

Article

Introgression of *qDTY1.1* Governing Reproductive Stage Drought Tolerance into an Elite Basmati Rice Variety “Pusa Basmati 1” through Marker Assisted Backcross Breeding

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Abstract: Drought stress at the reproductive stage in rice is one of the most important cause for yield reduction, affecting both productivity and quality. All Basmati rice varieties, including the popular cultivar “Pusa Basmati 1 (PB1)” is highly sensitive to reproductive stage drought stress (RSDS). We report for the first time, improvement of a Basmati cultivar for RSDS tolerance, with the introgression of a major quantitative trait locus (QTL), “*qDTY1.1*” into PB1. The QTL was sourced from an *aus* variety, Nagina 22 (N22). A microsatellite (simple sequence repeat (SSR)) marker “RM 431” located at telomeric end (38.89 mb) of chromosome 1, and located within a 1.04 mb QTL region was employed for foreground selection for *qDTY1.1* in the marker assisted backcross breeding process. A set of 113 SSR markers polymorphic between N22 and PB1 were utilized for background selection to ensure higher recurrent parent genome recovery. After three backcrosses followed by five generations of selfing, eighteen near isogenic lines (NILs) were developed, through combinatory selection for agromorphological, grain and cooking quality traits. The NILs were evaluated for three consecutive *Kharif* seasons, 2017, 2018 and 2019 under well-watered and drought stress conditions. RSDS tolerance and yield stability indicated that P1882-12-111-3, P1882-12-111-5, P1882-12-111-6, P1882-12-111-7, P1882-12-111-12, P1882-12-111-15 and P1882-12-111-17 were best in terms of overall agronomic and grain quality under RSDS. Additionally, NILs exhibited high yield potential under normal condition as well. The RSDS tolerant Basmati NILs with high resilience to water stress, is a valuable resource for sustaining Basmati rice production under water limiting production environments.

Keywords: Basmati rice; QTL introgression; near isogenic lines; yield under drought; climate resilience

1. Introduction

India is gifted with a vast rice varietal diversity spread across diverse ecosystems. The region spanning from the Himalayan foothills traversing through north-western Indo-Gangetic plains is particularly bestowed with rices of incredible quality, popularly known as Basmati [1]. Over the time, Basmati has become pride possession of Indian subcontinent, serving exquisite cuisine and thereby an export commodity for trade in the

world market. Basmati export from India alone, has earned foreign exchange worth of INR 310,255.6 million (US\$ 4330.6 million) during 2019–2020 from an annual milled rice production of more than eight million tons [2]. Besides this, almost an equal amount of as much as exported, is consumed in the domestic market. Since Basmati rice is confined to a specific geographic boundary earmarked as Basmati geographical indication (GI) area, their natural genetic diversity remains low rendering them sensitive to all major biotic and abiotic stress factors. With the ensuing threat of global climate change, rice is poised to suffer the onslaught of several stresses, particularly abiotic stresses. Among these, drought would be a major factor as global warming is recognized as one of the important causes of climate change [3]. Global warming results from atmospheric increase in greenhouse gases propelling vagaries of temperature and rainfall [4]. Furthermore, climate change envisages exacerbation of drought in the coming years [5] and the strength and frequency of drought are expected to become worse [6]. Rice requires about 3000 to 5000 L of water to yield one kilogram of rice [7], and erratic rainfall can drive rice production ambivalent in rainfed regions. Estimates indicate that almost 34 million ha of rice in rainfed lowland areas in Asia alone recurrently suffers from water stress, in addition to an area of eight million ha under upland ecology [8].

Rice is grown throughout the year in India depending on the geographical region, in different seasons such as *Kharif* and *Rabi*. *Kharif* season coincides sowing in late summer, and is the principal rice growing season. *Rabi* which coincides with the winter sowing, is however limited to peninsular India, where winter is mild. *Rabi*, however, forms the second most important rice growing season with plantings restricted to southern and eastern India. Depending on geography, the weather parameters of *Kharif* and *Rabi* season vary, including intermittent spells of stress such as drought. Onset of drought can happen in all stages of rice crop. However, sensitivity to the drought stress depends on its duration and intensity. Although rice is affected badly in all phenological stages of growth, the stress occurring during the reproductive stage is particularly perilous, leading to significant reduction in grain yield [9,10]. The impact on rice plant is multifaceted, ranging from the reduction in dry matter accumulation to poor apportioning of metabolites from the source (stem and leaves) to sink (grain) [11]. This results in reduced number of filled grains per panicle, reduced grain weight and, ultimately, the grain yield. Varietal diversity in rice embodies various mechanisms for drought tolerance particularly at the reproductive stage. Tolerance is established through manifestations such as reduction in the tiller number, leaf area, shortening and thickening of leaves, leaf rolling and promotion leaf senescence [12,13]. These mechanisms offer opportunities for varietal improvement targeting drought prone areas. Additionally, breeding of futuristic cultivars resilient to multiple stresses requires enhanced drought tolerance, especially at the reproductive stage. Drought stress at reproductive stage leads to delay in flowering which slows down the panicle development, impairs flowering and grain development leading to crop loss, that can be total at severe situations. Stress occurring at anthesis stage leads to sterile panicle, because the panicle that is still inside the leaf sheath, fails to develop. Failure of reproduction ultimately translates into significant yield decline. The reproductive stage drought stress (RSDS) tolerance in rice is considered as a complex and multifarious trait, governed by several genes, major and minor. These may include a large number of quantitative trait loci (QTLs) that may comprise of structural and functional genes as well as regulatory elements such as transcription factors, controlling a multitude of morpho-physiological and biochemical responses [14].

Exploiting molecular markers of various kind, several studies have reported QTLs governing drought tolerance in rice, primarily using grain yield under drought stress (DTY) as the surrogate trait. Among these, *qDTY1.1* is a major QTL for grain yield under drought stress (explaining about 12.6% to 16.9% of phenotypic variance), mapped on chromosome 1 independently from two tolerant cultivars such as Nagina 22 (N22) [15] and Dhagaddeshi [16]. *qDTY1.1* was the first QTL reported to have consistent effect in different genetic backgrounds and was shown to affect many drought related traits like root length, relative water content, root thickness, osmotic adjustment and biomass [16,17]. Previous

studies have demonstrated that *qDTY1.1* was flanked by markers RM431 on telomeric end and RM11943 on the centromeric end. Additionally, Bernier et al. [17] identified a different major QTL on chromosome 12, *qDTY12.1*, from the cross between Vandana and Way Rarem explaining approximately 51% of phenotypic variation. There are also other major QTLs reported which were demonstrated effective either under upland or lowland situations [14,18].

Marker assisted introgression of major-effect QTLs could be a proficient and rapid approach for breeding rice varieties tolerance to drought stress [17]. Consequent attempts to introgress/pyramid these QTLs by marker assisted selection, primarily into mega-varieties has found significant advancements towards breeding climate-adaptive cultivars [19,20], such as Sabitri (*qDTY3.2* and *qDTY12.1*) [21], IR 64 (*qDTY2.2* and *qDTY4.1*), Vandana (*qDTY12.1*) [22] and Pusa 44 [23]. In the past twelve years, there has been about sixty-six RSDS tolerant varieties released around the world, which involve several of these QTLs [14]. Although important, there has not been a previous attempt to improve Basmati cultivars for RSDS tolerance. One of the major reasons for this hiatus was the lack of donors from the Basmati group. Use of donors from the non-Basmati backgrounds for Basmati improvement, present a major challenge of loss of grain quality of the Basmati parent, while introgression [24]. However, marker assisted backcross breeding coupled with phenotypic selection, has been demonstrated to effectively address this problem [25–27].

