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Drought Tolerant near Isogenic Lines (NILs) of Pusa 44 Developed through Marker Assisted Introgression of *qDTY2.1* and *qDTY3.1* Enhances Yield under Reproductive Stage Drought Stress

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Abstract: Reproductive stage drought stress (RSDS) is detrimental for rice, which affects its productivity as well as grain quality. In the present study, we introgressed two major quantitative trait loci (QTLs), namely, *qDTY2.1* and *qDTY3.1*, governing RSDS tolerance in a popular high yielding non-aromatic rice cultivar, Pusa 44, through marker-assisted backcross breeding (MABB). Pusa 44 is highly sensitive to RSDS, which restricts its cultivation across drought-prone environments. Foreground selection was carried out using markers, RM520 for *qDTY3.1* and RM 521 for *qDTY2.1*. Background selection was achieved with 97 polymorphic SSR markers in tandem with phenotypic selection to achieve faster recurrent parent genome (RPG) recovery. Three successive backcrosses followed by three selfings aided RPG recoveries of 98.6% to 99.4% among 31 near isogenic lines (NILs). Fourteen NILs were found to be significantly superior in yield and grain quality under RSDS with higher drought tolerance efficiency (DTE) than Pusa 44. Among these, the evaluation of two promising NILs in the multilocational trial during *Kharif* 2019 showed that they were significantly superior to Pusa 44 under reproductive stage drought stress, while performing on par with Pusa 44 under normal irrigated conditions. These di-QTL pyramided drought-tolerant NILs are in the final stages of testing the All India Coordinated Rice Improvement Project varietal trials for cultivar release. Alternately, the elite drought-tolerant Pusa 44 NILs will serve as an invaluable source of drought tolerance in rice improvement.

Keywords: drought tolerance; QTL introgression; marker-assisted backcross breeding; near isogenic lines; reproductive stage drought stress; climate resilience

1. Introduction

Rice (*Oryza sativa* L.) is an important food crop of Asian countries, including India. Globally, rice is widely consumed as a staple food by more than half of the human population, contributing over 20 percent of the total calorie intake. To meet the growing food demand, rice production has to be boosted by 0.6–0.9% annually [1]. Among the rice-growing countries of the world, India holds the first position in acreage (44 mha), but is second in rice production. India witnessed a 226% increase in rice production with a corresponding 28.3% increase in area, and a 152% increase in productivity during the green revolution, mainly due to the substantial increase in rice productivity in India through the cultivation of modern semi-dwarf high-yielding rice cultivars. However, the average annual productivity is still low compared to the world average. One of the major factors for lower productivity is the uncertain water availability in major rice-growing regions, followed by biotic stresses.

During the past sixty years, over 1200 rice varieties have been released for commercial cultivation; however, only a few of them have gained large-scale popularity among the farmers and other stakeholders associated with rice. One such variety is Pusa 44, developed and released in 1994 by the ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. Even though Pusa 44 was released for commercial cultivation in Karnataka and Kerala, this variety became immensely popular elsewhere, growing into a megavariety in northern India [2], particularly due to its higher productivity and suitability for mechanical harvesting. Pusa 44 is a semi-dwarf *indica* rice variety, possessing sturdy culm, with long slender grains and high head rice recovery. With average productivity of 8–10 tonnes/hectare, Pusa 44 became popular in Punjab and quickly spread across to neighboring states of Haryana and Uttar Pradesh. Pusa 44 contributes a significant share in supply towards the public distribution system (PDS) from Punjab, and also contributes to the non-Basmati rice export from Northern India [3].

Water scarcity for agriculture purposes is a growing concern in the contemporary world. The increasing demand for available water is exacerbating water shortage in irrigated ecosystems, predisposing drought-like situations while bolstering the intensity of the prevailing drought in rainfed ecosystems [4]. Rice is vulnerable to drought stress, and drought has been predicted to occur more frequently due to climate change. Drought stress is detrimental to rice production and yield stability. Therefore, there is a need to develop and disseminate ecosystem-specific technologies that can reduce the excessive use of groundwater for rice production without compromising the productivity. To enable this, breeding rice varieties with resilience to drought is an important step forward in addressing this challenge. One of the major bottlenecks in analyzing the drought-related response in rice genotypes is the inherent complexity of the experimental system. This is because the drought itself is manifested in several ways, and is often complicated with the presence of high temperatures [5]. Additionally, the quick drought progression also affects the chance of crop survivability, as the plants get relatively less time to recover against the rapid damage induced due to drought stress. Screening for drought tolerance under artificially managed stress levels is comparatively hard compared to managing stress during the dry season, as well as under rainfed upland situations [6–9]. Therefore, screening for drought tolerance is carried out under managed conditions where facilities for controlling irrigations are available. Additionally, rainout shelters are also used to prevent precipitation interference in the experimentation [2]. In recent times, northern India has been facing issues of receding water levels bringing in episodes of intermittent drought when rainfall patterns fluctuate. Since Pusa 44 is highly susceptible to drought, especially at the reproductive stage, its yield and grain quality are compromised when exposed to reproductive stage drought stress (RSDS). This has raised concerns on the continuity of Pusa 44 cultivation in its niche areas of adoption. On account of being a lowland cultivar suitable for the irrigated rice ecosystem, it is obvious that the productivity of Pusa 44 can be significantly reduced due to water stress.

Genetically, drought tolerance in rice is a complex response. Several large-effect and small-effect QTLs and meta-QTLs have been reported for grain yield under reproductive-stage drought stress [10,11]. A large-effect QTL, *qDTY2.2*, on chromosome 2 was identified in the MTU 1010/Kali Aus population under upland [12] and lowland [13] conditions.

Mishra et al. [14] reported another QTL, *qDTY12.1*, on chromosome 12 that was significantly associated with reproductive-stage drought stress. *qDTY12.1* explains 42% of the genetic variation with an additive effect of 172 kg/ha for grain yield under drought with no significant penalty under unstressed conditions [8]. Another major-effect QTL, *qDTY1.1* was identified on chromosome 1 between the marker intervals RM431 and RM12091 in the Swarna/IR 64 population [15]. Venuprasad et al. [16] identified two major QTLs, *qDTY2.1* and *qDTY3.1*, on chromosome 2 and 3, respectively, from the population of Apo/2*Swarna, that explained 13–16% and 31% of the genetic variances, respectively. The subsequent use of these QTLs, particularly of *qDTY3.1*, has been demonstrated to impart improved drought tolerance among several genetic backgrounds, such as improved White Ponni [17], MR219 [18], Samba Mahsuri [19], Sabitri [20], IR64 and Vandana [21], and several others [22].

Marker-assisted selection (MAS) approach to transferring these QTLs into drought-sensitive genetic backgrounds of high-yielding varieties has been widely adopted in rice [22]. Jongdee et al. [23] advocated the use of molecular breeding to accelerate product development, especially for characters which are complex and have low heritability, or if the breeding process is time-consuming and expensive. The marker-assisted incorporation of major- and minor-effect QTLs for grain yield under drought stress has been recognized as time- and cost-effective, as well as a fast-track approach for breeding drought-tolerant rice varieties [24]. Dixit et al. [25] developed drought-tolerant near isogenic lines (NILs) of Savitri (CR1009) with the combination of two large-effect QTLs, *qDTY3.2* and *qDTY12.1*. Marker-assisted backcross breeding (MABB) has been successful in transferring the genes/QTLs governing tolerance to biotic/abiotic stresses in rice [26–35].

Considering the popularity of Pusa 44 and its high sensitivity to RSDS, the present study aimed at the introgression of two QTLs, *qDTY2.1* and *qDTY3.1*, governing tolerance to RSDS into Pusa 44 through MABB (Supplementary Figure S1). The improved drought tolerant NILs with tolerance to RSDS were tested for two years on the station and for another two years in multilocation trials, the results of which are discussed below.

2. Materials and Methods

2.1. Plant Material

Pusa 44 used as the recurrent parent and a drought-tolerant NIL of Swarna, namely, IR81896-B-B-142 was used as a donor for the two QTLs, *qDTY2.1* and *qDTY3.1*, for tolerance to RSDS. Pusa 44 was crossed with the donor and the F_1 progenies were tested for hybridity. The true F_1 was backcrossed to the recurrent parent to generate BC_1 generation. Two more backcrosses were made in subsequent generations using selected progenies based on marker-assisted foreground selection for the two QTLs, *qDTY2.1* and *qDTY3.1*, followed by background and phenotypic selection to generate BC_3 generation. Further selections on the segregating populations of BC_3 were used to develop NILs of Pusa 44 possessing these two QTLs. All the early generations were developed by growing under irrigated conditions, with recommended agronomic maintenance. However, phenotypic selection was carried out starting with BC_1F_1, for various agro-morphological traits in comparison with Pusa 44. All the field experiments were conducted at the research farm of Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi (28°38′ N; 77°10′ E; 223 m AMSL), and the offseason crops were taken up at the Rice Breeding and Genetics Research Centre (RBGRC)- IARI, Aduthurai (11°00′ N; 79°28′ E; 19 m AMSL).

