-B-B-195 as QTL donors for developing improved Pusa 44 lines with reproductive stage

drought stress (RSDS) tolerance and higher yield potential that can be grown under drought-prone rice environments.

Materials and methods

The plant materials used in the present study includes, Pusa 44 as the recurrent parent for improving tolerance to RSDS, for which it shows high sensitivity. The donors used were IR81896-B-B-195 and IR81896-B-B-142 possessing the QTLs, *qDTY2.1* and *qDTY3.1* respectively, which are stable drought-tolerant genotypes with high grain yield. The breeding scheme adopted for introgression of the QTLs through marker assisted backcross breeding is given in Fig. 1. Both



Fig. 1. Breeding scheme for the development of Pusa 44 *qDTY* mono-QTL backcross derived lines through selfing of BC₃F₁ plants. Each generation was subjected to marker-assisted foreground selection. The numbers in the circles indicate final selections at each step

IR81896-B-B-142 and IR81896-B-B-195 are lines derived from the crosses Apo/Swarna*2 (Venuprasad et al. 2009; Fischer et al. 2012; Shamsudin et al. 2016). Two parallel backcross breeding programmes with the pedigree, Pusa 44/ IR 81896-B-B-142// Pusa 44*3 and Pusa 44/ IR 81896-B-B-195// Pusa 44*3 were used. The breeding scheme for developing the di-QTL lines carrying both *qDTY2.1*+*qDTY3.1* is provided in Dwivedi et al. (2021). The lines developed and used in the present study included a combination of near-isogenic lines (NILs) having a background recovery of more than 90% and backcross derived inbred lines (BILs) having background recovery of more than 85%, but lower than 90%. However, for uniformity, we address all the lines as backcross derived lines throughout this paper. All the plant materials were raised in the field following standard agronomic practices. Selective evaluation of drought response was carried out using controlled conditions in the field.

Marker Assisted Selection

For foreground selection of the target QTLs, SSR markers, RM521 and RM520 tightly linked to *qDTY2.1* and *qDTY3.1*, respectively, were used. Prior to the start of the experiment, validation of both the markers was done to confirm the polymorphism between the parents and were then used for effecting foreground selection in every backcross generation. Additionally, a genome-wide polymorphism survey was conducted between Pusa 44 and the donor parents IR81896-B-B-142 and IR81896-B-B-195 using 850 and 846 SSR markers respectively to identify correspondingly 72 and 67 polymorphic SSR markers. These markers were used for background selection as well as in estimating the recurrent parent genome recovery (RPGR) in the backcross derived lines.

Development of Pusa 44 mono- and di-QTL backcross derived lines

Pusa 44 was crossed with IR81896-B-B-142 during *Kharif* 2012 and with IR81896-B-B-195 during *Kharif* 2013, at the Genetics field of ICAR-Indian Agricultural Research Institute (ICAR-IARI), Pusa Campus, New Delhi. Pusa 44 was used as the female parent. The crosses were designated as Pusa 1823 for the former and Pusa 1969 for the latter. Development of backcross derived lines followed marker assisted backcross breeding (MABB) strategy, which involved foreground, phenotypic and background selections. From the cross, Pusa 1823, near-isogenic lines (NILs) were developed as described by Dwivedi et al. (2021). In the second population, Pusa 1969 F_1 s were field-grown during the ensuing season, *Rabi* of 2013-14, to identify true F_1 s using foreground markers, RM521 and RM520. True F_1 was chosen for backcrossing to the recurrent parent, Pusa 44 to develop BC_1F_1 generation. BC_1F_1 s carrying *qDTY2.1* heterozygote was identified using the marker, RM521. One of the BC_1F_1 s having the target QTL was selected for backcrossing with Pusa 44 to develop BC_2F_1 generation. Similarly, BC_2F_1 possessing *qDTY2.1* was further backcrossed to Pusa 44 to develop BC_3F_1 progeny. Phenotypic selection for agronomic performance on par or better than Pusa 44 was also

carried out. Selected BC_3F_1 plants possessing *qDTY2.1* were subjected to pedigree selection in successive generations to develop BC_3F_4 generation. The selection was exercised on the progenies at every selfing generations, to maximise recurrent parent phenome, while fixation of the target QTL was done during the BC_3F_2 stage. Background selection was carried out at BC_3F_4 stage to estimate the recovery of RPG (%).

Screening of Pusa 44 BILs for drought stress response

At BC_3F_4 generation, one set of Pusa 44 NILs carrying *qDTY2.1*, *qDTY3.1* and *qDTY2.1+qDTY3.1* were field-grown under both the drought stress and irrigated conditions, in New Delhi during *Kharif* 2018. Drought stress treatment was initiated at two weeks into the vegetative stage and continued throughout the reproductive stage. Soil moisture was monitored continuously with tensiometers from the imposition of drought stress and life saving irrigation was given when the soil moisture level fell below -70 kPa. No irrigation was provided during the reproductive stage. The irrigated plot was compared with drought screening plot as the standard check. The screening trial was laid out using a randomised complete block design with two replications. The trial was carried out with all the recommended agronomic management except for irrigation. The drought imposed (stressed) and irrigated (unstressed) plots were evaluated for various agronomic characters such as days to 50% flowering, plant height, tillers per hill and panicle length at the time of physiological maturity of the plants, along with yield and yield related traits such as grains per panicle and spikelet fertility. Days to maturity was recorded on the harvest date. These parameters were further used for the evaluation of drought stress response among the NILs. Additionally, the stress response of the NILs was evaluated using yield related indices such as stress tolerance (TOL), geometric mean productivity (GMP), stress susceptibility index (SSI), stress-tolerance index (STI), harmonic mean (HM), yield index (YI) and yield susceptibility index (YSI), as described by Ghobadi et al. (2012). Different indices were computed using the following formulae.

Relative stress tolerance (TOL)	=	$\frac{(Y_p - Y_s) \times 100}{Y_p}$	Rosielle and Hamblin (1981) (modified)
Geometric mean productivity (GMP)	=	$\sqrt{Y_p \times Y_s}$	Fernandez (1992)
Stress susceptibility index (SSI)	=	$\frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{Y_s}{\bar{Y}_p}}$	Fischer and Maurer (1978)

Stress tolerance index (STI)	=	$\frac{Y_p \times Y_s}{\bar{Y}_p^2}$	Fernandez (1972)
Harmonic mean (HAM)	=	$\frac{2 \times Y_p \times Y_s}{Y_p + Y_s}$	Kristin et al. (1997)
Yield stability index (YSI)	=	$\frac{Y_s}{\bar{Y}_p}$	Bousslama and Schapaugh (1984)
Yield index (YI)	=	$\frac{Y_s}{\bar{Y}_s}$	Lin et al. (1986)

Where Y_p and Y_s are the grain yield of each genotype respectively under unstressed and stressed conditions. \bar{Y}_p and \bar{Y}_s are the average grain yield of all the genotypes under unstressed and stressed conditions, respectively.

Data analyses

The RPG recovery was computed (Singh et al. 2018) from the background marker data, that showed gaining of Pusa 44 alleles among the BILs after selection. Graphical depiction of RPG recovery was carried out using the software Graphical GenoTypes 2.0 (Van Berloo 2008). The agronomic data were analysed for genetic variability and relative response parameters using standard computations. Statistical analyses including analysis of variance were carried out using the design analysis software, STAR (Version 2.0.1 2014). Rest of the analyses was conducted under the R statistical environment.