Developed by the Indian Council of Agricultural Research (ICAR)-Indian Agricultural Research Institute (ICAR-IARI), New Delhi and released for commercial cultivation during year 1989, Pusa Basmati 1 (PB1) is the world's first semi-dwarf (105 cm) Basmati variety with high yielding potential. This variety, that showcased a tremendous level of improvement over the traditional Basmati cultivars that were low yielding (~2.3 tons/ha), photosensitive, tall (>150 cm), lodging with weaker stem and of long duration (>150 days), became popular among the farmers in no time. Besides the high yield (5.0 tons/ha), PB1 possesses insensitivity to photoperiodism, resistance to lodging, superior grain quality, semi-dwarf stature and a duration of 135–140 days. The grain quality of PB1 was adorned with strong aroma with explicit cooking qualities such as high kernel elongation ratio of 1.8, an average milled grain length of 7.4 mm and cooked kernel length of 13.7 mm [19]. However, as that of traditional cultivars, PB1 too is highly sensitive to several stress factors such as pests and diseases as well as drought. This study forms the maiden attempt to improve any Basmati cultivar towards climate resilience, by introgressing RSDS tolerance into PB1. The augmented objectives were to recover the Basmati grain quality traits as well as the recovery of all the agronomic traits including yield in the near isogenic lines (NILs). Further, evaluation of the improved NILs across multiple locations and environments to ascertain the stability in yield and drought tolerance to deploy as commercial Basmati rice cultivar.

2. Materials and Methods

2.1. Plant Material and Experimental Sites

PB1, the elite Basmati cultivar was selected as the recurrent parent. An *aus* cultivar, N22, was used as donor parent for the QTL, *qDTY1.1*. N22 is a pure line selection from a landrace, Rajbhog. Well-known as a universal donor for high temperature tolerance, drought tolerance and grain dormancy [28], N22 is a tall (~120 cm), short duration (90–95 days) cultivar, with short bold non-aromatic grains. The entire study was conducted between two locations, New Delhi and Aduthurai. Experiments at New Delhi was conducted in the research fields of the Genetics Division, ICAR-IARI, situated at 28°35' N latitude, 77°12' E longitude with an altitude of 228.16 m above mean sea level. The other experimental site at Aduthurai was the research farm of IARI-Rice Genetics and Breeding Research Centre situated at 11°00' N latitude, 79°28' E longitude and 20 m above MSL. The whole generation advancement between New Delhi and Aduthurai was carried out through shuttle breeding approach. Final drought stress evaluation of NILs was done at New Delhi for three subsequent crop seasons.

2.2. Development of NILs through Marker Assisted Backcrossing

Hybridization was taken up between PB1 and N22 at New Delhi, using PB1 as recipient (female) and N22 as donor (male) in *Kharif* 2013. Hybrid seeds (F_1 s) were harvested and propagated in the late *Rabi* season of 2013–2014 at Aduthurai, Tamil Nadu. F_1 s were backcrossed to PB1 as recurrent parent (RP), to generate BC_1F_1 . During subsequent seasons, two successive backcrosses were done to generate BC_2F_1 and BC_3F_1 during 2014 and 2014–2015. In the BC_3 generation, from *Kharif* 2015, four successive selfings were carried out to generate BC_3F_5 NILs by 2016–2017 late *Rabi* season at Aduthurai. All through during different generations, selections were carried out to recover maximum recurrent parent genome recovery (RPGR) among the progenies. The selected NILs, beginning from the BC_3F_4 generation, were subjected to three consecutive drought screenings at New Delhi during *Kharif* seasons 2017 to 2019.

For molecular marker analyses, the genomic DNA was isolated from fresh leaf samples using CTAB method with minor modifications [29]. Polymerase chain reaction was performed with the volume of 10 μ L containing 20–30 ng template DNA, 5 pmol of each primer, 0.05 mM dNTPs (MBI, Fermentas, Lithuania, USA), 10 \times PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl and 1.8 mM $MgCl_2$) and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India), running a amplification profile consisting of one cycle of initial denaturation at 94 $^\circ$ C for 5 min; followed by 35 cycles containing denaturation with at 94 $^\circ$ C for 30 s, annealing at 55 $^\circ$ C for 30 s, extension at 72 $^\circ$ C for 1 min; and a final extension at 72 $^\circ$ C for 7 min. The amplified PCR products were resolved by electrophoresis having 3.5% agarose gel and fluoro-stained with ethidium bromide. The amplicon resolution was photographed using a Gel Doc XR+[®] (BioRad Laboratories, Hercules, CA, USA) gel documentation system.

For the marker analyses, marker sequences were downloaded from the marker database at Gramene (<http://www.gramene.org>) and the primers were custom synthesized from Sigma-Aldrich (Bengaluru, India). The *qDTY1.1* linked simple sequence repeat (SSR) marker RM431 (F: tctgcgaaactgaagagttg; R: agagcaaaacctggtcac) was polymorphic between the parents and was thus used for foreground selection [15]. For background analysis, the genome wide polymorphism between PB1 and N22 was determined using a genome wide survey employing 651 SSR markers, which resulted in identification of 113 markers that could distinguish both the genotypes. These polymorphic SSR markers spanned across the rice genome and were used for background selection in various backcross generations (Supplementary Table S1), using the reductive screening approach [30]. The recovery of PB1 type alleles among the backcross progenies during each generation was used for computing the RPGR [31]. For the graphical comparison of the genomes of NILs and parents, graphical genotypes were drawn using GGT v.2.0 [32].

2.3. Field Management under Stressed and Unstressed Conditions

In order to assess the efficiency of *qDTY1.1* introgression into PB1, the selected NILs along with their parents and checks were evaluated for three consecutive *Kharif* seasons of 2017 to 2019 period at ICAR-IARI, New Delhi, under an irrigated ecology with drought stress imposed at reproductive stage. The drought tolerant check was IR86918-B-B-305, a backcross inbred line carrying *qDTY1.1* in the background of IR64 developed from the cross IR64/N22. The sensitive check used in this experiment was IR64. Two treatments were maintained, stressed and unstressed. Experiments were laid out using a randomized complete block design (RCBD) with two replications for each stress treatment. The plot size was 6.5 m² with a spacing of 20 by 15 cm. Approximately 25 g of seeds were nursery sown to raise the population. In the plots designated for stress treatment, tensiometers were installed at every six NILs to monitor and characterize the soil moisture status. Initially, for both the treatments, seedlings were raised in a wet-bed nursery and after 21 days transplanted into a flooded field with 5 cm standing water. To ensure uniform establishment of the transplanted seedlings, all the plants were maintained under irrigation for 30 days post transplanting. At the 31st day after transplanting, water from the stress

treatment plots were drained to initiate the stress. The stressed plots were left un-irrigated until the soil moisture tension reached -70 kPa at 30 cm depth. Severe leaf rolling and leaf drying were observed at this soil moisture level. At this severe stress, a flash life-saving irrigation was provided, and the excess water was drained out approximately after 24 h. This cycle was constantly repeated until harvest [15,33]. The unstressed plots were maintained with normal irrigation and the plants were maintained in the standing water. Altogether, three irrigations were given into stressed plots, while the unstressed plots were irrigated six times. During the crop duration, from June to November, a total of 839.8, 913.4 and 608.1 mm of rainfall was received in the years 2017, 2018 and 2019, respectively.

2.4. Phenotypic Data Collection

From each of the NILs under both treatments, phenotypic data were recorded from five randomly tagged plants on days to 50% flowering (DF)—the number of days from sowing to flowering in 50% plants/tillers was recorded, plant height at maturity (PH)—was measured from ground level to the tip of main panicle, panicle length (PL)—average length of the primary panicles was taken from peduncle base to the tip, number of reproductive tillers (NT)—average number of tillers containing grain filled panicles per plant, spikelet fertility % (SF)—the proportion of filled grains to the total number grains per panicle and grain yield (GY)—the harvested grain from the plant were dried to optimum moisture level of approximately 14% after which they were weighed. The NILs were also characterized for kernel length and breadth before and after cooking. From these measurements, length/breadth ratio (LBR) and cooking quality characteristics, such as kernel length elongation ratio (KER) were computed. Further, alkali spreading value (AS) and aroma were determined using standard protocols [34].