2.2. Marker-Assisted Selection

For marker analysis, DNA was extracted from leaf samples collected from field-grown plants as per the standard procedure [36]. PCR amplification was conducted as per the protocol standardized in our laboratory [33]. Foreground selection was carried out with QTL-linked markers, RM520 for *qDTY3.1* and RM 521 for *qDTY2.1* (Table 1). The parental lines were screened for the genome-wide polymorphism using 460 microsatellite (SSR) markers distributed across the rice genome. Among these polymorphic markers

(Supplementary Figure S2), SSR markers on the target chromosomes 2 and 3 flanking the QTL linked markers and were used for effecting recombinant selection. After the generation of F_1, the hybridity of the F_1s was confirmed with the foreground markers by confirming the heterozygosity for the QTL-linked markers. One of the true F_1 plants was backcrossed to Pusa 44 to generate BC_1F_1 plants. The BC_1F_1 plants were initially subjected to foreground selection using RM520, and the heterozygous plants thus identified were subsequently genotyped using RM521 to identify plants heterozygous for both the markers. Such double heterozygotic BC_1F_1s identified from foreground selection were screened for the background recovery using the genome-wide polymorphic SSR markers. The recovery of Pusa 44-specific alleles in these polymorphic markers was used for computing the recovery of the recurrent parent genome (RPG). Additionally, the selected BC_1F_1s were also screened for morphological similarity to Pusa 44, for identifying plants with higher RPG and recurrent parent phenome (RPP). One BC_1F_1 plant with desirable RPG and RPP recovery was used to generate BC_2F_1, by backcrossing it with Pusa 44. The selection strategy followed earlier in BC_1F_1 was adopted until the BC_3F_1 was generated. From BC_3F_1 onwards, the plants were advanced to BC_3F_4 to identify genotypes which are homozygous for both the target QTLs. Schematic selection (Figure 1) for agronomical, morphological, grain and cooking quality traits, coupled with the genotypic selection at every selection stage, was done to ensure maximum recovery of RPP. In each step, background SSR markers that showed non-recovery/heterozygosity for recurrent parent alleles in the selected progenies were only used for background selections in the succeeding backcross generations. From the BC_3F_3 generation onwards, the selected families were evaluated in field trials under stressed as well as unstressed conditions. Finally, at the BC_3F_5 stage, the selected NILs were reconfirmed for the presence of two target QTLs, *qDTY2.1* and *qDTY3.1*. The agronomic evaluation was done for morphological and yield component traits as well as grain quality traits. RPG recovery in the NILs was depicted using Graphical GenoTypes (GGT) Version 2.0 software [37].

Table 1. *qDTY3.1* and *qDTY2.1* linked markers for foreground selection (FS) and recombinant selection (RS).

QTL	Marker	Chromosome	Physical Location	Type	Reference
qDTY3.1	RM520	3	30.71 Mb	FS	[16]
	RM15791		28.56 Mb	RS	
	RM16033		32.56 Mb	RS	
qDTY2.1	RM521	2	10.8 Mb	FS	[16]
	RM5791		10.74 Mb	RS	
	RM324		11.4 Mb	RS	

FS, foreground selection; RS, recombinant selection.

2.3. Evaluation of the NILs Under Stressed and Unstressed Conditions

A preliminary field evaluation of BC_3F_4 NILs was carried out along with both the parents, Pusa 44 and IR81896-B-B-142, together with two other checks, namely, IR81896-B-B-195 and IR87728-59-B-B. During *Kharif* 2016, agro-morphological evaluation was carried out using an augmented randomized block design (ARBD) with six blocks. Two treatments, stressed and unstressed, were maintained, with stressed plots having irrigation withheld during the entire heading period, beginning from 30 days after transplanting. The unstressed treatment had the normal recommended irrigation schedule. Barring irrigation, both the treatments had similar agronomic management. The NILs were grown in plots of 10.3 m^2 following a spacing of 20 cm $\times$ 15 cm. The data were recorded on five uniform-looking healthy plants from each of the NILs for the characteristics, namely, days to 50% flowering (DtF), plant height (PtH), number of productive tillers (NpT), panicle length (PnL), grain yield (GrY), number of filled grains per panicle (FdG), spikelet fertility (SpF) and thousand grain weight (GrW). Plants were harvested at physiological maturity. The mean of five plants constitutes yield per plant for the genotype and plot yield was also measured for all the NILs.

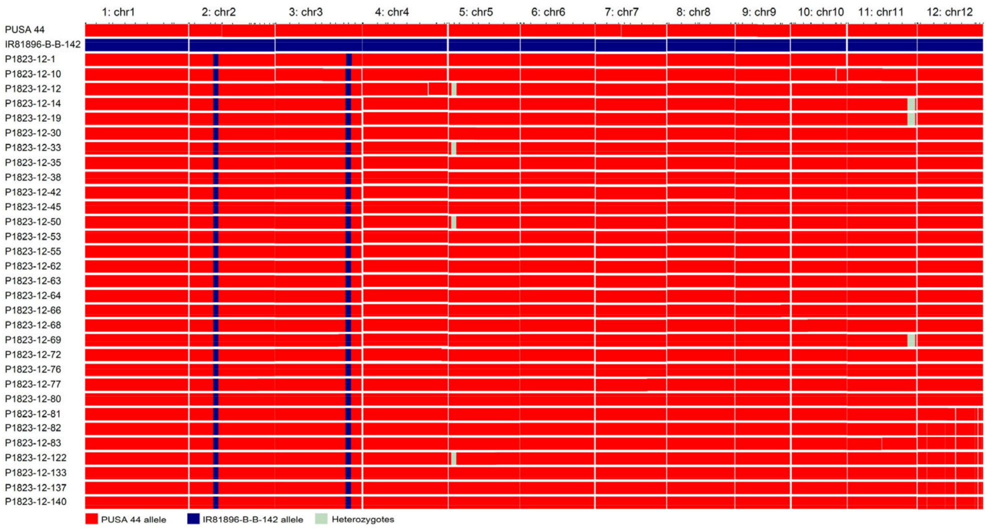

Figure 1. Graphical representation of recurrent parent genome recovery and quantitative trait locus (QTL) introgression among the near-isogenic lines of Pusa 44 in BC_3F_4 generation. Chromosome segments in red represent Pusa 44 type, while blue segments represent that from the donor, IR81896-B-B-142. Heterozygous segments are shown in a dull grey color. The complete recovery of recurrent parent genome underpins the importance of augmenting phenotypic selection with the marker-assisted background selection.

Based on the agronomic and quality performance of the NILs under both stress and non-stress conditions, the set of 31 best-performing NILs in BC_3F_5 generation was evaluated in RBD along with parents and two checks in two replications during *Kharif* 2017 with two treatments, namely, moisture stressed and normal irrigated (unstressed). The plot size was 10.3 m^2 with a spacing of 20 × 15 cm. Recommended agronomic management was followed in the trial, except for the irrigation schedule. Under unstressed conditions, irrigation was scheduled to maintain a 10 cm water level in the fields for the entire duration from transplanting to harvest. In the moisture-stressed plots, approximately 5 cm of standing water was maintained till panicle initiation, which coincided with 30 days after transplanting. After booting, irrigation was withheld totally until maturity. In the drought-stress plots, the moisture stress imposed was monitored using tensiometers and the irrigation was withheld till the stress level reached −70 KPa, to ensure stringent evaluation of the drought-tolerance in these NILs. Data recording was done for all the traits that were recorded earlier in the ARBD trial, from five random uniform-looking plants from each plot at maturity.

Both the trials during *Kharif* 2016 and *Kharif* 2017 were carried out at the research farm of Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. In New Delhi, the average temperature during *Kharif* 2017 ranged between 38 °C in June to 15 °C in November, with a total rainfall of 856.0 mm.

2.4. Drought Tolerance Indices

Based on the performance of NILs under both stressed and unstressed conditions, the following drought indices were worked out for a comprehensive evaluation of the drought tolerance.