Results

Marker-assisted development of Pusa 44 BILs

The polymorphism survey for background diversity between the recurrent parent, Pusa 44 and the two donors, revealed that 72 markers were polymorphic between IR81896-B-B-142 out of 850 markers surveyed, and 67 markers polymorphic between IR81896-B-B-195 out of 846 markers surveyed (Supplementary Fig. S1). In the case of Pusa 1823, more markers were used for polymorphism survey than reported by Dwivedi et al. (2021). The hybridization between Pusa 44 and IR81896-B-B-195 initially resulted in 100 F_1 seeds. By the marker-based hybridity testing using the foreground markers RM521, twenty true F_1 s were identified possessing drought-tolerant QTL, $qDTY2.1$. Of these, ten plants were backcrossed to the recurrent parent, Pusa 44 to develop BC_1F_1 progenies. Of these, 100 BC_1F_1 plants were raised, and the foreground selection identified 24 true BC_1F_1 plants. Based on the phenotypic observations for resemblance to Pusa 44, one of the BC_1F_1 s, Pusa 1969-13 was backcrossed to Pusa 44

in the next cycle of backcross. Of the 75 BC_2F_1 progenies grown, sixteen true BC_2F_1 s were identified through foreground selection which were heterozygous for $qDTY2.1$. Through phenotypic selection one of the BC_2F_1 's, Pusa 1969-13-21, was backcrossed to Pusa 44 to generate BC_3F_1 seeds. About 80 BC_3F_1 progenies were field-grown, and among which 13 were found positive for the QTL, $qDTY2.1$. One among these, Pusa 1969-13-21-10, which was phenotypically similar to Pusa 44 was selected and selfed to generate 240 BC_3F_2 seeds. Of the 200 BC_3F_2 plants grown in the field, foreground selection resulted in the identification of 25 plants possessing $qDTY2.1$ donor allele in the homozygous state. All these lines were self-pollinated to generate 220 BC_3F_3 progenies belonging to 25 families. Phenotypic selection among the BC_3F_3 plants helped in identification of 35 desirable progenies with the higher resemblance to Pusa 44. These lines were further selfed to generate 175 BC_3F_4 progenies. The entire selection scheme is presented in Fig. 1. Background recovery analysis on superior 41 BC_3F_4 plants has found to have resulted in an RPGR of 86.6% to 98.6% among the progenies (Table 2). Among these, the best five lines in terms of yield performance, and background recovery were used in screening for drought response.

Similarly, in the other cross, one of the BC_3F_1 lines, Pusa 1823-12 was used to select stable $qDTY$ carriers. Foreground selection was carried out using SSR marker, RM520 for selecting $qDTY3.1$ in successive backcrosses. From subsequent selfing of the BILs from the BC_3F_3 generation, Pusa 1823-12-31-12 carrying a single QTL, $qDTY3.1$ were generated. Further, advancement from the selected line has culminated in three near isogenic lines (NILs) that were used for the drought response screening. One of these lines, Pusa 1823-12-31-12-12 having a background recovery of 95% was further used for agronomic evaluation. Additionally, four di-QTL carrying NILs possessing both $qDTY2.1$ and $qDTY3.1$ were developed from a parallel breeding scheme of the same cross (Dwivedi et al. 2021). Five BILs possessing

qDTY2.1 alone, one BIL possessing *qDTY3.1* alone and four BILs with both *qDTY2.1* and *qDTY3.1* with an RPG recovery ranging from 86.6 to 99.1% were included in this study, for comparative phenotypic evaluation. The graphical genotypes of the recovered BILs using both the crosses are presented in Fig. 2.

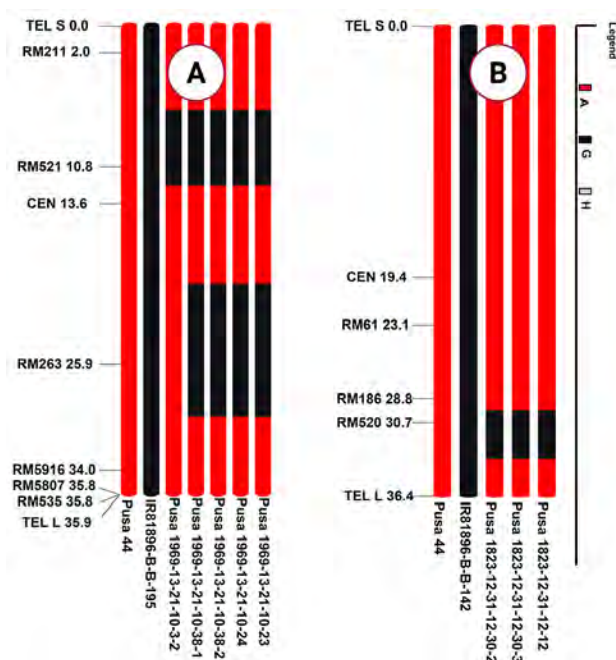


Fig. 2. Graphical depiction of marker-based chromosomal segments recovered from the Pusa 44 *qDTY* BILs on the respective carrier chromosome in BC_3F_4 generation, from the crosses (A) Pusa 1969 (Pusa 44*3//Pusa 44/IR81896-B-B-142) carrying *qDTY2.1* and (B) Pusa 1823 (Pusa 44*3//Pusa 44/IR81896-B-B-195) carrying *qDTY3.1*

The agronomic response of Pusa 44 *qDTY* BILs under varying growth conditions

The evaluation of Pusa 44 *qDTY* BILs, under irrigated (unstressed) and managed-drought (stressed) conditions, revealed significant variation between the BILs and Pusa 44 (Table 1). Flowering date of BILs was significantly variable for seasonal effect (years), genotype effects and for genotype x year interactions, under both stressed and unstressed conditions. Plant height under drought stress among the BILs was not significantly different between years, but it was significantly varying among the genotypes and also for the interaction of seasons and genotypes. Under unstressed conditions, however, plant height was significantly varying between genotypes, seasons, and their interactions. Effective tillers per hill were

significantly affected by seasons, genotypes and their interactions under stress. Whereas, under unstressed, tiller number per hill showed no significant influence by seasons, genotypes and their interactions. Similar to plant height, except for year wise variation, panicle length also indicated significant variation for all the sources of variation, both under stressed and unstressed treatments. BILs also showed non-significant yield variation under drought across years. However, it varied significantly between two years under unstressed conditions. Moreover, the genotype effect was significantly varying across both the stressed and unstressed conditions. There was also a significant variation for year x genotype interaction effects under drought and unstressed treatments (Table 1).

Response under drought

The average agronomic performance of BILs across two years indicated agreement on the response pattern for a few traits (Table 2). Under drought conditions, there was a strong agreement between two years for days to 50% flowering and plant height, while under irrigated conditions, only plant height showed agreement between the two seasons. Most of the mono- and di-QTLs were stable and higher yielding than Pusa 44 under stress condition during both the seasons. The grain yield of Pusa 44 under drought stress was 61.33 g.m^{-2} during the first season, which was reduced to 46.67 g.m^{-2} in the second season. In 2018, rice grain yield of mono-QTL BILs possessing, *qDTY2.1* and *qDTY3.1* namely, Pusa 1969-13-21-10-24 and Pusa 1823-12-31-12-30-2 were 182.22 g.m^{-2} and 175.23 g.m^{-2} which, however, was found significantly reduced in the next season. Di-QTLs BILs namely, Pusa1823-12-62 and Pusa1823-12-55 produced 151.26 g.m^{-2} and 120.14 g.m^{-2} in *kharif* 2018, respectively and those lines also significantly higher yields of 164.75 g.m^{-2} and 161.33 g.m^{-2} during *kharif* 2019. All the BILs outperformed Pusa 44 with significantly higher yield and with stable performance under drought stress during both the years. The yields of the BILs under stress taking into consideration the QTL classes shows that the average grain yield of BILs possessing *qDTY2.1* was 114.97 g.m^{-2} and 98.73 g.m^{-2} during 2018 and 2019, respectively. In similar terms, the *qDTY3.1* BIL yielded 175.24 g.m^{-2} and 152.87 g.m^{-2} during consecutive years. On the other hand, the di-QTL BILs possessing *qDTY2.1+qDTY3.1* yielded 128.61 g.m^{-2} in 2018, while producing 139.88 g.m^{-2} in 2019. By and large, a fall in the grain yield has been noticed under drought during 2019 season, which was

Table 1. Combined Analysis of variance among the Pusa 44 backcross derived lines carrying mono and di- QTLs of *qDTY2.1* and *qDTY3.1* under irrigated (US) and drought stress (S) during 2018 and 2019 seasons.