2.5. Statistical Analyses

Initially, independent analyses of variance (ANOVA) was carried for each season to identify significant responses among the NILs under stressed and unstressed situations and to compare them with the checks. A subsequent combined ANOVA was carried out using linear mixed model approach with genotypes as fixed factor and seasons and stress as random factors. The trait predictions from the model was saved as best linear unbiased predictors (BLUPs). The data were analyzed using STAR package version 2.0.1. (<http://bbi.irri.org/products>). To identify the genotypes with stable performance across the environments, seasons under drought condition, an additive main effective and multiplicative interaction (AMMI) model was constructed using BLUPs and AMMI stability value (AST) [35] and yield stability index (YSI) [36] were generated, as follows:

$$\text{AMMI stability value, AST} = \sqrt{\left[\frac{\text{SS}_{\text{IPC1}}}{\text{SS}_{\text{IPC2}}} \times \text{IPC1} \right]^2 + (\text{IPC2})^2} \quad (1)$$

$$\text{Yield stability index, YSI} = R^{\text{AST}} + R^{\text{Y}} \quad (2)$$

where, SS_{IPC1} and SS_{IPC2} are the sum of squares of interaction principal component axes (IPCA) 1 and 2, respectively, and IPC1 and IPC2 are the respective IPCA scores. Similarly, R^{AST} and R^{Y} are the respective genotypes ranks based on AST and yield (Y).

To compare the effect of drought on different traits, a forward stepwise regression analysis was carried out for delineating the traits which contributed significantly to yield. Based on the BLUPs for grain yield under stressed (S) and unstressed (NS) treatments, different indices namely, drought yield index [37], stress tolerance index [38], stress susceptibility index [39], and percent reduction in yield were worked out as follows:

$$\text{Drought yield index, DYI} = \frac{(Y_i)^{\text{NS}} / (Y_i)^{\text{S}}}{(Y_G)^{\text{NS}} / (Y_G)^{\text{S}}} \quad (3)$$

$$\text{Stress tolerance index, STI} = \frac{(Y_i)^{NS} \times (Y_i)^S}{(Y^{NS})^2} \quad (4)$$

$$\text{Stress susceptibility index, SSI} = \frac{(Y_i)^{NS} - (Y_i)^S}{Y^{NS} - Y^S} \times \frac{Y^{NS}}{(Y_i)^{NS}} \quad (5)$$

$$\text{Percent reduction in yield, \%R} = \frac{(Y_i)^{NS} - (Y_i)^S}{(Y_i)^{NS}} \times 100 \quad (6)$$

where, Y_i represents the mean yield of a genotype i on the untransformed scale, Y_G refers to geometric mean across genotypes, the suffices, NS and S represent unstressed and stressed conditions, respectively and Y refers to the arithmetic mean yield across genotypes.

3. Results

3.1. Parental Polymorphism

The genome wide polymorphism survey using 651 SSR markers, revealed a diversity of 17.4% between the parents PB1 and N22, identifying 113 markers to be polymorphic between them (Table 1). Out of 101 markers tested on chromosome 1, the carrier chromosome of *qDTY1.1*, 14 markers were observed polymorphic including the linked marker, RM431. The target chromosome diversity was 13.9%. Of the remaining chromosomes, highest diversity was found for chromosome 8 (40.9%) while the chromosome 6 (9.2%) indicated low diversity.

Table 1. Genome wide marker polymorphism between the recurrent parent, Pusa Basmati 1 (PB1) and donor parent, Nagina 22 (N22).

Chromosome	No. of Markers		Marker Diversity * (%)
	Total Surveyed	Polymorphic	
1	101 [¶]	14	13.86
2	74	12	16.21
3	64	12	18.75
4	61	10	16.39
5	71	9	12.67
6	87	8	9.19
7	55	13	23.63
8	22	9	40.90
9	18	4	22.22
10	13	5	38.46
11	34	9	26.47
12	49	8	16.32
Total	651	113	17.35

* Marker diversity = Number of polymorphic markers \times 100/ Total number of markers. [¶] Foreground marker, RM431 not counted among the chromosome 1 markers.

3.2. Introgression of *qDTY1.1* into PB 1

The breeding scheme for development of PB1 NILs carrying *qDTY1.1* is given in Figure 1. From a total of 23 seeds initially collected of the cross PB1/ N22, five plants were found to be pure hybrids showing heterozygosity for the foreground marker, RM431 linked to *qDTY1.1*. These F_1 s were backcrossed to the recurrent parent, PB1 to obtain 39 BC_1F_1 seeds. Out of these, 15 seedlings were confirmed heterozygous for *qDTY1.1* by foreground selection. These 15 plants were further analyzed with 113 polymorphic background SSRs (Table 2). Based on the recovery of PB1 alleles, the RPGR among the BC_1F_1 plants was estimated to range between 78.0% and 86.7%. The plant with highest recovery (86.7%) had 83 background markers in homozygous state for recurrent parent (RP) allele, while 30 markers were heterozygous. Further, these plants were also tested for their agronomic and grain quality similarities with PB1. The plant showing phenotypic resemblance and

high RPGR (86.7%) was used to backcross with PB1 to generate 37 BC₂F₁ seeds. However, only one BC₂F₁ plant was found to be heterozygous for RM431. Background analysis on this plant using 30 markers heterozygous from the previous backcross generation, showed the RPGR of 94.7%. Subsequent backcrossing of this plant to PB1 yielded 25 seeds. On raising the BC₃F₁ generation from these seeds, five plants were found to be heterozygous for *qDTY1.1* linked marker, RM431. Further the one F₁ out of five with maximum RPGR and excellent cooking quality was selected for further generation advancement.