Drought susceptibility index, DSI $= \frac{Y_{ns} - Y_s}{Y_{ns}}$ [38]

Relative decrease yield, RDY $= 100 - \left(\frac{(Y_i)_s}{(Y_i)_{ns}} \times 100\right)$ [39]

Drought tolerance efficiency, DTE (%) $= \frac{Y_s}{Y_{ns}} \times 100$ [40]

where Y_s and Y_{ns} represent the yields of all genotypes evaluated under drought stressed and unstressed conditions, respectively. $(Y_i)_s$ denotes the yield of the *i*th genotype under drought stress and $(Y_i)_{ns}$ the yield of the *i*th genotype under unstressed conditions.

2.5. Grain and Cooking Quality

The grain samples from the NILs were analyzed for the grain dimension and quality parameters as described by Ellur et al. [34]. The traits observed were hulling percentage (HgP), milling percentage (MgP), kernel length before cooking (KLBC), kernel width before cooking (KWBC) and length-width ratio (LWB), kernel length after cooking (KLAC), kernel width after cooking (KWAC), elongation ratio (ER) and alkali spreading value (ASV). All the measurements of physical parameters of the milled rice were done using a photo analyzer.

2.6. Statistical Analyses

The agro-morphological and grain quality data from the improved drought tolerant NILs of Pusa 44 were subjected to statistical analyses of variance and means [41] and analysis of variance (ANOVA). The mean comparison between the genotypes was carried out using the appropriate statistical assumption of normal distribution. The comparison was done using Tukey's honestly significant test at the 5% confidence level. The conformability of genotype performance across both the seasons was computed using the Pearson correlation coefficient. All the data analyses were carried out using Analysis Toolpack plugin in Microsoft Excel, and STAR statistical analysis package [42]. The hierarchical agglomerative clustering of the multi-location data was performed under the R statistical environment using the "Stats" package [43].

3. Results

3.1. Introgression of the QTLs, qDTY2.1 and qDTY3.1

Screening of the genome-wide polymorphism between the recurrent parent, Pusa 44, and the donor parent, IR81896-B-B-142, using 460 SSR markers revealed 97 markers to be polymorphic, indicating 21.1% of genetic variability between the parents (Supplementary Figure S1). These polymorphic markers were further used for background selection. Among the polymorphic markers, two markers from chromosome 3, RM15791 (28.56 Mb) and RM16033 (32.56 Mb), were identified to be closely flanking *qDTY3.1*. Similarly, RM5791 (10.74 Mb) and RM324 (11.4 Mb) were identified flanking *qDTY2.1* on chromosome 2. These markers were used for recombinant selection to help minimize linkage drag (Table 1).

The initial hybridization of Pusa 44 with IR81896-B-B-142 produced 11 seedlings, out of which 9 were found to be heterozygous for the QTL-linked markers during foreground selection (Table 2). The cross was designated as Pusa 1823. Two F_1 plants were selected out of the nine true F_1s and were backcrossed to the recurrent parent, Pusa 44, to generate forty-eight BC_1F_1 plants. Foreground selection using the RM520 identified 18 plants heterozygous for this marker. These plants on further genotyping using RM521 could identify eight plants heterozygous for both the QTLs. All the 8 BC_1F_1 plants were subjected to background selection with 97 polymorphic SSR markers, which indicated RPG recovery ranging between 73.08 and 82.87%. Further, the phenotypic selection of BC_1F_1 with Pusa 44 helped in identifying two BC_1F_1 plants with the RPG recovery of 82.0 and 82.7, respectively. These plants were also found to show the highest recurrent parent phenome (RPP) recovery. They were further backcrossed to Pusa 44 to generate 32 BC_2F_1 plants. Foreground selection using the marker RM520 among the BC_2F_1 plants revealed that nine plants were heterozygous for *qDTY3.1*. Additional foreground selection among these nine plants for the second QTL, *qDTY2.1*, identified three plants heterozygous for both the QTLs. Based on agro-morphological similarity to Pusa 44 and RPG recovery (98.5%), backcrossing was done using two BC_2F_1 plants. Among the 18 BC_3F_1 plants produced, only 1 BC_3F_1 plant was found to be heterozygous for both the QTLs, *qDTY2.1* and *qDTY3.1*. The single BC_3F_1 plant on background selection revealed that it had an RPG recovery of 98.6%. Being a single progeny, this line was not subjected to morphological selection. The selfing of this BC_3F_1 plant produced 400 BC_3F_2 plants, of which 54 plants were identified as homozygous for both the markers linked to *qDTY2.1* and *qDTY3.1*, and were selected from the BC_3F_2 population. From the selected plants, a large population of 9488 BC_3F_3 was raised, out of which a total of 242 single plant selections showing agronomic resemblance to Pusa 44 were initially made. Following the grain and cooking quality evaluations, the selections were subsequently reduced to 108 lines. In the BC_3F_4 generation, progenies from these selected lines were evaluated under both stress and non-stress conditions, reducing the selection to 31 BC_3F_5 families. Finally, the 31 NILs were further evaluated for drought stress response under replicated trials for two years.

Table 2. Progressive selection statistics of backcross generations and the recovery of the recurrent parent genome during the development of Pusa 44 near isogenic lines.

Generation	No. of Plants Raised	QTL Positive Progenies ¶	Plants Selected	RPG Recovery (%)
F_1	11	9	2	*
BC_1F_1	48	8	2	73.1–82.9
BC_2F_1	32	3	2	86.1–95.9
BC_3F_1	18	1	1	98.6
BC_3F_2	400	54	54	98.6–99.0
BC_3F_3	9488 (54 families)	242	108	98.8–99.4
BC_3F_4	108 NILs	108 NILs	31 NILs	>99.6

* Not estimated; RPG, recurrent parent genome; ¶ number of plants/ lines positive for both *qDTY3.1* and *qDTY2.1*.

Recombinant selection for the target QTLs was conducted to eliminate the undesirable effect of linkage drag, if any, that may adversely affect the agronomic recovery of the NILs. The recombinant selection conducted at BC_3F_4 generation revealed that the markers RM15791 and RM16033 on chromosome 3 have recovered Pusa 44 alleles at these loci, indicating a introgression of the QTL, *qDTY3.1* free from linkage drag. Similarly, for *qDTY2.1*, the recovery of Pusa 44 alleles at the closest flanking markers, RM5791 and RM324, was complete (Figure 1).

3.2. Performance of NILs under Stressed vis-à-vis Unstressed Conditions in BC_3F_4

The preliminary screening for agronomic performance under stressed and unstressed conditions, with BC_3F_4 generation NILs, revealed significant variation for yield and related agronomic traits (Figure 2). The analysis of variance (ANOVA) for the ARBD is given in Table 3. The variation among the NILs was higher for most of the traits than that under the unstressed conditions. The ratio of variances indicated greater variation for traits such as hulling and milling recovery. Importantly, grain yield and plant height showed higher variance under unstressed situations than under stress. Similarly, the increased variance for grain quality traits was also noticed under unstressed conditions. The variation in the panicle weights of the NILs, under both the treatments, was relatively similar.

The average performances of NILs under stress and unstressed conditions (Table 4) indicated that NILs regained almost all agronomic parameters of the recurrent parent, Pusa 44 (Supplementary Table S1). The average days to fifty percent flowering among the NILs without stress was 110.5 days, with a range of 106 to 114 days, as against 108.5 days in Pusa 44. Under stress, however, there was a marginal increase of about 2–3 days for flowering in all the genotypes, including the recurrent parent. In the case of plant height, NILs varied between 92.7 cm and 109.9 cm under unstressed situations, as against the height of 97.8 cm for Pusa 44. Under drought stress, there was a decrease in height averaging about 4.0 cm. However, the decrease in tillering was remarkable under drought, to the tune of 15.4% over the tiller number under unstressed conditions. Similarly, other traits that showed significant reductions under stressed conditions were grain yield (32.9%) and weight of 1000 grains (16.7%). Grain yield per plant under unstressed treatment ranged between 15.2 g and 32.9 g among the NILs, with an average of 24.6 g as against 25.1 g in Pusa 44. Under RSDS, the grain yield in Pusa 44 was 40.6% of that under unstressed conditions, while among the NILs, 67.7% of the normal yield was realized under RSDS on an average. The yield under stress in NILs ranged between 7.7 g and 26.6 g, with a mean of 16.5 g. However, Pusa 44 yielded 10.2 g of grains per plant under RSDS.