Traits	Treatment	Year	Genotype	Genotype x Year	CV%	CD (5%)
Days to 50% flowering	S	82.68*	167.82**	56.50**	1.79	3.57
	US	123.52**	92.23**	77.70**	0.90	1.84
Plant height (cm)	S	0.53 ^{ns}	261.24**	148.99**	6.73	10.4
	US	1273.59**	742.14**	91.97**	3.39	6.01
Tillers/ hill	S	229.90**	3.71**	5.80**	6.46	1.42
	US	55.27 ^{ns}	10.08 ^{ns}	8.12 ^{ns}	17.8	2.63
Panicle length (cm)	S	3.97 ^{ns}	7.61**	5.40**	6.27	2.85
	US	42.00**	4.47**	3.06**	4.32	1.97
Grain yield/ plant (g)	S	0.03 ^{ns}	27.14**	10.09**	8.16	5.41
	US	16.11*	15.54**	8.09**	4.52	4.19

*,** Significant at 0.05 and 0.01 probability level, respectively; ns = non-significant; S = stressed; US = unstressed; CV = coefficient of variation; CD = the critical difference at 5% level probability, by Tukey's honestly significant test

more apparent among the *qDTY2.1* BILs except Pusa 1969-13-21-10-23. However, in *qDTY* BILs possessing *qDTY3.1* and *qDTY2.1+qDTY3.1* were seen as almost yield stability across the seasons.

For the other agronomic traits, the average performance of BILs under drought, for days to 50% flowering indicated that they ranged between 84.0 and 110.5 days as against 96.0 days of Pusa 44 during the first season, and between 86.5 and 105.0 days as against 90.5 days in the second season. Plant height of *qDTY* BILs ranged from 62.00 cm (Pusa 1969-13-21-10-23) to 96.25 cm (Pusa 1969-13-21-10-3), both *qDTY2.1* BILs. Plant height of Pusa 44 was 76.00 cm under drought condition during 2018 season, while it was 69.30 cm during 2019. During 2019, under drought, plant height of BILs ranged between 63.95 cm (Pusa 1969-13-21-10-3) and 97.83 cm in Pusa 1823-12-31-12-12. Similarly, panicle length of BILs ranged between 16.00 cm to 23.75 cm, while Pusa 44 had panicles of length 23.12 cm during 2018. There was no significant variation in panicle length during 2019 among the BILs and parents. Effective tillers per hill also indicated variation during both the seasons. With a range from 6.87 to 12.5 during 2018 season, tiller numbers of the *qDTY* BILs were better than Pusa 44, except for two di-QTL BILs, Pusa 1823-12-14 and Pusa 1823-12-55. Whereas, during 2019, except one *qDTY2.1* BIL, the effective number for tillers per hill was higher than that of Pusa 44 (11.50) and ranged between 11.9 to 15.5.

Response under irrigation

The grain yield of Pusa 44 under the unstressed

condition wherein the plants were sufficiently irrigated varied between 763.0 g.m⁻² and 782.5 g.m⁻² during 2018 and 2019, respectively. As in the case of drought stress treatment, a slight fall in the average performance of Pusa 44 was observed under 2018. In 2018, rice grain yield of BILs such as Pusa 1969-13-21-10-23 (*qDTY2.1*) was observed to be the highest with 855.5 g.m⁻² followed by Pusa 1969-13-21-10-24 (*qDTY2.1*) with 832.5 g.m⁻². All the BILs were either on par or marginally superior to Pusa 44 during this season. In 2019 season, Pusa 1823-12-31-12-12 (*qDTY3.1*) performed best with a grain yield of 971.0 g.m⁻², followed by Pusa 1969-13-21-10-23 (*qDTY2.1*) with 937.0 g.m⁻² of grain yield. During this year too, all the BILs either performed better than or on par to Pusa 44. Besides, these BILs were found to perform similarly for other agronomic traits such as plant height, effective tillers per hill, panicle length in both the seasons (Table 2).

Under the unstressed condition, average grain yield of the mono-QTL BILs carrying *qDTY2.1* varied between 804.5 g.m⁻² and 880.13 g.m⁻², during 2018 and 2019, respectively. Corresponding grain yield for the *qDTY3.1* BIL under the two seasons was 755.5 g.m⁻² to 971.0g g.m⁻², while the di-QTL BILs, produced 733.38 g.m⁻² to 879.0 g.m⁻² of grain yield. As observed under drought, when compared to 2018, there is no reduction in grain yield observed during 2019 under irrigated conditions (Table 2).

The agronomic performance of the BILs was significantly better than Pusa 44 under drought stress. Height of the plants during 2018 season, among the

qDTY BILs ranged from 61.00 cm (Pusa 1969-13-21-10-23) to 96.25 cm (Pusa 1969-13-21-10-3), both *qDTY2.1* BILs. During 2019, however, plant height varied between 79.10 cm (Pusa 1823-12-55) and 105.10 cm (Pusa 1969-13-21-10-38). In both the years, the average height of Pusa 44 plants was 80.87 cm and 79.70 cm, respectively. Although there was some variation in the length of the panicle during 2018, no significant difference for this trait was observed in 2019. During 2018, Pusa 44 had a panicle length of 20.87 cm, while that among the BILs ranged between 16.75 cm and 22.87 cm. The effective number of tillers per hill among the BILs were on par with Pusa 44 in both the seasons.

Drought tolerance indices

Seven indices related to drought response were used for constructing the tolerance pattern of the BILs vis-a-vis parents (Table 3), and their interrelations indicated that all the indices showed a high degree of concurrence between the two seasons (Table 4). During 2018, the relative stress tolerance (TOL) of the BILs ranged between 76.81 and 90.49 as against the TOL of 91.96 for Pusa 44. During 2019, TOL ranged between 80.03 to 92.19 among the BILs, with Pusa 44 having TOL of 94.04. In both the seasons, high TOL values were shown by Pusa 44. Geometric mean productivity (GMP) of *qDTY* BILs ranged from 235.99 (Pusa 1969-13-21-10-38) to 389.48 (Pusa 1969-13-21-10-24) in 2018 GMP of Pusa 44 was 216.32 during 2018, while it was 191.1 during the second season (2019). During 2019, all the BILs had GMP higher than that of Pusa 44, with high statistical difference. The stress susceptibility index (SSI) of the BILs during 2018 varied from 0.90 in Pusa 1823-12-31-12-12 to 1.06 in Pusa 1969-13-21-10-23 and Pusa 1969-13-21-10-38 as against 1.08 of Pusa 44. Whereas in 2019 season, SSI among the BILs was lowest in Pusa 1823-12-62 (0.91) followed by 0.94 in Pusa 1823-12-55. Highest SSI among the BILs was recorded in Pusa 1969-13-21-10-38 (1.05), while Pusa 44 was having an SSI of 1.07. Stress tolerance index (STI) during 2018, ranged from 0.09 in Pusa 1969-13-21-10-38 to 0.25 in Pusa 1969-13-21-10-24 while that of Pusa 44 was 0.07. A similar trend was observed in 2019, with the recording of highest STI in Pusa 1823-12-31-12-12 (0.20) followed by Pusa 1823-12-55 (0.19) and Pusa 1823-12-62 (0.18). During this season, Pusa 44 recorded an STI of 0.04. The harmonic mean (HAM) of the BILs was highest in Pusa 1969-13-21-10-24 (298.99) during 2018 and lowest in Pusa 1969-13-21-10-38 (134.25). Pusa 44 had a HAM of 113.53 during