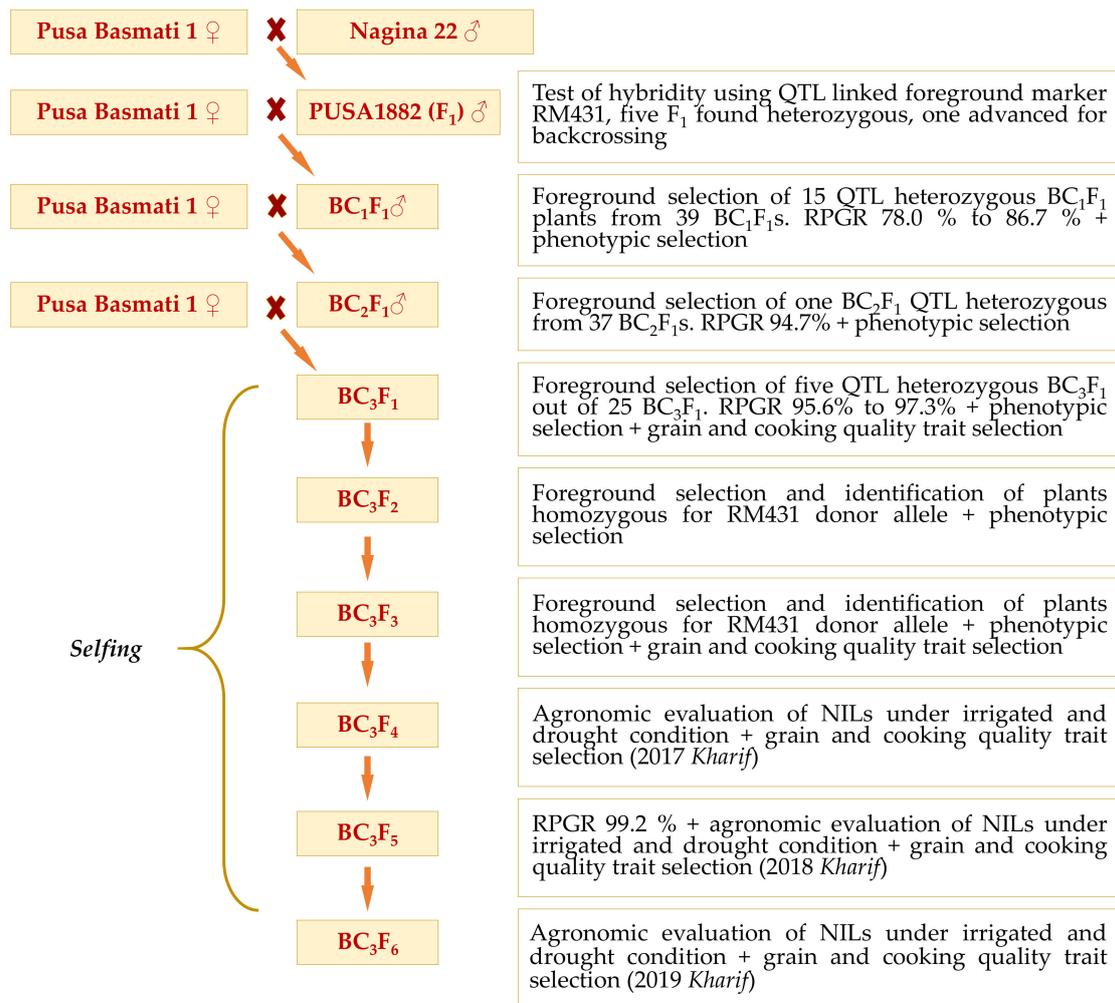


Figure 1. Breeding scheme used in the marker-assisted backcross program for the transfer of *qDTY1.1* quantitative trait locus (QTL) in the background of the elite rice variety, Pusa Basmati 1. Recurrent parent genome recovery (RPGR) denotes recurrent parent genome recovery.

Background selection with the remaining unrecovered heterozygous markers among the BC₂F₁ plant, resulted in an RPGR ranging from 95.6% to 97.4%. These BC₃F₁ plants were further subjected to phenotype matching with PB1, and the plant with maximum genome recovery (97.4%) as well as phenotype similarity was used for further step of selfing. Selfing the selected BC₃F₁, yielded 229 BC₃F₂ seeds. Through foreground followed by background selections of these BC₃F₂ plants, 55 plants homozygous for *qDTY1.1* with an RPGR of 97.8–98.7% were identified. All these 55 plants were advanced to BC₃F₃ families which underwent a rigorous phenotype selection for agro-morphological and grain quality traits to select 24 families for further advancement to BC₃F₄ generation. The BC₃F₄ families were grown under both irrigated and stressed conditions. Subsequent selection among the 24 BC₃F₄ families was carried out for agronomic performance, drought response and grain quality. This resulted in 18 families of near isogenic lines, having close similarity to

PB1 and comparable grain and cooking qualities. Phenotype matching of these 18 NILs with PB1, both in terms of agronomic features and grain quality, identified them closer to PB1. All the 18 NILs had target marker pattern of the donor (250 bp) parent for *qDTY1.1* (Figure 2). The RPGR at BC₃F₅ generation, ranged between 98.2% and 99.1%. All along the selfing generation, selection was carried out with major emphasis on recovery of grain quality, agro-morphological features, and yield of PB1, among the NILs. The graphical genotype of targeted segment for *qDTY1.1* from donor and recovery of background genome on chromosome 1 in 18 NILs are depicted in Figure 3.

Table 2. Progressive selection statistics for the development of PB1 near isogenic lines introgressed with the reproductive stage drought stress (RSDS) tolerance QTL, *qDTY1.1*.

Generation	No. of Seedlings/Families Tested			RPGR (%)		
	Total	QTL Positive	Selected	Range	Mean	Average Gain
F ₁	23 (s)	5	1	50.0	50.0	0.0
BC ₁ F ₁	39 (s)	15	1	78.0–86.7	73.4	23.4
BC ₂ F ₁	37 (s)	1	1	94.7	94.7	21.3
BC ₃ F ₁	25 (s)	5	1	95.6–97.4	96.5	1.8
BC ₃ F ₂	229 (s)	95	55	97.8–98.7	98.2	1.7
BC ₃ F ₃	55 (f)	55	24	*	-	-
BC ₃ F ₄	24 (f)	24	18	*	-	-
BC ₃ F ₅	18 (f)	18	18	98.2–99.1	99.0	0.8

* Not tested; RPGR, Recurrent parent genome recovery; s, seeds; f, families.

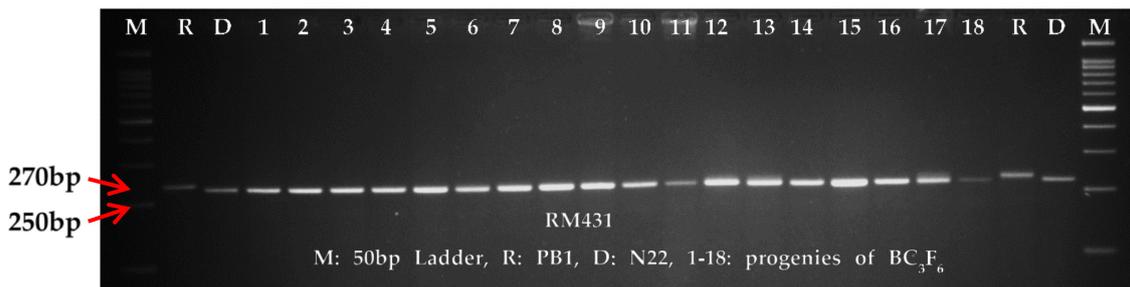


Figure 2. Foreground selection of PB1 near isogenic lines (NILs) for *qDTY1.1* using the marker, RM 431.

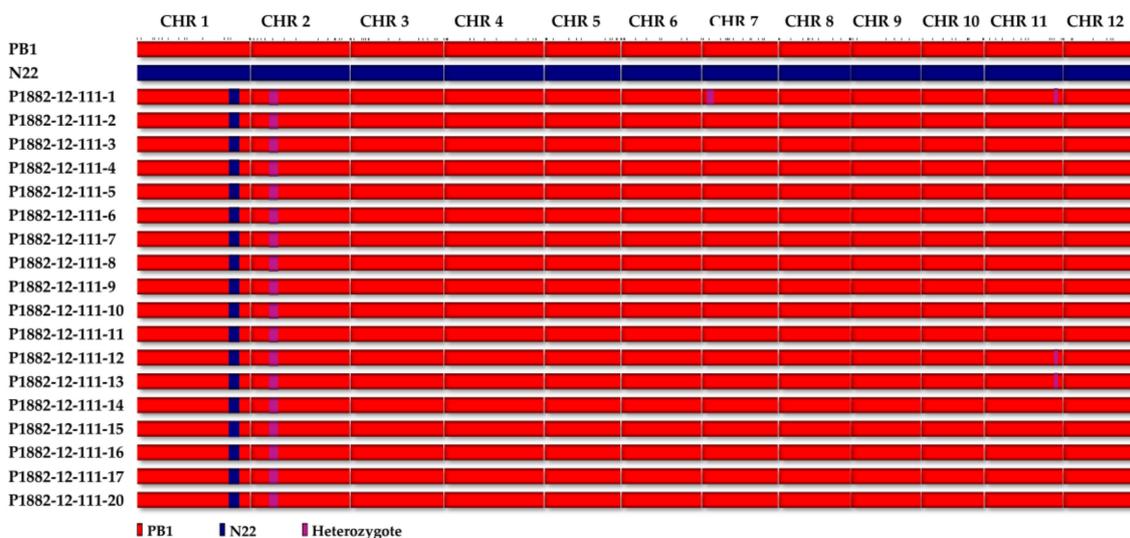


Figure 3. Graphical representation of the genotypes of 18 *qDTY1.1* introgressed NILs of PB1. All the NILs had maximum recovery on the carrier chromosome 1. CHR: Chromosome.