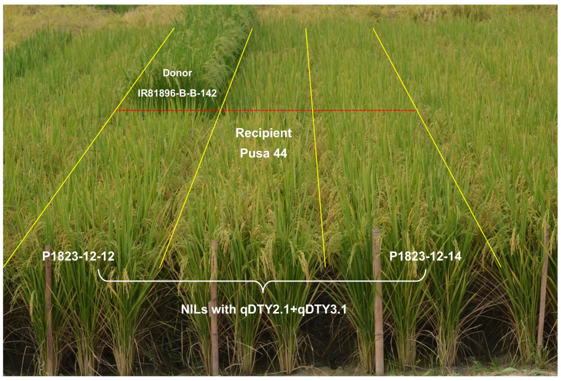

Figure 2. Field view of the selected Pusa 44 *qDTY* NILs in BC_3F_4 generation under irrigated conditions. NILs, in general, showed agronomic superiority over the recurrent parent, Pusa 44 under drought compared to irrigated conditions. The grain quality of NILs was simialr to Pusa 44, in most of the NILs.

Table 3. The analysis of variance (ANOVA) for agronomic and grain quality traits showing significant components of variance under stressed and unstressed treatments in BC_3F_4 generation.

Trait	Variance under Stress (Vs)			Variance under Unstress (Vus)			(Vs/Vus)
	NILs	Checks	NIL × Check	NILs	Checks	NIL × Check	NIL
DtF	2.40 *	906.89 *	41.08 *	2.20 *	844.15 *	91.01 *	1.09
PtH	6.90 ns	1315.62 *	1487.98 *	8.64 *	1861.47 *	2818.28 *	0.80
NpT	2.56 *	22.72 *	1.61 *	1.92 *	11.60 *	0.96 ns	1.33
PnL	1.07 ns	16.57 *	41.66 *	0.97 ns	25.06 *	3.39 *	1.10
BmP	110.34 *	611.94 *	1998.34 *	69.36 *	181.41 *	3.34 ns	1.59
PnW	29.92 ns	156.80 *	65.03 ns	30.68 *	158.37 *	811.58 *	0.98
FdG	668.29 *	1610.25 *	28,600.08 *	535.97 *	9693.43 *	1771.72 *	1.25
SpF	55.60 *	248.72 *	241.68 *	37.45 *	43.02 *	1059.19 *	1.48
GrY	9.79 *	11.00 *	511.57 *	11.22 *	63.72 *	364.31 *	0.87
GrW	1.50 *	45.37 *	27.56 *	2.66 *	14.54 *	31.54 *	0.56
HgP	84.27 *	12.87 *	75.44 *	0.73 *	7.92 *	0.09 ns	115.44
MgP	60.58 *	42.97 *	242.92 *	1.31 *	6.58 *	11.06 *	46.24
KLBC	0.04 *	9.01 *	0.00 ns	0.03 *	9.36 *	0.85 *	1.33
KWBC	0.01 *	0.15 *	0.02 *	0.15 *	0.17 *	0.16 *	0.07
LWR	0.03 *	1.87 *	0.02 *	0.06 *	1.78 *	0.01 ns	0.50
KLAC	0.12 *	21.37 *	0.19 *	0.94 *	23.48 *	0.09 ns	0.13
KWAC	0.04 *	0.54 *	1.19 *	0.13 *	0.84 *	1.50 *	0.31
ER	0.01 *	0.02 *	0.00 ns	0.03 *	0.01 *	0.09 *	0.33

DtF, days to 50% flowering; PtH, plant height in cm; NpT, number of panicle-bearing tillers; PnL, length of panicle in cm; BmP, biomass per plant in g; PnW, panicle weight in g; FdG, number of whole grains per panicle; SpF, spikelet fertility in %; GrY, grain yield per plant in g; GrW, weight of 1000 grains in g; HgP, hulling percent; MgP, milling percentage; KLBC, kernel length before cooking in mm; KWBC, kernel width before cooking in mm; LWR, length/width ratio; KLAC, kernel length after cooking in mm; KWAC, kernel width after cooking in mm; ER, elongation ratio; * Significant at 5% level; ns, non-significant.

Table 4. Comparison of average agronomic and grain quality of the NILs *vis-à-vis* parents in the BC_3F_4 generation under stressed and unstressed treatments.

Trait	Env	RP (P_1)	DP (P_2)	NILs		R_{Us}	CV	LSD
				Mean	Range			
DtF	US	108.5	120.3	110.5	106.2–114.2	−2.44	0.6	1.7
	S	111.6	122.6	113.2	108.7–115.2	-	0.5	1.3
PtH	US	97.8	136.6	102.6	92.7–109.9	4.29	1.2	3.2
	S	93.3	126.7	98.2	88.4–102.4	-	2.5	6.1
NpT	US	13.7	11.4	11.7	8.2–14.6	15.38	7.3	2.1
	S	9.0	8.0	9.9	7.2–13.4	-	4.6	1.1
PnL	US	28.2	28.3	26.5	24.3–29.1	−0.38	2.6	1.7
	S	26.3	27.0	26.6	23.7–30.1	-	3.2	2.1
GrY	US	25.1	19.9	24.6	15.2–32.9	32.93	6.8	4.2
	S	10.2	13.4	16.5	7.7–26.6	-	4.0	1.7
FdG	US	202.6	168.5	145.4	75.6–194.5	−7.69	7.2	36.0
	S	128.6	123.2	156.6	88.9–215.3		4.7	24.0
SpF	US	89.6	88.1	81.7	64.3–92.7	2.94	3.7	7.6
	S	67.9	73.5	79.3	54.1–93.8	-	2.6	5.2
GrW	US	22.4	20.5	22.2	17.4–25.2	16.67	3.4	1.9
	S	18.2	16.5	18.5	15.0–21.2	-	4.3	2.0
HgP	US	79.7	78.3	78.5	75.9–81.4	2.55	0.3	0.6
	S	76.7	79.9	76.5	71.4–81.6	-	0.4	0.8
MgP	US	72.8	71.7	72.2	67.1–75.1	6.23	0.5	0.9
	S	67.0	72.8	67.7	62.4–72.4	-	1.8	3.0
KLBC	US	6.3	6.9	5.9	5.5–6.3	5.08	1.1	0.2
	S	6.2	6.7	5.6	5.1–6.3	-	2.1	0.3
LWR	US	3.4	3.5	3.0	2.8–3.4	−3.33	1.9	0.1
	S	3.5	3.7	3.1	2.7–3.4	-	1.6	0.1
KLAC	US	10.7	11.2	9.5	9.0–10.6	3.16	2.1	0.5
	S	10.1	10.6	9.2	8.3–9.8	-	1.1	0.3
ER	US	1.7	1.6	1.6	1.4–1.8	−6.25	2.2	0.1
	S	1.6	1.6	1.7	1.5–1.9	-	2.1	0.1

RP, recurrent parent (Pusa 44); DP, donor parent (IR81896-B-B-142); Env, environments (stressed and unstressed); DtF, days to 50% flowering; PtH, plant height in cm; NpT, number of panicle-bearing tillers; PnL, length of panicle in cm; BmP, biomass per plant in g; PnW, panicle weight in g; FdG, number of whole grains per panicle; SpF, spikelet fertility in %; GrY, grain yield per plant in g; GrW, weight of 1000 grains in g; MgP, milling percentage; HgP, hulling percent; KLBC, kernel length before cooking in mm; LWR, length/width ratio; KLAC, kernel length after cooking in mm; EgR, elongation ratio; R_{Us}, relative reduction under drought over unstressed in % (R_{Us} = (trait under stress—trait under unstress) × 100/trait under unstress); CV, coefficient of variation; SE, standard error of difference; LSD, least significant difference at 5% level of significance.

Another yield component that was found largely affected under RSDS was grain weight. However, comparative grain weight reduction among the NILs and the recurrent parent was found to be almost similar, with a reduction of around 4.0 g per 1000 grains. Interestingly, grain dimension traits including raw and cooked kernel traits were found to show an inconspicuous difference between stressed and unstressed treatments, whereas milling recovery had an apparent reduction under drought. Milling percentage was found reduced by around 5% under RSDS in both NILs and Pusa 44, which however was considerably less in the donor parent, IR81896-B-B-142 (Supplementary Table S2).