2018, and 88.08 during 2019. However, among the BILs, Pusa 1823-12-62 recorded the highest HAM of 274.65 followed by Pusa 1823-12-55 (274.43). Two yield indices, yield index (YI) and yield stability index (YSI) were used to emphasize the yield under drought conditions. Among the BILs, during 2018, YI ranged from 1.55 in Pusa 1969-13-21-10-24 to 0.63 in Pusa 1969-13-21-10-38, only to be followed by Pusa 44 having the lowest YI of 0.52. Whereas, during 2019, BILs with best YI values were Pusa 1969-13-21-10-24 (1.54) followed by Pusa 1823-12-31-12-12 (1.48), Pusa 1823-12-82 (1.29) and Pusa 1823-12-62 (1.28). Pusa 1969-13-21-10-38, however, recorded the lowest YI value of 0.58, just above that of the recurrent parent, Pusa 44 (0.52). The seventh index, yield susceptibility index (YSI) also showed a similar trend. In 2018, a maximum value of 0.23 for YSI was recorded in Pusa 1823-12-31-12-12, followed by 0.22 in Pusa 1823-12-62. While during 2019, highest YSI of 0.19 was recorded in Pusa 1823-12-62 followed by Pusa 1823-12-55 (0.17). YSI values for Pusa 44 during 2018 and 2019 seasons, were 0.08 and 0.05, respectively (Table 3).

The interrelations between drought tolerance indices (Table 4) indicated high significance for all the data pairs. Except for TOL and SSI, the indices showed a similar pattern for identifying tolerance and sensitive genotypes, while TOL and SSI showed an opposite pattern. These parameters indicated tolerance when the index value was the lowest, while the high value indicated sensitive behaviour of the BILs. For the remaining indices, lower values suggested sensitivity and higher values the tolerance.

Comparing the overall pattern of drought response among the BILs and Pusa 44 (Fig. 3), it is

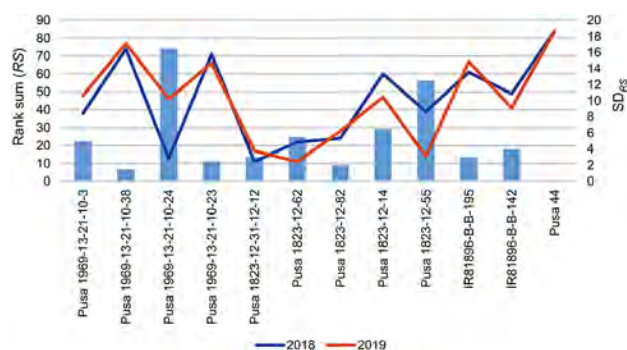


Fig. 3. Mean rank of Pusa 44 *qDTY* backcross derived lines under stress and unstress across the seasons. Blue line represents 2018, red line represents 2019 and the blue bar denotes standard deviation across the seasons

Table 2. Average agronomic performance of Pusa 44 mono- and di- QTL backcross derived line carrying *qDTY2.1* and *qDTY3.1* under drought stress (S) and irrigated (US) conditions during wet seasons of 2018 and 2019