3.3. Recovery of RP Alleles in Carrier Chromosome

The polymorphism survey between PB1 and N22 on chromosome 1, the carrier chromosome of *qDTY1.1*, was performed using 102 markers, of which 14 were found polymorphic including the foreground marker, RM431. There were two flanking markers for *qDTY1.1* in the marker array, RM431 and RM11943. Among these, only RM431, the telomeric end marker was found polymorphic between the parents. RM11943 was found monomorphic. Further, among the downstream markers towards the telomeric end, only one marker RM6840 was polymorphic. However, RM6840 was at the distal end, 4.27 Mb away from RM431. On the centromeric end, however, the next upstream polymorphic marker was RM3825. RM3825 was used for recombinant selection. These markers, except for RM431, were included in the whole genome background recovery analysis. All the 14 polymorphic markers in chromosome 1 showed complete recovery of RP alleles among the 18 NILs by BC₃F₅ generation (Supplementary Figure S1).

3.4. Per se Performance of the NILs under Reproductive Stage Drought Stress and Unstress Treatments

Agronomic evaluation of NILs along with parents and checks over the three *Kharif* seasons under field-imposed drought stress as well as under normal conditions, indicated significant variation for several traits (Table 3). These evaluations belonged to BC₃F₄, BC₃F₅ and BC₃F₆ generations. ANOVA over individual years revealed significant variation for genotypes, treatment and genotype \times treatment components, for most of the agronomic characters studied. However, only two traits, SF and GY showed consistently significant variation for genotype \times treatment component in all the seasons. For the remaining traits, during 2018, genotype effects for NT and genotype \times treatment effects for DF, NT and PL were found non-significant, as that of the genotype \times treatment effect of NT in 2017. Combined ANOVA, indicated a uniform pattern across the traits, particularly for GY and SF. NT had non-significant effects for various sources of variation except genotype component. Traits that showed significant variation for all the sources except for one component, included DF and PH where in year \times treatment interaction was non-significant, while PL had non-significant year \times genotype effect. Except for PL and NT, year \times treatment \times genotype interaction was significant for all the other traits. Least significant difference (LSD) values generated from the individual ANOVA in different years were utilized for mean comparison of NILs with checks.

Considering the individual traits (Supplementary Table S2), DF exhibited uniform pattern across three experimental years, but a general delay was observed in flowering under reproductive stage drought stress. The delay was conspicuous in IR64 and was particularly apparent during 2019 period. Among the NILs, P1882-12-111-9 and P1882-12-111-10 showed significant delay in DF under stress during year 2017 than rest of the NILs and PB1. In the same year, there were five NILs that showed significant late flowering than PB1. During 2018, both under stressed as well unstressed conditions, DF of all the NILs were at par with PB1. Similarly, during 2019 season too, DF of all the NILs were similar to PB1, except for P1882-12-111-1 under stress, that showed delayed flowering. On an average, the flowering delay under stress was between one to five days among the NILs. Overall, it was observed that drought stress increased the vegetative period in most of the NILs and checks across all the three years of evaluation (Table 4).

Table 3. Analysis of variance (ANOVA) for agronomic performance of NILs in individual years and combined under two stress treatments, stressed and unstressed.

Traits	Seasons	C.V (%)	Component Variance								
			Block	Genotype (G)	Stress (S)	G × S	Error	Year (Y)	Y × S	Y × G	Y × S × G
DF	2017	1.1	2.6	148.5 **	102.6 **	27.9 **	1.1				
	2018	1.9	1.9	175.2 **	23.0 *	5.0	3.7				
	2019	1.1	2.9	278.2 **	192.0 **	2.6 *	1.4				
	Combined	1.3	1.2	276.1 *	548.5 **	15.6 **	1.6	1443.4 **	20.7	26.7 **	9.9 **
PH	2017	4.8	9.0	220.8 **	1.7E+04 **	26.3	18.1				
	2018	2.8	2.4	458.1 **	1.1E+04 **	22.5 **	7.2				
	2019	2.0	8.2	325.0 **	1.2E+04 **	14.8 *	3.4				
	Combined	2.8	6.4	3.9E+04 **	930.2 **	20.0 **	7.2	3850.5 **	204.2	36.9 **	21.7 **
NT	2017	14.7	17.0	4.8 *	46.8 **	2.2	2.6				
	2018	13.2	25.8	2.5	255.7 **	2.7	3.5				
	2019	10.6	14.6	4.9 *	207.1 **	4.1 *	2.1				
	Combined	11.9	9.7	184.8	5.3 **	2.1	2.3	243.8	162.3	3.5	3.4
PL	2017	2.6	0.0	19.8 **	58.5 **	2.0 **	0.5				
	2018	4.3	0.1	10.9 **	536.1 **	1.4	1.0				
	2019	3.4	1.7	14.7 **	253.6 **	0.6	0.8				
	Combined	3.3	0.7	728.0 **	42.2 **	1.8 **	0.7	34.0 **	60.1 **	1.5	1.1
SF	2017	5.0	126.3	89.4 **	2.1E+04 **	37.0 **	11.9				
	2018	6.8	23.9	86.9 **	1.1E+04 **	58.3 **	23.8				
	2019	3.7	9.5	56.4 **	4866.1 **	21.5 **	5.3				
	Combined	7.2	1.7E+05	6.7E+08 **	2.5E+05 **	1.1E+05 **	2.5E+04	3.0E+08 **	1.7E+08 **	3.4E+05 **	1.6E+05 **
GY	2017	7.4	3.3E+05	2.3E+05 **	6.1E+08 **	1.8E+05 **	5.8E+04				
	2018	5.7	2.2E+04	6.9E+05 **	4.1E+08 **	2.5E+05 **	3.2E+04				
	2019	3.1	3.1E+04	4.4E+05 **	3.9E+08 **	3.8E+05 **	1.9E+04				
	Combined	4.8	1.2E+04	1.3E+08 **	1.5E+05 **	1.3E+05 **	3731.6	3.9E+08 **	1.3E+08 **	1.5E+05 **	1.3E+05 **

DF, days to 50% flowering; PH, plant height in cm; NT, number of tillers; PL, panicle length in cm; SF, spikelet fertility in %; GY, grain yield in kg/ha. *, ** indicates significance at 5% and 1% level, respectively.

Table 4. Average agronomic performance of NILs, parents and checks under drought stressed (S) and unstressed (NS) conditions over three seasons.