The relative influence of RSDS on BC_3F_4 NILs as determined by three drought indices, namely, drought tolerance efficiency (DTE), relative decrease in yield (RDY) and drought susceptibility index (DSI), reflected that the 31 lines selected from 108 NILs were better performing over the recurrent parent, Pusa 44. The DTE of the NILs ranged between 62.74% (P1823–12-15) and 98.15% (P1823-12-83), which was significantly improved over Pusa 44, having a DTE of 40.60%. Similarly, RDY was in the range from 1.85% to 37.26% among the NILs, and DSI was ranging between 0.02% and 0.37%. These parameters again emphasized that the selection of 31 BC_3F_4 NILs for better adaptability and performance under RSDS was effective, whereas Pusa 44 indicated sensitive values for these parameters. The RDY of Pusa 44 was 59.4, while it showed DSI of 0.59. The NILs carrying the QTLs *qDTY2.1* + *qDTY3.1* therefore indicated that they can tolerate RSDS significantly as compared to the recipient parent (Supplementary Table S3), indicating that these lines can be further tested for their RSDS endurance. However, as far as the grain dimensions and cooking quality characteristics are concerned, the selected NILs were on par with the recipient parent Pusa 44 except for five NILs which possessed significantly lower kernel widths.

3.3. Yield under Drought and No Drought Conditions in Large-Scale Screening

The large-scale evaluation carried out during the 2017 *Kharif* season explicated the agronomic response of 31 NILs under both RSDS and unstressed treatments. Under unstressed conditions, the agronomic performances of the 31 NILs did not show any significant differences among themselves, or between the recurrent parent, Pusa 44, for traits such as plant height, number of productive tillers or panicle length. There was no significant variation for grain quality characteristics such as grain weight and kernel breadth before cooking as well. Similarly, under RSDS, the grain quality parameters showed non-significant deviation from Pusa 44. However, agronomic performance of NILs and Pusa 44 was on par for most of the traits under unstressed conditions, which under stress situation showed up a significant variation. This enabled the comparison of NILs for yield-related traits, unravelling the drought response patterns of different yield component traits. Moreover, the pattern of yield response of NILs both under stressed and unstressed conditions showed a good agreement between BC_3F_4 and BC_3F_5 generations (Table 5).

Table 5. Statistics of yield performance of Pusa 44 NILs along with parents and checks during *Kharif* 2017 under stressed (S) and unstressed (US) conditions.

Trait	Env	RP (P_1)	DP (P_2)	CH1	CH2	NILs	Range	CV	$(R_{Us})_{NIL}$	Pr
YdP	US	6186.1	4581.2	5427.4	4014.2	5747.0 *	4235.0–6543.0	8.6	88.0	0.45 **
	S	466.7	766.7	1050.0	416.7	690.8 **	410.0–1200.0	18.9	-	0.34 **
GrY	US	21.4	14.4	14.0	15.7	18.3 *	15.1–23.4	12.3	79.2	0.54 **
	S	2.9	5.0	6.2	3.4	3.8 **	1.7–5.9	23.7	-	0.46 **
DtF	US	113.5	120.0	119.0	100.5	113.9 **	111.0–116.0	0.5	−4.0	0.85 **
	S	119.5	123.5	121.5	108.0	118.4 **	117.0–120.5	0.5	-	0.82 **
SpF	US	84.0	79.3	81.6	78.8	79.5 **	66.7–88.1	2.2	29.9	0.81 **
	S	48.7	61.2	59.5	43.0	55.7 **	43.8–79.3	11.3	-	0.81 **
YdR	S	86.7	65.7	56.1	78.4	78.9	-	-	-	-

RP, recurrent parent (Pusa 44); DP, donor parent (IR81896-B-B-142); CH1, check 1 (IR81896-B-B-195); CH2, check 2 (IR64); Env, environments (stressed and unstressed); YdP, grain yield in kg/ha; GrY, grain yield per plant in g; DtF, days to 50% flowering; SpF, spikelet fertility in %; YdR, percentage of yield reduction under stress; $(R_{Us})_{NIL}$, relative reduction under drought over unstressed among NILs expressed in % ($(R_{Us})_{NIL}$ = (trait under stress—trait under unstress) × 100/trait under unstress); Pr, Pearson correlation between 2016 and 2017 data; CV, coefficient of variation; *,** Significant at 5% and 1% P levels, respectively.

This table clearly indicates that the percent yield reduction under stress conditions was highest in the RP and was lower in the DP, as well as other checks. Moreover, the reduction in yield among the NILs (78.9%) was lower as compared to the RP (86.7%). The relative reduction under drought compared to unstressed among NILs was found to be 79.2% for yield per plant, and 88% for grain yield, which is very high, indicating the severity of the drought during *Kharif* 2017. The correlation coefficient for both the years of trial, *Kharif* 2016 and *Kharif* 2017, was found to be highly significant for all the characteristics, such as yield per plant, grain yield, days to 50% flowering and spikelet fertility, which reveals that the NILs were showing consistent performance during both the years of evaluation.

3.4. Performance under Stress Conditions

Under drought stress conditions, significant phenotypic variations were found for all morphological traits, particularly in some of the NILs (Supplementary Table S4). Besides this, no significant differences could be observed for grain and cooking quality traits for almost all the lines, denoting the recovery of quality parameters in the selected NILs. For days to 50% flowering, eleven NILs were found to be significantly earlier than Pusa 44, with earliness values of 1.5 to 2.5 days. In the case of plant height, most of the NILs had similar heights to Pusa 44, except a few that showed slightly taller plant stature. Most of the NILs possessed longer panicles than Pusa 44, whereas, only a few NILs showed panicle length similar to or lower than the RP. In general, the NILs were found to perform better than the recipient parent, Pusa 44, for other phenotypic characteristics, such as productive tiller number, plant biomass, spikelet fertility and panicle weight. A similar situation could be noticed with all the quality parameters. For grain yield per plant, all the NILs recorded significantly higher values than Pusa 44, except 4 NILs, and 17 NILs significantly out-yielded the recipient parent for plot yield (Table 6). Among the outperforming NILs, P1823-12-82 and P1823-12-122 were the top yielders under RSDS, which had shown 157% and 111% more yield than Pusa 44 (Figure 3). Besides this, there were 15 other NILs that showed more than a 50% increased yield over Pusa 44 under RSDS. The NILs that performed well under drought also showed a significantly high number of productive tillers and high spikelet fertility, indicating their improved adaptability. The grain quality parameters, however, did not show significant deviation from those of Pusa 44 under drought conditions (Supplementary Table S5). However, there was a significant reduction in the milling properties of grains when compared to unstressed conditions, specifically for traits such as hulling and milling percentages and head rice recovery.

Table 6. Drought indices and yield performance of the Pusa 44 *qDTY* NILs during *Kharif* 2017.