Pedigree	<i>qDTY</i>	DTH		PTH		PNL		TPH		YLD		RPGR %
		2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	
Under drought stress conditions (Stressed)												
Pusa 1969-13-21-10 - 3	2.1	84.00 ^c	98.00 ^{bc}	96.25 ^a	63.95 ^c	22.75 ^a	20.44 ^a	7.50 ^{bc}	10.70 ^d	122.61 ^c	102.87 ^{de}	98.59
Pusa 1969-13-21-10 - 38	2.1	112.00 ^a	97.50 ^{bc}	79.00 ^{a-d}	76.48 ^{bc}	19.37 ^{ab}	20.97 ^a	8.50 ^{bc}	12.50 ^{cd}	73.67 ^{ef}	64.94 ^{fg}	88.03
Pusa 1969-13-21-10 - 24	2.1	96.00 ^b	95.50 ^{cd}	67.37 ^{cd}	74.44 ^{bc}	19.75 ^{ab}	22.22 ^a	8.50 ^{bc}	13.40 ^{bc}	182.22 ^a	104.89 ^{de}	86.62
Pusa 1969-13-21-10 - 23	2.1	113.00 ^a	105.00 ^a	62.00 ^d	75.21 ^{bc}	16.00 ^b	22.49 ^a	12.50 ^a	12.60 ^{cd}	81.38 ^{ef}	86.21 ^{ef}	90.14
Pusa 1823-12-31-12-12	3.1	94.00 ^b	90.00 ^{def}	72.25 ^{bcd}	79.83 ^{bc}	23.75 ^a	24.42 ^a	7.50 ^{bc}	16.10 ^a	175.23 ^{ab}	152.87 ^{ab}	95.00
Pusa 1823-12-62	2.1+3.1	94.00 ^b	96.00 ^{cd}	71.62 ^{bcd}	65.54 ^c	23.50 ^a	22.87 ^a	8.62 ^{bc}	11.90 ^{cd}	151.26 ^b	164.75 ^a	99.06
Pusa 1823-12-82	2.1+3.1	92.00 ^b	93.50 ^{cde}	68.62 ^{cd}	69.80 ^{bc}	22.37 ^a	23.11 ^a	7.50 ^{bc}	12.00 ^{cd}	152.27 ^b	130.27 ^{bc}	99.06
Pusa 1823-12-14	2.1+3.1	94.00 ^b	86.50 ^f	71.25 ^{bcd}	69.70 ^{bc}	21.37 ^a	21.40 ^a	6.87 ^c	12.50 ^{cd}	90.75 ^{de}	103.16 ^{de}	86.63
Pusa 1823-12-55	2.1+3.1	95.00 ^b	89.00 ^{ef}	69.75 ^{bcd}	67.90 ^c	23.12 ^a	21.50 ^a	6.87 ^c	15.50 ^{ab}	120.14 ^c	161.33 ^a	98.02
IR81896-B-B-195	3.1	94.00 ^b	99.00 ^{abc}	88.12 ^{ab}	101.70 ^a	21.62 ^a	22.46 ^a	9.00 ^{bc}	13.10 ^{bcd}	88.96 ^{de}	85.33 ^{ef}	-
IR81896-B-B-142	2.1+3.1	110.50 ^a	102.50 ^{ab}	81.62 ^{abc}	87.50 ^{ab}	23.50 ^a	21.90 ^a	9.50 ^b	12.10 ^{cd}	112.07 ^{cd}	108.33 ^{cd}	-
Pusa 44		96.00 ^b	90.50 ^{def}	76.00 ^{bcd}	69.30 ^{bc}	23.12 ^a	23.38 ^a	8.50 ^{bc}	11.50 ^{cd}	61.33 ^f	46.67 ^g	-
CV%		1.79	1.79	6.73	6.73	6.27	6.27	6.46	6.46			
LSD (5%)		8.80	5.74	9.68	11.71	2.46	1.77	1.55	1.64	11.63	23.63	
Correlation between years		0.553**		0.275		0.217		-0.220		0.037		
Under irrigated conditions (Unstressed)												
Pusa 1969-13-21-10 - 3	2.1	118.00 ^a	95.00 ^{cd}	96.25 ^b	82.70 ^{bd}	22.87 ^{ab}	22.70 ^a	10.00 ^a	14.70 ^a	774.0 ^{ab}	834.5 ^{a-d}	-
Pusa 1969-13-21-10 - 38	2.1	113.00 ^b	98.50 ^{ab}	84.50 ^c	105.10 ^b	20.75 ^{ab}	23.60 ^a	8.00 ^a	13.00 ^a	756.0 ^{ab}	831.5 ^{bcd}	-
Pusa 1969-13-21-10 - 24	2.1	94.00 ^{de}	95.50 ^{bcd}	68.75 ^{ef}	83.60 ^{cd}	20.00 ^{bc}	23.80 ^a	12.96 ^a	13.30 ^a	832.5 ^a	917.5 ^{abc}	-
Pusa 1969-13-21-10 - 23	2.1	97.00 ^d	99.00 ^a	61.00 ^f	80.70 ^{cd}	16.75 ^c	21.60 ^a	13.83 ^a	13.20 ^a	855.5 ^a	937.0 ^{ab}	-
Pusa 1823-12-31-12-12	3.1	92.00 ^e	96.00 ^{a-d}	76.37 ^{cde}	85.70 ^{cd}	21.12 ^{ab}	23.10 ^a	13.00 ^a	12.40 ^a	755.5 ^{ab}	971.0 ^a	-
Pusa 1823-12-62	2.1+3.1	96.00 ^d	94.00 ^d	76.00 ^{cde}	84.10 ^{cd}	20.75 ^{ab}	23.30 ^a	10.88 ^a	13.10 ^a	675.0 ^b	825.0 ^{bcd}	-
Pusa 1823-12-82	2.1+3.1	94.00 ^{de}	94.00 ^d	72.87 ^{de}	89.80 ^c	22.62 ^{ab}	23.50 ^a	10.62 ^a	18.00 ^a	760.0 ^{ab}	872.0 ^{a-d}	-
Pusa 1823-12-14	2.1+3.1	91.00 ^e	96.50 ^{a-d}	68.00 ^{ef}	83.90 ^{cd}	19.75 ^{bc}	22.90 ^a	11.25 ^a	14.00 ^a	763.0 ^{ab}	901.0 ^{abc}	-
Pusa 1823-12-55	2.1+3.1	97.00 ^d	99.00 ^a	68.75 ^{ef}	79.10 ^d	22.25 ^{ab}	22.40 ^a	13.75 ^a	17.40 ^a	735.5 ^{ab}	918.0 ^{abc}	-
IR81896-B-B-195	3.1	107.00 ^c	97.50 ^{abc}	103.25 ^{ab}	117.10 ^a	21.50 ^{ab}	22.80 ^a	12.12 ^a	9.20 ^a	795.0 ^{ab}	789.0 ^{cd}	-
IR81896-B-B-142	2.1+3.1	106.50 ^c	98.00 ^{abc}	107.75 ^a	116.50 ^a	24.00 ^a	22.80 ^a	9.62 ^a	11.10 ^a	752.0 ^{ab}	755.5 ^d	-
Pusa 44		92.00 ^e	96.00 ^{a-d}	80.87 ^{cd}	79.70 ^{cd}	20.87 ^{ab}	23.20 ^a	12.50 ^a	14.90 ^a	763.0 ^{ab}	782.5 ^{cd}	-
CV%		0.90	0.90	3.39	3.39	4.32	4.32	17.88	17.88	4.67	4.67	
LSD (5%)		8.85	1.93	14.68	13.84	1.95	0.83	2.63	2.90	138.3	138.3	
Correlation between years		0.222		0.781**		0.320*		0.113		0.007		

**Pearson's correlation, significant at 5% level. RPGR = Recurrent parent genome recovery in percentage. DTG = Days to 50% flowering; PTH = Plant height in cm; PNL = Panicle length in cm; TPH = Tiller per hill; YLD = Grain yield in g.m⁻². Trait means followed by same letters are statistically not different at 5% level by Turkey's honestly significant test

seen that BILs showed near similar performance across the seasons, with high stability across both the seasons. This was indicated by almost similar rank sums except for two BILs, Pusa 1969-13-21-10-24 and Pusa 1823-12-55. Having lowest rank sums indicate drought tolerance response, and during 2018 season, the BILs, Pusa 1969-13-21-10-24 and Pusa 1823-12-31-12-12 showed the lowest rank sums based

on all the indices. In 2019, Pusa 1823-12-31-12-12 was preceded by Pusa 1823-12-62 and followed by Pusa 1823-12-55 showed high tolerance response. All the BILs had lower cumulative rank sum during both the seasons, than Pusa 44 indicating overall tolerance response among the BILs. During 2019 season, some of the BILs, such as Pusa 1969-13-21-10-38 underperformed along with their parent, IR81896-B-B-

Table 3. Drought tolerance indices for Pusa 44 backcross derived lines computed for two seasons, 2018 and 2019.