NILs	DF		PH		NT		PL		SF	
	S	NS	S	NS	S	NS	S	NS	S	NS
P1882-12-111-1	108.5 ^a	103.7 ^{ab}	78.2 ^{cd}	108.0 ^c	12.0 ^a	14.6 ^a	24.1 ^b	28.9 ^a	50.1 ^c	80.1 ^{bc}
P1882-12-111-2	103.5 ^{bc}	100.8 ^{cd}	80.9 ^c	104.9 ^{cd}	12.4 ^a	14.0 ^a	26.6 ^a	28.5 ^a	62.3 ^{ab}	83.7 ^{abc}
P1882-12-111-3	103.7 ^{bc}	100.2 ^d	79.9 ^c	103.2 ^{cd}	11.8 ^a	13.7 ^a	25.7 ^{ab}	28.2 ^a	60.1 ^{ab}	82.8 ^{abc}
P1882-12-111-4	104.7 ^{bc}	104.5 ^a	80.6 ^c	106.6 ^c	11.5 ^a	14.9 ^a	25.3 ^{ab}	28.2 ^a	64.1 ^{ab}	82.6 ^{abc}
P1882-12-111-5	103.3 ^{bc}	102.5 ^{a-d}	80.7 ^c	105.0 ^c	11.3 ^a	14.0 ^a	25.5 ^{ab}	29.0 ^a	65.1 ^a	85.0 ^{ab}
P1882-12-111-6	102.2 ^c	101.2 ^{b-d}	80.0 ^c	105.9 ^c	11.6 ^a	14.7 ^a	25.5 ^{ab}	28.5 ^a	57.7 ^b	86.7 ^{ab}
P1882-12-111-7	102.7 ^{bc}	101.2 ^{b-d}	81.8 ^c	105.6 ^c	13.5 ^a	15.7 ^a	26.4 ^a	29.1 ^a	57.8 ^b	84.8 ^{abc}
P1882-12-111-8	105.0 ^{bc}	102.7 ^{a-d}	79.5 ^c	105.7 ^c	12.3 ^a	14.5 ^a	25.6 ^{ab}	28.5 ^a	59.9 ^{ab}	79.9 ^{bc}
P1882-12-111-9	103.7 ^{bc}	103.0 ^{a-d}	80.2 ^c	106.9 ^c	11.6 ^a	13.8 ^a	25.4 ^{ab}	28.7 ^a	59.5 ^{ab}	83.2 ^{abc}
P1882-12-111-10	105.5 ^b	103.3 ^{a-c}	78.7 ^{cd}	103.3 ^{cd}	10.9 ^a	14.1 ^a	24.9 ^{ab}	28.4 ^a	63.3 ^{ab}	83.1 ^{abc}
P1882-12-111-11	104.5 ^{bc}	103.2 ^{a-c}	79.0 ^c	104.9 ^{cd}	10.6 ^a	15.5 ^a	25.3 ^{ab}	29.5 ^a	57.2 ^b	81.4 ^{abc}
P1882-12-111-12	103.3 ^{bc}	102.0 ^{a-d}	82.2 ^c	106.4 ^c	10.6 ^a	13.4 ^a	25.9 ^{ab}	29.4 ^a	62.5 ^{ab}	80.7 ^{bc}
P1882-12-111-13	103.7 ^{bc}	101.5 ^{b-d}	81.3 ^c	104.4 ^{cd}	10.6 ^a	13.4 ^a	26.3 ^a	29.0 ^a	57.0 ^b	80.1 ^{bc}
P1882-12-111-14	103.0 ^{bc}	100.7 ^{cd}	77.4 ^{cd}	100.1 ^d	11.1 ^a	13.9 ^a	25.5 ^{ab}	28.8 ^a	60.3 ^{ab}	82.2 ^{abc}
P1882-12-111-15	102.3 ^c	101.3 ^{b-d}	81.5 ^c	104.2 ^{cd}	11.2 ^a	13.1 ^a	25.1 ^{ab}	28.6 ^a	65.5 ^a	80.5 ^{bc}
P1882-12-111-16	103.8 ^{bc}	103.7 ^{ab}	80.8 ^c	106.1 ^c	10.9 ^a	12.8 ^a	25.0 ^{ab}	29.4 ^a	57.3 ^b	82.0 ^{abc}
P1882-12-111-17	103.8 ^{bc}	102.7 ^{a-d}	81.4 ^c	107.1 ^c	12.1 ^a	15.2 ^a	25.7 ^{ab}	29.6 ^a	63.6 ^{ab}	84.1 ^{abc}
P1882-12-111-20	104.7 ^{bc}	104.0 ^{ab}	82.0 ^c	106.2 ^c	10.8 ^a	13.8 ^a	26.0 ^a	29.0 ^a	61.7 ^{ab}	81.7 ^{abc}
PB1	102.3 ^c	100.7 ^{cd}	80.4 ^c	103.9 ^{cd}	10.2 ^a	14.3 ^a	25.2 ^{ab}	29.1 ^a	50.9 ^c	77.6 ^c
N22	84.2 ^e	82.3 ^f	103.2 ^b	126.5 ^b	13.4 ^a	14.3 ^a	18.2 ^d	23.0 ^b	62.4 ^{ab}	88.4 ^a
IR86918-B-B-305	79.5 ^f	78.8 ^g	115.0 ^a	136.2 ^a	12.2 ^a	14.1 ^a	21.2 ^c	24.3 ^b	62.0 ^{ab}	86.4 ^{ab}
IR64	98.7 ^d	87.7 ^e	73.9 ^d	89.9 ^e	11.1 ^a	14.3 ^a	21.9 ^c	23.9 ^b	60.7 ^{ab}	82.5 ^{abc}

DF, days to 50% flowering; PH, plant height in cm; NT, number of tillers; PL, panicle length in cm; SF, spikelet fertility in %; S, stressed; NS, unstressed. Means followed by same letters are statistically at par based on the least significant difference test at 95% confidence level.

Significant reduction in PH was observed under drought stress in all the years. When stressed, among the NILs, PH ranged from 69 cm to 79 cm during 2017, 83 cm to 92 cm during 2018 and 74 cm to 86 cm during 2019. However, in the first two seasons, the PH among NILs were on par with PB1, but during 2019, six NILs showed significantly low PH. Whereas, under normal conditions all the NILs showed PH on par with PB1 during all the seasons, except for P1882-12-111-1 and P1882-12-111-9 during 2017. However, the average performance across the seasons revealed no significant difference among the NILs under both the treatments. No significant difference was noticed for PL and NT during 2017 and 2018 seasons under both unstressed and stressed situations. However, during 2019, differences were noticed in few of the NILs, for NT alone. In case of PL too, average values within each of the treatments showed no difference among the NILs. Reduction in SF percentage of all the NILs and checks was found under stress during all three years. Two NILs out of 18, P1882-12-111-14 and P1882-12-111-15, had significantly better SF than PB1 under stress of 2017, which turned insignificant during 2018. Whereas during 2019, significantly better SF was found among thirteen NILs than PB1 under drought stress, which was on par for all the NILs and PB1 during unstressed treatment.

Most of the NILs out-yielded PB1 under stress in all the experimental years 2017, 2018 and 2019 under drought stress clearly reflecting the effect of the *qDTY1.1* introgression (Supplementary Table S3). The GY of NILs under stress during 2017 ranged from 463 kg/ha (P1882-12-111-10) to 1015 kg/ha (P1882-12-111-20) in comparison to PB1 (309 kg/ha). In the same season, under unstressed treatment, the yield ranged from 5541 kg/ha (P1882-12-111-9) to 6355 kg/ha (P1882-12-111-5) in comparison to PB1 yield of 5581 kg/ha. When subjected to drought stress during 2018 season, the yield of NILs ranged from 325 kg/ha (P1882-12-111-1) to 1654 kg/ha (P1882-12-111-17) in comparison to PB1 (573 kg/ha), however, under unstressed field the yield was in the range of 4733 kg/ha (P1882-12-111-9) to 5955 kg/ha (P1882-12-111-3) as against PB1 (5291 kg/ha). During the next season, 2019, the yield of NILs was in the range of 727 kg/ha (P1882-12-111-1) to 2516 kg/ha (P1882-12-111-17) under stress as against the yield of 692 kg/ha for PB1. However, under the unstressed condition, the yield of all the NILs was at par with PB1. The GY of donor parent N22 was found 889 kg/ha 1796 kg/ha and 2632 kg/ha under stress during year 2017, 2018 and 2019, respectively.

3.5. Drought Tolerance Level of NILs Judged through Percent Reduction of Yield and Stress Indices

In order to assess the true tolerance of NILs, percent reduction in yield (%R) and stress indices were calculated. The average values are provided in Table 5. Further, the data for individual lines across different years is provided in Supplementary Table S3. The unstressed yield levels of the 18 NILs were non-significantly different from that of the recurrent parent, PB1 and the donor parent N22 in several cases. However, under drought, the yield level was significantly superior than PB1 in seventeen NILs, and seven NILs showed yield similar to N22. The %R was found to be maximum in PB1 in all the experimental years. The minimum and maximum %R among NILs was found to be 82% in P1882-12-111-20 and 93% in P1882-12-111-8 during year 2017. The donor parent, N22 maintained the least %R of 64% and minimum and maximum %R among NILs was for P1882-12-111-17 (72%) and P1882-12-111-1 (93%) in year, 2018. The %R was the lowest in 2019, among NILs as compared to previous years 2017 and 2018; it ranged from minimum 59% of P1882-12-111-17 to maximum 86% of P1882-12-111-1. The drought yield index (DYI) ranged from 0.98 (P1882-12-111-20) to 3.14 (PB1) in year 2017; DYI was between 0.49 (N22) to 2.79 (P1882-12-111-1) in year 2018 and in year 2019 DYI range was from 0.35 (N22) to 1.37 (PB1). Lowest DYI (0.7) was found for P1882-12-111-17 followed by six other NILs (0.9). The stress tolerance index (STI) was in the range 0.05 (PB1) to 0.19 (P1882-12-111-17) during 2017; during 2018 STI was ranged from 0.05 (P1882-12-111-1) to 0.31 (P1882-12-111-17) and during 2019 its range was from 0.12 (PB1) to 0.47 (P1882-12-111-17). P1882-12-111-17 had the highest STI in all the experimental seasons. SSI values are in range of 1.04 (P1882-12-111-17) to 1.18 (PB1) during 2017; while its range in 2018 was 0.90 (P1882-12-111-17) to 1.17

(P1882-12-111-1) and during 2019 it was 0.75 (P1882-12-111-17) to 1.09 (PB1). The SSI values have shown same pattern during 2018 and 2019, and on average P1882-12-111-17 showed the lowest SSI value of 0.90 followed by P1882-12-111-6 (0.94) and P1882-12-111-7 (0.95).