ENTRIES	GrY ¶		YdP ¶		DTE	RDY	DSI
	US	S	US	S			
P1823-12-1	16.84 $^{d–i}$	4.80 $^{a–f}$	6176.00 $^{a–d}$	816.66 $^{b–e}$	28.50	71.50	0.71
P1823-12-10	18.81 $^{b–h}$	5.65 ^{abc}	6330.65 ^{ab}	766.66 $^{c–f}$	30.04	69.96	0.70
P1823-12-12	19.18 $^{a–g}$	2.20 ^{ij}	6235.99 ^{abc}	700.00 $^{d–h}$	11.47	88.53	0.89
P1823-12-14	18.40 $^{b–i}$	4.85 $^{a–f}$	6539.12 ^{a}	733.33 $^{c–g}$	26.36	73.64	0.74
P1823-12-19	17.39 $^{c–i}$	4.35 $^{a–g}$	5851.04 $^{a–e}$	616.66 $^{e–i}$	25.01	74.99	0.75
P1823-12-30	18.56 $^{b–i}$	4.00 $^{c–i}$	6064.90 $^{a–d}$	666.66 $^{d–i}$	21.55	78.45	0.78
P1823-12-33	23.45 ^{a}	3.00 $^{f–j}$	6543.06 ^{a}	750.00 $^{c–f}$	12.79	87.21	0.87
P1823-12-35	17.73 $^{c–i}$	3.50 $^{d–j}$	6090.25 $^{a–d}$	733.33 $^{c–g}$	19.74	80.26	0.80
P1823-12-38	16.86 $^{d–i}$	2.45 ^{hij}	5324.64 $^{c–f}$	716.66 $^{d–h}$	14.53	85.47	0.85
P1823-12-42	15.11 ^{ghi}	3.20 $^{e–j}$	5442.00 $^{b–f}$	716.66 $^{d–h}$	21.18	78.82	0.79
P1823-12-45	19.20 $^{a–g}$	3.15 $^{e–j}$	5850.59 $^{a–e}$	750.00 $^{c–f}$	16.41	83.59	0.84
P1823-12-50	19.25 $^{a–g}$	4.25 $^{b–h}$	6335.81 ^{ab}	816.66 $^{b–e}$	22.08	77.92	0.78
P1823-12-53	20.94 $^{a–d}$	3.75 $^{d–i}$	5959.70 $^{a–e}$	733.33 $^{c–g}$	17.91	82.09	0.82
P1823-12-55	18.05 $^{b–i}$	4.00 $^{c–i}$	6153.59 $^{a–d}$	666.67 $^{d–i}$	22.16	77.84	0.78
P1823-12-62	15.42 ^{ghi}	3.25 $^{e–j}$	5279.02 $^{c–f}$	816.66 $^{b–e}$	21.08	78.92	0.79
P1823-12-63	20.68 $^{a–d}$	3.95 $^{c–i}$	5979.95 $^{a–e}$	616.66 $^{e–i}$	19.10	80.90	0.81
P1823-12-64	20.48 $^{a–e}$	4.00 $^{c–i}$	5594.44 $^{a–e}$	650.00 $^{d–i}$	19.53	80.47	0.80
P1823-12-66	20.31 $^{a–e}$	3.15 $^{e–j}$	5971.05 $^{a–e}$	700.00 $^{d–h}$	15.51	84.49	0.84
P1823-12-68	22.30 ^{ab}	3.90 $^{c–i}$	6272.35 ^{abc}	600.00 $^{e–i}$	17.49	82.51	0.83
P1823-12-69	16.76 $^{d–i}$	1.70 ^{j}	6202.13 $^{a–d}$	433.33 ^{i}	10.14	89.86	0.90
P1823-12-72	20.61 $^{a–e}$	4.00 $^{c–i}$	6059.02 $^{a–d}$	483.33 ^{ghi}	19.41	80.59	0.81
P1823-12-76	20.05 $^{a–f}$	3.17 $^{e–j}$	6222.65 ^{abc}	533.33 $^{f–i}$	15.81	84.19	0.84
P1823-12-77	18.23 $^{b–i}$	5.35 $^{a–d}$	5492.99 $^{b–f}$	883.33 ^{bcd}	29.35	70.65	0.71
P1823-12-80	17.23 $^{c–i}$	2.40 ^{hij}	5917.69 $^{a–e}$	410.00 i	13.93	86.07	0.86
P1823-12-81	20.02 $^{a–f}$	3.15 $^{e–j}$	5605.51 $^{a–e}$	433.33 i	15.73	84.27	0.84
P1823-12-82	19.44 $^{a–g}$	5.90 ^{ab}	5454.75 $^{b–f}$	1200.00 a	30.35	69.65	0.70
P1823-12-83	16.77 $^{d–i}$	3.15 $^{e–j}$	5208.29 $^{d–g}$	600.00 $^{e–i}$	18.78	81.22	0.81
P1823-12-122	15.24 ^{ghi}	4.55 $^{a–g}$	4235.52 ^{gh}	983.33 ^{abc}	29.86	70.14	0.70
P1823-12-133	15.24 ^{ghi}	4.25 $^{b–h}$	5573.36 $^{a–f}$	666.67 $^{d–i}$	27.89	72.11	0.72
P1823-12-137	16.06 $^{e–i}$	3.40 $^{e–j}$	5028.36 ^{efg}	733.33 $^{c–g}$	21.17	78.83	0.79
P1823-12-140	19.14 $^{a–h}$	4.85 $^{a–e}$	5943.33 $^{a–e}$	550.00 $^{f–i}$	25.34	74.66	0.75
PUSA 44	21.43 ^{abc}	2.85 $^{g–j}$	6186.13 $^{a–d}$	466.67 ^{hi}	13.30	86.70	0.87
IR81896-B-B-142	14.41 ^{hi}	4.95 $^{a–e}$	4581.15 ^{fgh}	766.66 $^{c–f}$	34.35	65.65	0.66
IR81896-B-B-195	14.00 ^{i}	6.15 ^{a}	5427.44 $^{b–f}$	1050.00 ^{ab}	43.93	56.07	0.56
IR64	15.71 $^{f–i}$	3.40 ^{ef}	4014.19 ^{h}	416.67 ^{i}	21.64	78.36	0.78

GrY, grain yield per plant in grams; YdP, grain yield in kg per hectare; DTE, drought tolerance efficiency in percent; RDY, relative decrease in yield in percent; DSI, drought susceptibility index; US, unstressed; S, stressed.
¶ Means followed by similar letters are statistically non-significant at 5% level of confidence as detected by Tukey's honestly significant difference (HSD) test.

3.5. Performance under Unstressed Conditions

Under unstressed conditions, plants were maintained under sufficient moisture with recommended irrigation schedules and did not experience any stress at any crop stage. The agronomic performance of the majority of the NILs showed no statistically significant deviation from Pusa 44 for all the traits (Supplementary Table S6). The on-par performance as compared to Pusa 44 indicated that the NILs have acquired all the agronomic characteristics of the recurrent parent. Concerning grain yield per plant, the plot yield of the NILs showed an advantage over Pusa 44 under unstressed conditions that was also, however, insignificant. However, there were two NILs that had lower plot yields than Pusa 44. A similar pattern was also found with single plant yield, as expected. The grain quality under unstressed conditions showed a significant increase in hulling and milling percentage and head rice recovery (Supplementary Table S7). Other grain-related parameters, such as the number of filled grains per panicle, grain weight and spikelet fertility, had significant deviations among some of the NILs, as compared to Pusa 44. In general, the plant height of the NILs was greater under unstressed conditions when compared to that under stressed situations. Similarly, there was a reduction in days to heading under drought. Twelve NILs showed significant deviation for days to 50% flowering, of which five showed a reduction in the days. In the case of filled grains per panicle, 23 out of 31 NILs released lesser grain number; however, spikelet fertility was better among the 17 NILs.

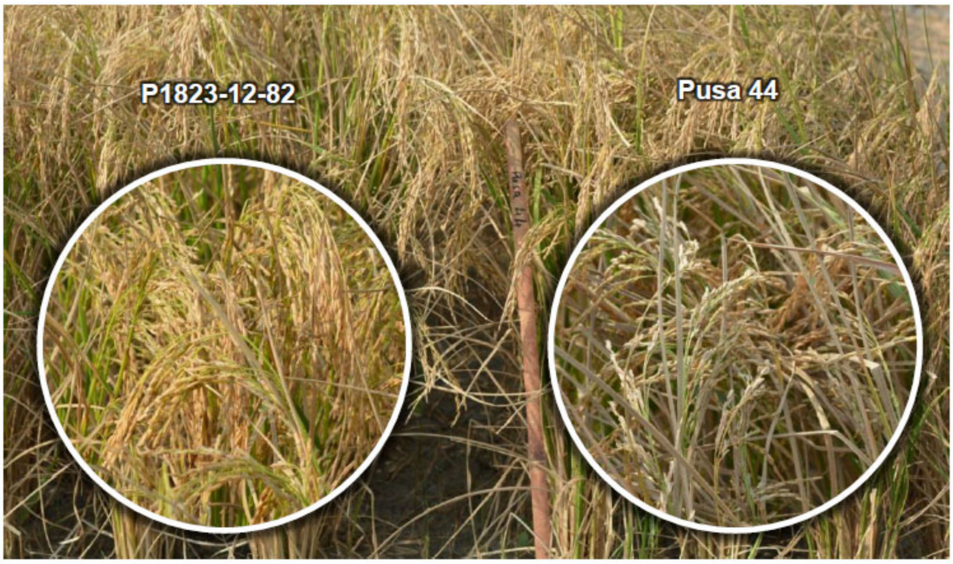

Figure 3. Comparative field view of Pusa 44 near isogenic line P1823-12-82 carrying two QTLs for reproductive stage drought stress tolerance under drought-stressed conditions. Note that P1823-12-82 had better grain filling and spikelet fertility than Pusa 44 under drought, and yielded 157% more than Pusa 44.