Pedigree	Stressed	Unstressed	TOL	GMP	SSI	STI	HAM	YI	YSI	Rank sum
(a) 2018 Season										
Pusa 1969-13-21-10-3	122.61 ^c	774.0 ^{ab}	84.16 ^{cd}	308.05 ^{cd}	0.99 ^d	0.16 ^{cd}	211.67 ^{cd}	1.04 ^{cd}	0.15 ^c	38.00
Pusa 1969-13-21-10-38	73.67 ^{ef}	756.0 ^{ab}	90.26 ^{ab}	235.99 ^{fg}	1.06 ^{ab}	0.09 ^f	134.25 ^{fg}	0.62 ^f	0.09 ^e	74.00
Pusa 1969-13-21-10-24	182.22 ^a	832.5 ^a	78.11 ^e	389.48 ^a	0.92 ^f	0.25 ^a	298.99 ^a	1.54 ^a	0.21 ^a	13.00
Pusa 1969-13-21-10-23	81.38 ^{ef}	855.5 ^a	90.49 ^{ab}	263.85 ^e	1.06 ^{ab}	0.11 ^{ef}	148.62 ^f	0.69 ^f	0.09 ^e	71.00
Pusa 1823-12-31-12-12	175.23 ^{ab}	755.5 ^{ab}	76.81 ^e	363.84 ^{ab}	0.90 ^f	0.22 ^{ab}	284.47 ^{ab}	1.48 ^{ab}	0.23 ^a	11.00
Pusa 1823-12-62	151.26 ^b	675.0 ^b	77.59 ^e	319.53 ^c	0.91 ^f	0.17 ^c	247.14 ^{bc}	1.28 ^b	0.22 ^a	22.00
Pusa 1823-12-82	152.27 ^b	760.0 ^{ab}	79.96 ^e	340.18 ^{bc}	0.94 ^{ef}	0.19 ^{bc}	253.70 ^b	1.29 ^b	0.20 ^{ab}	24.00
Pusa 1823-12-14	90.75 ^{de}	763.0 ^{ab}	88.11 ^{bc}	263.13 ^{ef}	1.04 ^{bc}	0.11 ^{ef}	162.20 ^e	0.77 ^e	0.11 ^{de}	60.00
Pusa 1823-12-55	120.14 ^c	735.5 ^{ab}	83.67 ^d	297.26 ^d	0.98 ^{de}	0.14 ^d	206.54 ^d	1.02 ^d	0.16 ^{bc}	39.00
IR81896-B-B-195	88.96 ^{de}	795.0 ^{ab}	88.81 ^{ab}	265.93 ^e	1.04 ^{bc}	0.11 ^{ef}	160.01 ^{ef}	0.75 ^{ef}	0.11 ^{de}	61.00
IR81896-B-B-142	112.07 ^{cd}	752.0 ^{ab}	85.10 ^{cd}	290.30 ^{de}	1.00 ^{cd}	0.14 ^{de}	195.06 ^{de}	0.95 ^{de}	0.14 ^{cd}	49.00
Pusa 44	61.33 ^f	763.0 ^b	91.96 ^a	216.32 ^g	1.08 ^a	0.07 ^f	113.53 ^g	0.52 ^f	0.08 ^e	84.00
LSD (p<0.05)	11.63	52.52	43.22	34.07	0.04	0.04	39.86	0.23	0.04	
(b) 2019 season										
Pusa 1969-13-21-10-3	102.87 ^{de}	834.5 ^{a-d}	87.67 ^{de}	292.99 ^d	1.00 ^{cd}	0.11 ^d	183.16 ^d	0.94 ^d	0.12 ^c	48.00
Pusa 1969-13-21-10-38	64.94 ^{fg}	831.5 ^{bcd}	92.19 ^{ab}	232.37 ^f	1.05 ^{ab}	0.07 ^{ef}	120.47 ^{ef}	0.59 ^{ef}	0.07 ^{ef}	77.00
Pusa 1969-13-21-10-24	104.89 ^{de}	917.5 ^{abc}	88.57 ^{cd}	310.21 ^c	1.01 ^c	0.12 ^c	188.25 ^{cd}	0.95 ^d	0.11 ^{cd}	46.00
Pusa 1969-13-21-10-23	86.21 ^{ef}	937.0 ^{ab}	90.80 ^{bc}	284.21 ^{de}	1.03 ^b	0.10 ^d	157.88 ^d	0.78 ^{de}	0.09 ^{de}	66.00
Pusa 1823-12-31-12-12	152.87 ^{ab}	971.0 ^a	84.26 ^{fg}	385.27 ^a	0.96 ^e	0.20 ^a	264.15 ^{ab}	1.39 ^{ab}	0.15 ^b	17.00
Pusa 1823-12-62	164.75 ^a	825.0 ^{bcd}	80.03 ^h	368.67 ^{ab}	0.91 ^f	0.18 ^{ab}	274.65 ^a	1.50 ^a	0.19 ^a	11.00
Pusa 1823-12-82	130.27 ^{bc}	872.0 ^{a-d}	85.06 ^{efg}	337.03 ^{bc}	0.97 ^{de}	0.15 ^{bc}	226.67 ^{bc}	1.19 ^{bc}	0.14 ^{bc}	28.00
Pusa 1823-12-14	103.16 ^{de}	901.0 ^{abc}	88.55 ^{cd}	304.87 ^{cd}	1.01 ^c	0.12 ^{cd}	185.12 ^d	0.94 ^d	0.11 ^{cd}	47.00
Pusa 1823-12-55	161.33 ^a	918.0 ^{abc}	82.43 ^{gh}	384.84 ^a	0.94 ^{ef}	0.19 ^a	274.43 ^a	1.47 ^a	0.17 ^{ab}	14.00
IR81896-B-B-195	85.33e ^f	789.0 ^{cd}	89.18 ^{cd}	259.47 ^{ef}	1.02 ^{bc}	0.09 ^{de}	154.00 ^{de}	0.78 ^{de}	0.10 ^d	67.00
IR81896-B-B-142	108.33 ^{cd}	755.5 ^d	85.66 ^{ef}	286.08 ^d	0.98 ^d	0.11 ^d	189.49 ^c	0.99 ^{cd}	0.14 ^{bc}	41.00
Pusa 44	46.67 ^g	782.5 ^{cd}	94.04 ^a	191.10 ^g	1.07 ^a	0.04 ^f	88.08 ^f	0.42 ^f	0.05 ^f	84.00
LSD (p<0.05)	23.63	71.64	41.33	39.58	0.03	0.03	39.36	0.23	0.03	

TOL = Relative stress tolerance; GMP = Geometric mean productivity; SST = Stress susceptibility index; STI = Stress tolerance index; HM = Harmonic mean; YI = Yield Index; YSI = Yield susceptibility index; RPGR = Recurrent parent genome recovery. The rank-sum is the sum of individual ranks for different tolerance indices for each line. Trait means followed by same letters are statistically not different at 5% level by Turkey's honestly significant test

195. Considering the QTL carried by the BILs, it is generally seen that, BILs with *qDTY2.1* were relatively less drought-tolerant, as compared to the BILs possessing *qDTY3.1* and the di-QTL combination of *qDTY2.1* and *qDTY3.1*.

Grain quality of the BILs

Since grain quality is an important parameter in selecting lines for adaptive cultivation, the grain quality of the BILs was tested during 2019 (Fig. 4). Kernel length before cooking (KLBC) of *qDTY* BILs varied from 5.80 mm (Pusa 1969-19-21-10-23) to 6.71mm (Pusa 1823-12-14), as against 6.42 mm in Pusa 44

(Table 5). Except for two lines, all the BILs had KLBC at par with that of Pusa 44. No significant variation was found between Pusa 44 and BILs for kernel width before cooking, except for one BIL, Pusa 1969-19-21-10-23. Similarly, the length and breadth ratio were also found non-significant except for the line, Pusa 1969-19-21-10-23. After cooking, kernel length increased 1.66 times than the uncooked kernel in Pusa 44. Statistically, similar elongation ratio was found among seven out of the nine BILs studied, with Pusa 1969-19-21-10-23 and Pusa 1969-19-21-10-24 showing relatively lower elongation.

Table 4. Pearson's correlation between the drought indices for the genotypes for the two seasons of 2018 (upper diagonal) and 2019 (lower diagonal). Diagonal elements are the correlation between both the seasons for the respective index

	TOL	GMP	SSI	STI	HAM	YI	YSI
TOL	0.643**	-0.860**	1.000**	-0.865**	-0.967**	-0.976**	-1.000**
GMP	-0.714**	0.638**	-0.860**	0.998**	0.960**	0.948**	0.860**
SSI	0.973**	-0.753**	0.600**	-0.865**	-0.967**	-0.976**	-1.000**
STI	-0.716**	0.998**	-0.752**	0.621**	0.962**	0.953**	0.865**
HAM	-0.898**	0.941**	-0.929**	0.940**	0.700**	0.998**	0.967**
YI	-0.923**	0.913**	-0.955**	0.914**	0.997**	0.675**	0.976**
YSI	-0.973**	0.753**	-1.000**	0.752**	0.929**	0.955**	0.600**

TOL = Relative stress tolerance; GMP = Geometric mean productivity; SST = Stress susceptibility index; STI = Stress tolerance index; HAM = Harmonic mean; YI = Yield Index and YSI = Yield susceptibility index

**Significant at 1% confidence level

Discussion

Imparting drought stress tolerance in rice varietal development forms an



Fig. 4. Grain morphology of Pusa 44, donor parents (row 1) and *qDTY* backcross derived lines (rows 2-4)

integral component, especially while targeting rainfed lowland and upland environments. Owing to unfavourable conditions, drought stress in particular, rice production and productivity is compromised in these environments (Vinod et al. 2019). Sustaining rice cultivation, specifically involving drought-sensitive but high yielding mega varieties, is becoming increasingly difficult due to the adverse effect of global climate change. Pusa 44 is a popular high yielding rice cultivar in northern India that has almost gained the position of a mega variety because of its stable high yield, translucent and attractive slender grain, acceptable cooking quality, strong stem, good tillering, medium plant height, and long panicle length. It is highly suitable for mechanical harvesting, making it a choice cultivar suitable for rice-wheat cropping system (Bhatt et al. 2016). Recent indications of receding water levels in north Indian plains are one of the major concerns for sustaining rice production in this region, because Pusa 44 is a highly drought-sensitive cultivar. Therefore, in order to sustain production, it is