Table 5. Drought tolerance and stability indices for grain yield among the NILs and checks averaged across seasons, with stability for spikelet fertility under stress.

Genotypes	Grain Yield (kg ha ⁻¹)		Indices						Spikelet Fertility (%)	
	Stressed	Unstressed	%R	DYI	STI	SSI	AST	YSI	Mean	AST
P1882-12-111-1	572.3 ^h	5545.0 ^{abc}	89.8	1.9	0.1	1.1	45.8	42.0	52.6	6.1
P1882-12-111-2	1090.6 ^{d-g}	5757.1 ^{abc}	80.9	1.1	0.2	1.0	9.7	19.0	62.3	6.2
P1882-12-111-3	1295.8 ^{a-f}	5943.7 ^{abc}	78.2	0.9	0.2	1.0	9.1	12.0	60.2	3.0
P1882-12-111-4	901.6 ^{d-g}	5542.2 ^{abc}	83.4	1.3	0.2	1.0	19.4	27.0	64.1	8.6
P1882-12-111-5	1236.6 ^{b-g}	5839.8 ^{abc}	78.3	1.0	0.2	1.0	11.0	17.0	65.1	2.3
P1882-12-111-6	1408.8 ^{a-d}	5841.0 ^{abc}	75.7	0.9	0.3	0.9	29.2	18.0	57.7	1.8
P1882-12-111-7	1393.3 ^{a-e}	5871.8 ^{abc}	76.4	1.0	0.3	1.0	34.9	23.0	57.8	5.4
P1882-12-111-8	879.6 ^{efg}	5656.5 ^{abc}	84.0	1.4	0.1	1.0	3.1	19.0	59.9	3.3
P1882-12-111-9	806.9 ^{fg}	5324.4 ^{bc}	84.8	1.3	0.1	1.1	16.7	28.0	59.5	4.4
P1882-12-111-10	725.9 ^g	5765.5 ^{abc}	87.3	1.6	0.1	1.1	17.5	30.0	63.3	5.9
P1882-12-111-11	1122.3 ^{d-g}	5678.1 ^{abc}	80.1	1.0	0.2	1.0	2.8	12.0	57.2	2.4
P1882-12-111-12	1254.6 ^{a-f}	5722.2 ^{abc}	78.0	0.9	0.2	1.0	6.5	12.0	62.5	0.6
P1882-12-111-13	866.2 ^{fg}	5582.3 ^{abc}	84.4	1.3	0.2	1.1	13.1	26.0	57.0	0.9
P1882-12-111-14	1050.0 ^{d-g}	5547.9 ^{abc}	81.0	0.9	0.2	1.0	33.4	30.0	60.4	7.5
P1882-12-111-15	1238.8 ^{b-g}	5879.9 ^{abc}	78.9	0.9	0.2	1.0	5.1	12.0	65.5	3.8
P1882-12-111-16	955.8 ^{d-g}	5938.4 ^{abc}	83.8	1.1	0.2	1.0	34.7	32.0	57.4	5.3
P1882-12-111-17	1715.8 ^{ab}	6122.9 ^a	72.0	0.7	0.3	0.9	27.8	15.0	63.5	5.9
P1882-12-111-20	1105.2 ^{d-g}	5622.7 ^{abc}	80.4	0.9	0.2	1.0	29.8	27.0	61.8	0.8
PB1	531.0 ^h	5475.6 ^{abc}	90.3	2.0	0.1	1.1	27.8	34.0	49.5	2.3
N22	1772.8 ^a	5228.6 ^c	65.8	0.6	0.3	0.8	38.0	20.0	62.4	4.6
IR86918-B-B-305	1706.3 ^{abc}	5748.6 ^{abc}	71.1	0.8	0.3	0.9	69.1	25.0	62.0	1.1
IR64	1188.9 ^{c-g}	6109.2 ^{ab}	80.7	1.4	0.2	1.0	38.3	26.0	59.9	5.1

%R, percentage reduction in yield; DYI, drought yield index; STI, stress tolerance index; SSI, stress susceptibility index; AST, additive main effects and multiplicative interaction (AMMI) stability value; YSI, yield stability index. Means followed by same letters are statistically at par based on the least significant difference test at 95% confidence level.

3.6. Stability Analysis of NILs under Stress Situation for Yield and Spikelet Fertility

To identify the NILs which could maintain superior performance under drought stress, an additive main effects and multiplicative interactions stability analysis across the season under drought condition, particularly for plot yield and spikelet fertility and stability indices, AMMI stability value and yield stability index was generated based on the AMMI model formulated (Table 5). For plot yield, the AST ranged from 2.80 (P1882-12-111-11) to 69.10 (IR86918-B-B-305) and the YSI range was from 12 (P1882-12-111-3, P1882-12-111-11, P1882-12-111-12, P1882-12-111-15) to 42 (P1882-12-111-1). Considering AST and YSI values with above average plot yields, P1882-12-111-11, P1882-12-111-8, P1882-12-111-15 and P1882-12-111-12 are the most stable among all NILs. For spikelet fertility, the AST range was from 0.61 (P1882-12-111-12) to 8.61 (P1882-12-111-4) and the YSI range was from 7 (P1882-12-111-15) to 40 (P1882-12-111-12). Considering AST, YSI and above average spikelet fertility values, NILs P1882-12-111-12, P1882-12-111-20 and P1882-12-111-13 were found most stable. Keeping in perspective the above two traits, it is apparent that the P1882-12-111-12 could maintain superior plot yield coupled with spikelet fertility across seasons under drought situation.

3.7. Stepwise Forward Regression Analysis to Identify the Significantly Affected Yield Contributing Trait

To find out the trait which has contributed significantly to yield and hence conditioned primarily by the QTL transferred, a stepwise forward regression was done with BLUP

values for individual years and across years by taking the grain yield as the dependent variable (Table 6). SF stood out as the major determinant trait contributing to grain yield consistently across seasons, particularly in 2017, where it alone contributed for 51% of the variation in grain yield under drought conditions. It was also clear that the contribution of SF was more apparent under drought stress than unstressed conditions, from its significant positive coefficients across the seasons. Remaining yield contributing traits did not show strong and stable influence on yield.

Table 6. Stability model for PB1 NILs along with checks under stressed and unstressed treatments during *Kharif* seasons of 2017, 2018 and 2019.

Treatment	Season	Model	R ²	F-Value	RMSE
Unstressed	2017	1874.43 + 35.49DF – 15.32PH + 25.66SF	0.71	14.8	210.5
	2018	14079.10 – 43.43DF – 52.14SF	0.35	5.3	430.2
	2019	3621.95 – 13.90PH – 44.44TN + 51.84PL + 34.79SF	0.52	4.7	161.2
	Pooled	7453.59 – 11.62PH – 36.23TN	0.17	6.4	383.4
Stressed	2017	–920.62 + 29.31SF	0.51	21.4	146.9
	2018	3619.85 – 46.53DF – 17.27PH + 229.72TN + 16.22SF	0.51	4.6	303.5
	2019	–998.34 – 62.25DF + 139.83TN + 113.11PL + 71.43SF	0.82	20.6	267.3
	Pooled	–4850.56 + 11.73PH + 165.36TN + 50.29SF	0.63	36.8	348.3

F-value, variance ratio; RMSE, root mean square error; DF, days to 50% flowering; PH, plant height in cm; TN, number of tillers; PL, panicle length in cm; SF, spikelet fertility in %; R², proportion of variation explained by the model.