3.6. Comparative Performance in Terms of Drought Indices

A clear pattern of the drought endurance features of the NILs was revealed on comparative evaluation using the drought-related indices (Table 6). Among the 31 NILs that were selected during the previous season, 28 NILs showed better DTE values when subjected to a large-scale replicated trial in *Kharif* 2017. The DTE of Pusa 44 was only 13.3%, while the tolerant donor (IR81896-B-B-142) and the tolerant check (IR81896-B-B-195) had DTE values of 34.4% and 43.9%, respectively. Among the NILs, the DTE ranged between 10.1% and 30.4%, with an average of 20.7%. The highest DTE among the NILs was recorded in P1823-12-82 followed by P1823-12-10. Further, there were 15 NILs that showed above-average DTE values. The other drought impact measure, the relative decrease in yield (RDY), showed a decrease of 86.7% in Pusa 44, while IR 64 had a reduction of 78.4%. The tolerance checks had RDY values ranging between 56.1 and 65.7%. Among the NILs, however, the RDY values ranged between 69.7% and 89.9%, with an average RDY of 79.3%. When compared to the previous season, the reduction in yield was higher in *Kharif* 2017, which was due to the higher severity of drought in the later season. The third measure, DSI, that ranges between zero (0) and one (1), indicated a continuous measure of drought response from sensitivity to tolerance. However, the DSI showed the same pattern as RDY. Relatively, the NILs had an average advantage of more than 100% over Pusa 44. Although there were few NILs that showed drought responses similar to or lower than that of Pusa 44, the proportion of such NILs was relatively very low among the NILs. Based on the agronomic performance and grain quality parameters, as well as considering the drought response indices, two NILs, Pusa 1823-12-62 and Pusa 1823-12-82, were nominated under drought screening trials under the All India Coordinated Rice Improvement Project (AICRIP) for national testing. These NILs showed DTE values of 21.1% and 35.4%, respectively, with plot yields of 5279.0 kg ha^{-1} and 5454.8 kg ha^{-1} under unstressed conditions. Additionally, these NILs also showed better or similar grain quality compared to Pusa 44, with an added advantage of acceptable head rice recovery.

3.7. Multilocation Evaluation for Varietal Identification

Two of the top performing NILs that were evaluated in the national testing of drought tolerant rice cultivars performed better than the recurrent parent, Pusa 44, at two locations when grown in the two rainfed locations which are prone to drought stress for two years (Figure 4). During *Kharif* 2019, the average performance of the NILs at both Hazaribagh and Rewa was significantly superior to the recurrent parent, Pusa 44. However, under a rainout shelter, screening during 2018 at Hazaribagh indicated a slightly different pattern of RSDS response among the NILs. This notwithstanding, during both the seasons and at both the locations, the NILs yields were significantly better than Pusa 44. Among the locations, the performance of the NILs was better than those of both the parents, Pusa 44 and IR81896-B-B-142, except for Hazaribagh in 2018. Although the NILs produced significantly higher yields as compared to the recurrent Pusa 44 at Hazaribagh during 2018, there was a marginally but insignificantly lower yield than the donor parent, IR81896-B-B-142.

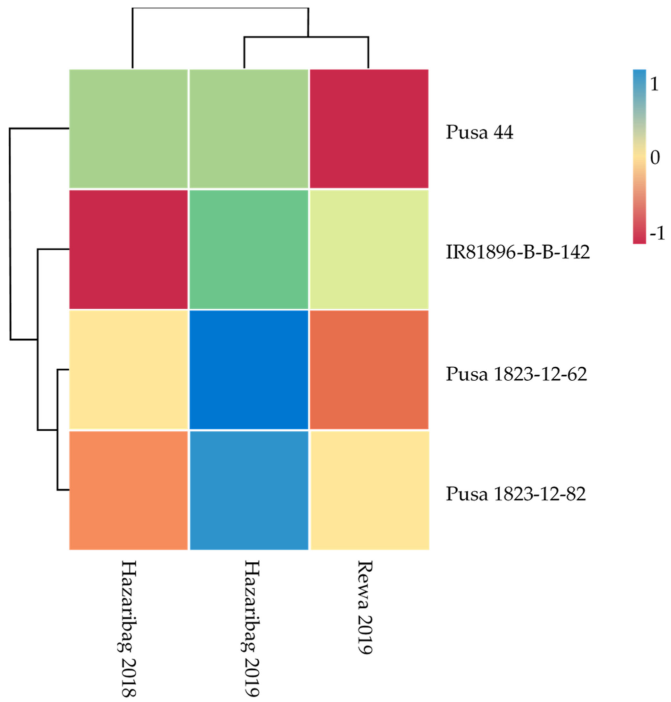

Figure 4. Heatmap of relations between the selected NILs for yield under drought stress, at two test locations, Hazaribag and Rewa, under varietal testing.

4. Discussion

In the wake of climate change, drought has undoubtedly become the most severe production constraint of all food crops, especially rice. In recent years, there have been frequent episodes of water scarcity in rice production worldwide, that have resulted in adverse effects. The consistent occurrence of drought challenges the sustenance of rice cultivation, particularly of grain yield. Drought is often dubious and occurs either with a sudden shift in climatic pattern, especially in rainfall pattern, or with a prolonged interval without rains. Coupled with high temperatures, the adverse effects of drought become multifold, often leading to crop failures. Without adequate resources, which is a major constraint in the economically weaker and agriculture-dependent nations, managing drought in physical terms is an almost impractical proposition. Harnessing external sources of water supply, which requires huge infrastructural improvements, becomes seldom practicable under erratic weather occurrences. Therefore, a more sustainable solution is to alter the cropping pattern to coincide with the probable drought occurrence, or to grow varieties that have more drought endurance features.

Drought tolerance in crops can be of two types—escape or endurance. Escape is often managed with the use of short duration varieties of crops that can complete their life cycle before the onset of drought. However, a lack of adequate short duration varieties is a major challenge in annual crops such as rice, and is not an option for perennial species. A prolonged period without precipitation may also affect the escape mechanism, leading to failure. Therefore, endurance can be considered as a better alternate for drought management in a wider perspective. Drought endurance can assure some grain production, where nothing is expected, by growing a drought-tolerant cultivar. Fortunately, there are several mechanisms of drought endurance in crops that are genetically determined. This offers us the opportunity of transferring the corresponding genes into a sensitive variety. With the characterization of these genes, or the genomic locations they reside in, it is now possible to integrate them into a new varietal background using DNA-based detectable markers. Marker-assisted breeding, particularly using the backcross method, has been extensively used for introgressing genes governing resistance/tolerance biotic as well as abiotic stresses in rice [17,44].

In rice, drought can affect crop growth in various stages, ultimately resulting in different outcomes. In the seedling stage, drought hinders population establishment, while that at the vegetative stage affects crop proliferation and tillering. Although drought at early growth stages may indirectly affect the crop yield, drought stress at the reproductive stage is more crucial because it directly affects grain development and ultimately the yield. Therefore, to breed rice varieties with drought endurance, RSDS tolerance is the most important trait to be harnessed. There are many QTLs governing tolerance to RSDS that have been mapped in rice [15–17,24], and many of them have been transferred into the well-known varieties [2]. Among these, *qDTY3.1* and *qDTY2.1* are two large-effect QTLs governing tolerance under severe lowland drought stress [16]. Identified on chromosome 3 from Apo, *qDTY3.1* was found to explain 31% of genetic variance for grain yield, while *qDTY2.1* on chromosome 2 had a significant effect on grain yield (13–16%). In this study, we have targeted these two QTLs for introgression into the popular rice variety, Pusa 44, using a NIL developed in the genetic background of Swarna with both *qDTY2.1* and *qDTY3.1* (IR81896-B-B-142) as the donor parents [16]. However, QTLs, such as *qDTY3.1*, have been reported as linked to undesirable traits, such as yield decline under non-stress [16,45,46]. This necessitates the breaking of such linkages for the effective transfer of the trait by marker-assisted backcross breeding (MABB) program [25]. The use of combined selection for the marker as well as the phenotype on a large breeding population can ensure the breaking of such linkages.

4.1. The severity of Drought Stress

During the two years of field evaluation of the Pusa 44 NILs, the severity of the drought was much higher during *Kharif* 2017 than during the previous season of *Kharif*

2016. During 2017, the RDY was found to be more than 69.7% for all the NILs, while during *Kharif* 2016 the maximum RDY was found to be 35.8%, indicating an almost twofold reduction during 2017. During 2016, however, the NILs showed better yield, suggesting a lesser drought impact during this year. Although less, the relative mean yield reduction was apparent in 2016 also, under the stress conditions; however, it was more prominent during 2017, indicating the more severe nature of drought during this season. Indicating this, during this experiment, the soil developed deep cracks due to insufficient soil moisture. In another study, Yambao et al. [47] reported a reduction of up to 70% in yield upon imposing drought for 15 days at the panicle initiation stage, and 88 and 52% reductions during stress imposition in flowering and grain filling stage, respectively. Kumar et al. [48] recommended that a screening method that could lessen the mean yield of the lines under study by at least 65% under severe stress as compared to irrigated non-stress is necessary to identify true drought-tolerant lines.