Table 5. Grain quality data of Pusa 44 backcross derived lines

Pedigree	KLBC (mm)	KBBC (mm)	L/B	KLAC (mm)	KBAC (mm)	ER	HULL (%)	MILL (%)
Pusa 1969-19-21-10-3	6.51 ^{abc}	2.38 ^{ab}	2.73 ^{abc}	10.35 ^{abc}	3.07 ^{ab}	1.59 ^{ab}	77.37	70.6
Pusa 1969-19-21-10-38	5.90 ^{def}	2.20 ^{ab}	2.68 ^{abc}	9.37 ^{cd}	2.83 ^{abc}	1.59 ^{ab}	79.15	72.63
Pusa 1969-19-21-10-24	6.37 ^{bcd}	2.12 ^{ab}	3.02 ^{abc}	9.14 ^{de}	2.54 ^c	1.44 ^b	77.94	70.80
Pusa 1969-19-21-10-23	5.80 ^{ef}	2.56 ^a	2.26 ^c	8.25 ^e	3.24 ^a	1.42 ^b	81.66	75.66
Pusa 1823-12-31-12-12	6.19 ^{cde}	2.18 ^{ab}	2.83 ^{abc}	9.23 ^{de}	2.63 ^{bc}	1.49 ^{ab}	78.71	71.74
Pusa 1823-12-62	6.55 ^{abc}	2.21 ^{ab}	2.96 ^{abc}	10.43 ^{abc}	2.80 ^{abc}	1.59 ^{ab}	83.45	75.08
Pusa 1823-12-82	6.66 ^{abc}	2.16 ^{ab}	3.08 ^{ab}	10.21 ^{abcd}	2.70 ^{bc}	1.53 ^{ab}	81.89	74.12
Pusa 1823-12-14	6.71 ^{ab}	2.02 ^b	3.37 ^a	10.66 ^{ab}	2.70 ^{bc}	1.58 ^{ab}	83.38	74.58
Pusa 1823-12-55	6.46 ^{abc}	2.30 ^{ab}	2.81 ^{abc}	10.81 ^a	3.06 ^{ab}	1.67 ^{ab}	83.38	76.53
IR81896-B-B-195	5.70 ^f	2.26 ^{ab}	2.52 ^{bc}	9.70 ^{bcd}	2.90 ^{abc}	1.71 ^a	79.63	71.7
IR81896-B-B-142	6.88 ^a	2.06 ^b	3.33 ^a	10.04 ^{abcd}	2.97 ^{abc}	1.46 ^{ab}	80.34	72.94
Pusa 44	6.42 ^{abc}	2.04 ^b	3.16 ^{ab}	10.64 ^{ab}	2.83 ^{abc}	1.66 ^{ab}	78.84	72.12
CV%	1.87	5.4	6.78	2.69	4.22	4.12		
LSD (5%)	0.37	0.17	0.34	0.79	0.22	0.1		

KLBC = Kernel Length Before Cooking (mm), KBBC = Kernel Breadth Before Cooking (mm), L/B = Length/ Breadth ratio, KLAC = Kernel Length After Cooking (mm), KBAC = Kernel Breadth After Cooking (mm), KER = Kernel Elongation Ratio, HULL = Hulling Recovery Percentage, MILL = Milling Recovery Percentage. Trait means followed by same letters are statistically not different at 5% level by Turkey's honestly significant test

pertinent to improve the drought tolerance of Pusa 44.

There are several QTLs reported for drought stress tolerance in rice, that can be suitably used for marker-assisted backcross breeding (Sagare et al. 2020; Oo et al. 2021), as we have undertaken in this study. Among these QTLs, *qDTY2.1* and *qDTY3.1* (Venuprasad et al. 2009) have been introgressed into multiple backgrounds such as Swarna, Anjali, TDK1 (Kumar et al. 2014, 2018). Both these QTLs were earlier validated for their functionality over multiple backgrounds and were chosen for introgression into Pusa 44. Pusa 44 being drought-sensitive cultivar, it is hypothesised that introgression of *qDTY* QTLs would improve their productivity under drought stress. We used two donors namely, IR81896-B-B-195 and IR81896-B-B-142 for sourcing the two QTLs, *qDTY2.1* and *qDTY3.1*. Developed from a common cross, Apo/Swarna, both the donor lines possessed *qDTY2.1*, as well as *qDTY3.1*, originating from Apo (Sandhu et al. 2019). However, in the current study, *qDTY2.1* alone was sourced from IR81896-B-B-195, while both *qDTY2.1* and *qDTY3.1* were sourced from IR81896-B-B-142.

Following the well-demonstrated marker assisted breeding approach we could introgress the target QTLs,

qDTY2.1 and *qDTY3.1* into Pusa 44. The BILs either possessed the QTLs alone or together. Having brought into a common background, this provided us with the opportunity to evaluate the drought response effects of the QTLs singly or in combination. The initial diversity between Pusa 44 and the donors, IR81896-B-B-142 and IR81896-B-B-195 was 8.47% and 7.92%, respectively. To bridge the gaps in genomic regions, that were left unscreened in a previous study (Dwivedi et al. 2021), we have used a different set of simple sequence repeat (SSR) markers. Therefore, combining both, the estimated diversity between the parents was about 12.4%. However, RPGR among the BILs ranged from 86.62 to 99.06%. This may be due to the delay in exercising background selection till BC₃F₄ generation. In MABB programmes, high recovery of RPG is generally achieved by integrating phenotypic selection with background selection (Rai et al. 2018). The results from the present study reaffirm the requirement of combined genotypic and phenotypic selection, although phenotypic selection alone may help amass recurrent parent phenome.

Agronomic performance of Pusa 44 *qDTY* BILs such as rice grain yield, tillers per hill, panicle length, plant height and days to 50% flowering varied significantly between the seasons as well as under

stressed and unstressed conditions. This was expected because all the lines used in the evaluation are genetically near similar as they carried a significant proportion of the genome of recurrent parent, Pusa 44. By totally relying on phenotypic selection, we have deferred the background selection until BC₃F₄ generation in this study. By this way, we could realise a gain of more than three lines having over 90% of RPGR, out of five BILs studied belonged to the crosses, Pusa 1969 and Pusa 1823. Two other lines (Pusa 1823-12-62 and Pusa 1823-12-82) used were NILs developed from a parallel programme (Dwivedi et al. 2021). These results imply an encouraging step in MABB programmes, wherein laborious background selection can be deferred if backed up by a stringent phenotypic selection and with a sufficient number of lines selected.

The ranking is used for comparing the genotypic response under drought and irrigated conditions in the study. The ranking is a non-parametric method which compares the subjects without any bias on the size of the difference. Since the present study was used indices for comparing the performance, it did not require a definite distribution pattern for concluding the genotypic performance, hence opting for the non-parametric method (Hettmansperger 2014). Seven indices were used for evaluating the performance but found that all of them are equally useful for ranking the genotypes. Although they are related as they are computed from the same data, each of the indices had its emphasis. For instance, TOL helped to identify the line that was relatively similar performing under drought as that under unstressed conditions (Ghobadi et al. 2012). Here the BILs with lower TOL values were judged tolerant and *vice versa*. Similarly, SSI laid emphasis on the relative stress index on the overall population. The stress index was the ratio between yield under stress to normal yield that deviated from unity, indicating that tolerant genotypes will have lower values than the sensitive ones. Therefore, SSI measures the advantage each tested line has over the average stress index of the population, and therefore, the lower SSI values indicate tolerance (Fischner and Maurer 1978). The remaining indices have a reverse pattern of indication of tolerant lines since all of them emphasized the *per se* yield of the lines. Higher index values are assigned for tolerant ones and lower values indicated sensitiveness. While GMP provided weight on high yield, STI laid weight on a larger ratio of productivity products related to stress yield (Fernandez 1992). In the case of HAM, the stress was on the

yield, but with the ratio of productivity product over productivity sum (Kristin et al. 1997). The last index used was YSI, which is the ratio of stress yield to the unstressed yield (Bousslama and Schapaugh 1984), the reverse of stress index.

In this context, it is interesting to note that, traits such as plant height and panicle length did not vary significantly across the seasons, particularly under drought stress. This indicated that the lines have attained stability in agronomic performance, apparently under drought conditions, where the stress could have resulted in general growth suppression. However, it is worth highlighting that the yield performance of the BILs was significantly higher than that of Pusa 44 under drought in both the seasons. On an average, the BILs had yield advantage over Pusa 44, 2.0 times during 2018 and 2.55 times during 2019. Examining the overall pattern of drought response of the backcross derived lines of Pusa 44 using various drought indices also indicated a high level of performance stability of lines except for two. It was noticed that drought in 2018 was relatively less severe than during 2019, because two among the BILs and their respective parent showed yield reduction on par with Pusa 44 during 2019. These lines, Pusa 1969-13-21-10-38 and Pusa 1969-13-21-10-23 were found to have higher rank-sum values than Pusa 44, along with their donor, IR81896-B-B-195. By further examination, it was revealed that these two lines shared ranks of 10 and 11 for almost all the indices used, just to be succeeded by Pusa 44 with 12th rank among all the twelve genotypes tested including parents. Also, it was noticed that their parent, IR81896-B-B-195 preceded these lines in the ranking.

Evaluating the performance of BILs based on the QTL(s) they carried brought out some interesting observations. Among the lines possessing *qDTY2.1*, Pusa 1969-13-21-10-24 produced the highest yield during 2018, which was, however, found low during 2019. Generally, we could find that the BILs with *qDTY2.1* was having relatively lesser drought tolerance than those with *qDTY3.1* and their combination, except for Pusa 1969-13-21-10-24. The best line in terms of stable drought tolerance was, however, Pusa 1823-12-31-12-12 which had only *qDTY3.1*, which was the second-best line under drought during 2018, and the third-best during 2019. Sandhu et al. (2019) reported that mono-QTL carriers for *qDTY3.1* performed the best with highest rice grain yield coupled with agronomic traits under severe to moderate drought stress and as well as under

unstressed environments. They found that yield advantage for the di-QTL NILs having $qDTY2.1+qDTY3.1$ ranged from 396 to 2376 kg.ha⁻¹ under unstress and 284 to 2086 kg.ha⁻¹ under reproductive stage drought stress. Similarly, in the current experiment also, Pusa 1823-12-31-12-12 was found yielding relatively high under unstress conditions. Notwithstanding, unlike during the 2018 season, the severity of the drought was found increased during 2019, wherein the soil moisture level dipped to -70 KPa during the reproductive stage. During this extreme stress, $qDTY2.1$ lines were found to show a low in performance relative to the lines having $qDTY3.1$ and the di-QTL combination of $qDTY2.1+qDTY3.1$. Among these, the di-QTL carriers showed better yield advantage in at least among two lines, Pusa 1823-12-62 and Pusa 1823-12-55, which showed the highest yield under drought during 2019. This implied that QTL combinations work better under severe drought than under moderate stress situations. A complementary effect of $qDTY3.1$ with $qDTY6.1$ in articulating stable and high grain yield across the different level of drought stress was earlier reported by Dixit et al. (2014). In the current study, $qDTY2.1$ and $qDTY3.1$ were shown to have a complementary effect on high yield under severe stress than under mild stress at the reproductive stage. Kumar et al. (2014) found that the combination of $qDTY1.1$, $qDTY2.1$ and $qDTY3.1$ provided a greater advantage over the respective mono-QTL lines in Swarna-Sub1 background when exposed to severe drought. The complementation effect of two QTL observed in this study also demonstrates that these QTL combinations provides effective drought stress response at the reproductive stage in a different genetic background, such as Pusa 44. When mapped from the Apo/Swarna*2 cross, $qDTY2.1$ and $qDTY3.1$ have emerged as large effect QTLs having a phenotypic contribution of 16.3% and 30.7% respectively towards yield under drought (Dixit et al. 2014). Derived from the upland rice variety, Apo, these QTLs can be advantages for configuring high grain yield under both lowland and upland rice under drought stress. In a similar study, Shamsudin et al. (2016) had reported that two $qDTY$ combinations, $qDTY3.1+qDTY2.2$ and $qDTY3.1+qDTY12.1$ performed better than their three- $qDTY$ combination, indicating that QTL complementation is not associated to the number of QTLs integrated. In our study, we could observe that mono-QTL lines performed relatively better under mild stress than under severe stress indicating that QTL complementation works better under severe drought. This is analogous to the findings of Kumar et al. (2018),

who reported grain yield advantage of the QTLs combination of $qDTY2.1+qDTY3.1$ under high drought stress.

Grain quality remains as one of the integral traits in modern rice breeding. Hence, we have tested the grain quality of the BILs used in this study in comparison to the recurrent parent, Pusa 44. Most of the BILs had similar grain physical dimensions as well as cooking quality as that of Pusa 44. The BILs, in general, were similar in kernel length before cooking, having comparable length breadth ratio and elongation ratio. There was no undesirable effect of QTLs on grain quality parameters.

Marker-assisted backcross breeding has been successfully adopted for the development of improved Pusa 44 $qDTY$ BILs with stable increased grain yield under reproductive stage drought stress. These improved lines would cater to the immediate requirement of drought-tolerant rice cultivars in the areas of adoption of Pusa 44, in the event of any unforeseen drought incidence. Several of these lines can therefore, be used for expansive cultivation in the eastern part of India as well, where drought occurrence is a regular feature. We could further realise that on an average, the QTL introgressed could garner a yield advantage ranging from 2.0 to 2.6 times than Pusa 44 under reproductive stage drought stress. Depending on the QTLs carried by the BILs, the response to drought varied, with $qDTY3.1$ providing better performance under milder stress and the di-QTL combination of $qDTY2.1$ and $qDTY3.1$ showing better tolerance to severe stress. Development of the improved lines could be concluded as a step towards developing climate resilient rice varieties for the future.

Authors' contribution

Conceptualization of research (GKS, AKS); Designing of the experiments (GKS, KSO, AKS); Contribution of experimental materials (GKS); Execution of field/lab experiments and data collection (KSO, GD, PD, VP, PKB); Analysis of data and interpretation (KSO, KKV, GKS); Preparation of manuscript (KSO, KKV, GKS, AKS).

Declaration

The authors declare no conflict of interest.

Acknowledgement

KSO acknowledges the financial support he received from the Indian Council of Cultural Relations,

Government of India during his doctoral program at ICAR-IARI, New Delhi. He also acknowledges the Advanced Centre for Agricultural Research and Education, Government of Myanmar for providing his deputation and scholarship during the programme. The study was carried out through the funding received from ICAR under the National Innovations for Climate Resilient Agriculture.

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