4.2. Agronomic Performance of Pusa 44 NILs under Stress and Non-Stress Conditions

Days to fifty percent flowering is a characteristic which is extremely sensitive to drought. The shift in flowering duration under stress conditions in the present experiment indicates that water stress at the initiation of the reproductive stage affected flowering time. Similar results were reported by earlier studies [15,23,45,49–56]. Bernier et al. [9] and Venuprasad et al. [16] reported that *qDTY12.1* and *qDTY3.1* influenced both grain yield and days to flowering under stress conditions, evincing that genes present within the QTLs are likely to be allied with early flowering. In the present experiment, stress was imposed 30 days after transplanting at the initiation of reproductive stage to ensure that the lines with the earliest flowering cannot escape drought, and only genuine drought-tolerant lines are selected. Similarly, there was also a significant suppression of plant height under severe stress, particularly during the 2017 season. The growth suppression further indicated that the plants did begin to experience stress much before entering into the reproductive phase. This could help us in divulging the relative endurance of the plants under prolonged drought, beginning from floral primordial initiation. We therefore presume that the drought tolerance exhibited by the NILs in the present study would be more pragmatic and would not be accounted for due to drought escape, as escape can occur solely under a transient drought rather than a prolonged one.

4.3. Relative Drought Tolerance of the Improved NILs

In the present study, all the selected lines showed a significant yield advantage over the recurrent parent under severe drought conditions. They also possessed grain and cooking quality on par with Pusa 44 during both years of evaluation. The performance of the NILs under unstressed situations proved that they recovered the maximum agronomic and grain quality attributes of Pusa 44 during the selection process. As successfully reported earlier [50–53], a stringent phenotypic selection along with marker-based background selection ensured an RPG recovery ranging between 98.6 and 99.4% after three successive backcrosses. This was further apparent from the recombinant selection data obtained later in the selection cycle, during the BC_3F_4 stage, that indicated complete recovery of carrier chromosomes.

Another trait that showed significant improvement among the Pusa 44 NILs was spikelet fertility. There were eight NILs that showed better, higher spikelet fertility than Pusa 44. A higher spikelet fertility in combination with a greater number of spikelets per panicle can ultimately result in better yield under RSDS, as observed in some of the NILs in this study. Although the relative drought response of the NILs was tested using three independently derived parameters, in practice, only two parameters were employed. This is because two of the indices, DSI and RDY, were the same, having been expressed by similar formulae. The DTE of the NILs showed significantly higher values as compared to Pusa 44, indicating the advantage they acquired by integrating both the QTLs, *qDT2.1* and *qDTY3.1*. Further, we could achieve an effective transfer of two QTLs together in three years of successive backcrossing along with phenotypic selection for agromorphological as well as grain quality traits. Additionally, selection among a large BC_3F_3 population could aid us in eliminating the undesirable effects associated with QTL, such as *qDTY3.1*, which was reported to be associated with yield decline under normal conditions. Among the NILs tested, except for eight NILs, all the remaining lines showed improved yield compared to Pusa 44, most of which was statistically insignificant. Compared by drought indices, 14 NILs out of 31 showed significantly superior performance as compared to the Pusa 44 parent, among which P1823-12-1, P1823-12-14, P1823-12-30, P1823-12-55, P1823-12-62, P1823-12-77, P1823-12-82, P1823-12-122, P1823-12-133, P1823-12-137 and P1823-12-140 showed the least RDI and DSI during both years of evaluation. Furthermore, P1823-12-14, P1823-12-77, P1823-12-82, P1823-12-122, P1823-12-137 and P1823-12-140 also had significantly higher spikelet fertility under stress conditions, and significantly lower days to flowering than the recipient parent. The NIL P1823-12-82 produced the highest yield per plant, as well as plot yield, in the replicated trial under the stress conditions. Additionally, P1823-12-14, P1823-12-55 and P1823-12-62 also produced comparatively good yields under both drought-stressed as well as unstressed conditions, along with equivalent grain and cooking as compared to Pusa 44.

We could demonstrate that QTL introgression using MABB is the most efficient way to transfer RSDS tolerance into mega/popular varieties, such as Pusa 44. Since they have acquired most of the recurrent parent features together with the target trait, these lines can be directly tested under the target environments to which the recurrent parent was originally released or widely cultivated. Moreover, the drought-tolerant NILs developed in the present study could be effectively used as potential donors for future breeding, and can be evaluated in multi-location trials to be released for commercial cultivation.

5. Conclusions

The marker-assisted transfer of two major QTLs, *qDTY2.1* and *qDTY3.1*, for grain yield under reproductive stage drought stress, into the variety Pusa 44 was accomplished, which will help in managing yield losses under drought stress, which is a major stress affecting rice production, especially under the changing climate and water limitations imposed by this change. These NILs are potential candidates for release as a variety after the required testing for replacing Pusa 44, which is highly susceptible to drought and still occupies a substantial area under cultivation. Additionally, these NILs can be used as donor lines for drought tolerance and as a component of a multiline in breeding programs. The deployment of drought-tolerant Pusa 44 NILs could reduce the water requirement and could practically cope with the present scenario of drastic climatic changes without compromising the yield and grain quality of the variety. The improved lines will also serve as an invaluable source of drought tolerance in rice improvement.

Supplementary Materials: Supplementary Materials can be found at https://www.mdpi.com/2077-0472/11/1/64/s1. Supplementary Figure S1: Marker-assisted backcross breeding scheme adopted for introgression of qDTY3.1 and qDTY2.1 in Pusa 44; Supplementary Figure S2: Graphical representation of all polymorphic markers used for background recovery; Supplementary Table S1: Yield performance of Pusa 44 qDTY NILs under drought-stressed and unstressed conditions in the BC3F4 generation (Kharif 2016); Supplementary Table S2: Grain dimensions and quality characteristics of Pusa 44 qDTY NILs under BC3F4 generation; Supplementary Table S3: Yield differential under stressed and unstressed conditions and drought indices of Pusa 44 qDTY NILs; Supplementary Table S4: Performance of selected NILs under drought stress conditions in Kharif 2017 season (BC3F5 generation); Supplementary Table S5: Quality data of Pusa 44 qDTY NILs under drought stress during Kharif 2017; Supplementary Table S6: Performance of Pusa 44 qDTY NILs under unstressed conditions in Kharif 2017; Supplementary Table S7: Quality parameters of selected NILs under unstressed conditions in Kharif 2017.

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writing—original draft preparation, P.D., K.K.V., S.G.K.; writing—review and editing, K.K.V., S.G.K.; visualization, K.K.V., P.D., S.G.K.; supervision, S.G.K., A.K.S., N.R.; project administration, S.G.K., M.P.S., A.K.S.; funding acquisition, S.G.K., M.P.S. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

ANOVA	Analysis of variance
ARBD	Augmented randomized block design
ASV	Alkali spreading value
BmP	Biomass per plant
DP	Donor parent
DSI	Drought susceptibility index
DTE	Drought tolerance efficiency
DtF	Days to 50% flowering
EgR	Elongation ratio
FdG	Number of whole grains per panicle
GGT	Graphical Genotypes
GrW	Weight of 1000 grains
GrY	Grain yield
HgP	Hulling percent
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
KLAC	Kernel length after cooking
KLBC	Kernel length before cooking
KWAC	Kernel width after cooking
KWBC	Kernel width before cooking
LWR	Length/width ratio
MABB	Marker-assisted backcross breeding
MAS	Marker-assisted selection
MgP	Milling percentage
NIL	Near isogenic lines
NpT	Number of panicle-bearing tillers
PDS	Public distribution system
PnL	Length of panicle
PnW	Panicle weight
PtH	Plant height
QTL	Quantitative trait loci

RBD	Randomized block design
RBGRC	Rice Breeding and Genetics Research Centre
RDY	Relative decrease in yield
RP	Recurrent parent
RPG	Recurrent parent genome
RPP	Recurrent parent phenome
RSDS	Reproductive stage drought stress
S	Stressed
SpF	Spikelet fertility
SSR	Simple sequence repeat
US	Unstressed
YdP	Yield per plant

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