3.8. Quality Assessment of NILs under Stressed and Unstressed Situations

The recovery grain and cooking quality traits is a major criterion in improvement of Basmati rice. Analysis of the grain quality recovered among the NILs under both stressed as well as unstressed conditions during 2019 season indicated that all the improved lines were as aromatic as PB1, the recurrent parent having a panel score of 2.0 for aroma (Table 7). Visually, the milled grains of the NILs appeared similar to that of PB1. However, some of the NILs showed non-significant variation for kernel length before cooking (KLBC) with respect to PB 1. By and large, similar pattern was observed for all the raw grain quality traits. After cooking, no significant variation was observed for kernel length after cooking (KLAC) than PB1 (Figure 4), among several of the NILs under unstressed conditions. However, under stress there were six NILs that showed significantly superior elongation than PB1, such as P1882-12-111-3, P1882-12-111-5, P1882-12-111-6, P1882-12-111-7, P1882-12-111-8 and P1882-12-111-11. Interestingly, under drought, the elongation ratio was found significantly good in all NILs except in seven NILs, that were on par with PB1. Furthermore, the alkali spreading value (ASV) of all the NILs were similar to PB1 (score of 7) as against the score of 5 recorded among the other checks.

Table 7. Grain and cooking quality traits of the NILs and the recurrent parent, PB1 along with checks during *Khariif* 2019 under stressed (S) and unstressed (NS) conditions.

Entry	KLBC		KBBC		LBR		KLAC		KER		ASV
	NS	S	NS	S	NS	S	NS	S	NS	S	NS/S
P1882-12-111-1	7.80 ^a	6.93 ^{b-e}	1.73 ^{cd}	1.60 ^{de}	4.50 ^{abc}	4.33 ^{ef}	13.47 ^{def}	12.53 ^{bc}	1.73 ^f	1.81 ^{cd}	7
P1882-12-111-2	7.53 ^{bcd}	7.2 ^{ab}	1.67 ^d	1.40 ^g	4.52 ^{ab}	5.14 ^{ab}	14.20 ^{a-d}	12.27 ^{b-e}	1.88 ^{cde}	1.70 ^{fgh}	7
P1882-12-111-3	7.53 ^{bcd}	6.6 ^{fg}	1.73 ^d	1.60 ^{de}	4.35 ^{a-d}	4.13 ^{fg}	14.27 ^{a-d}	12.67 ^b	1.89 ^{cd}	1.92 ^a	7
P1882-12-111-4	7.40 ^{c-f}	6.47 ^g	1.80 ^c	1.67 ^d	4.11 ^d	3.88 ^g	14.00 ^{a-e}	11.93 ^{cde}	1.89 ^{cd}	1.85 ^{bc}	7
P1882-12-111-5	7.67 ^{ab}	6.93 ^{b-e}	1.67 ^d	1.40 ^g	4.60 ^a	4.95 ^{abc}	14.67 ^{ab}	12.87 ^{ab}	1.91 ^{a-d}	1.86 ^{abc}	7
P1882-12-111-6	7.53 ^{bcd}	7.00 ^{bcd}	1.67 ^d	1.53 ^{ef}	4.52 ^{ab}	4.57 ^{de}	14.80 ^a	12.67 ^b	1.96 ^{ab}	1.81 ^{cd}	7
P1882-12-111-7	7.47 ^{b-e}	7.07 ^{abc}	1.67 ^d	1.40 ^g	4.48 ^{abc}	5.05 ^{ab}	14.80 ^a	13.53 ^a	1.98 ^a	1.92 ^a	7
P1882-12-111-8	7.27 ^{e-h}	7.13 ^{ab}	1.67 ^d	1.47 ^{fg}	4.36 ^{a-d}	4.86 ^{bcd}	14.13 ^{a-e}	12.93 ^{ab}	1.94 ^{abc}	1.81 ^{cd}	7
P1882-12-111-9	7.33 ^{d-g}	7.13 ^{ab}	1.67 ^d	1.40 ^g	4.40 ^{abc}	5.1 ^{ab}	13.93 ^{b-e}	11.93 ^{cde}	1.9 ^{bcd}	1.67 ^{ghi}	7
P1882-12-111-10	7.40 ^{c-f}	7.07 ^{abc}	1.67 ^d	1.47 ^{fg}	4.44 ^{abc}	4.82 ^{bcd}	13.80 ^{cde}	11.6 ^e	1.86 ^{de}	1.64 ^{hi}	7
P1882-12-111-11	7.47 ^{b-e}	7.27 ^a	1.67 ^d	1.40 ^g	4.48 ^{abc}	5.19 ^a	14.00 ^{a-e}	13.53 ^a	1.88 ^{cde}	1.86 ^{abc}	7
P1882-12-111-12	7.47 ^{b-e}	7.2 ^{ab}	1.67 ^d	1.47 ^{fg}	4.48 ^{abc}	4.91 ^{a-d}	13.80 ^{cde}	11.73 ^{de}	1.85 ^{de}	1.63 ⁱ	7
P1882-12-111-13	7.60 ^{abc}	7.07 ^{abc}	1.67 ^d	1.47 ^{fg}	4.56 ^a	4.82 ^{bcd}	13.87 ^{b-e}	12.53 ^{bc}	1.82 ^e	1.77 ^{de}	7
P1882-12-111-14	7.13 ^{gh}	7.00 ^{bcd}	1.67 ^d	1.60 ^{de}	4.28 ^{bcd}	4.38 ^{ef}	13.60 ^{c-f}	12.4 ^{bcd}	1.91 ^{a-d}	1.77 ^{de}	7
P1882-12-111-15	7.20 ^{fgh}	6.93 ^{b-e}	1.67 ^d	1.67 ^d	4.32 ^{a-d}	4.16 ^{fg}	13.33 ^{ef}	11.87 ^{de}	1.85 ^{de}	1.71 ^{efg}	7
P1882-12-111-16	7.07 ^h	7.07 ^{abc}	1.67 ^d	1.53 ^{ef}	4.24 ^{cd}	4.61 ^{cde}	12.87 ^f	12.33 ^{b-e}	1.82 ^e	1.75 ^{def}	7
P1882-12-111-17	7.20 ^{fgh}	6.53 ^{fg}	1.67 ^d	1.53 ^{ef}	4.32 ^{a-d}	4.26 ^{ef}	13.60 ^{c-f}	12.47 ^{bcd}	1.89 ^{cd}	1.91 ^{ab}	7
P1882-12-111-20	7.27 ^{e-h}	6.8 ^{def}	1.60 ^d	1.53 ^{ef}	4.54 ^{ab}	4.43 ^{ef}	14.33 ^{abc}	11.6 ^e	1.97 ^a	1.71 ^{efg}	7
PB1	7.47 ^{b-e}	7.07 ^{abc}	1.67 ^d	1.47 ^{fg}	4.48 ^{abc}	4.82 ^{bcd}	14.13 ^{a-e}	11.93 ^{cde}	1.89 ^{cd}	1.69 ^{f-i}	7
N22	5.53 ^j	4.93 ^h	2.33 ^a	2.33 ^a	2.37 ^g	2.11 ^j	8.00 ^h	6.67 ^h	1.45 ^h	1.35 ^k	5
IR86918-B-B-305	5.67 ^j	5.2 ^h	2.00 ^b	1.93 ^{bc}	2.83 ^f	2.69 ⁱ	9.00 ^g	8.13 ^g	1.59 ^g	1.56 ^j	5
IR64	6.53 ⁱ	6.53 ^{fg}	2.00 ^b	2.00 ^b	3.27 ^e	3.27 ^h	9.67 ^g	9.00 ^f	1.48 ^h	1.38 ^k	5
S.E _m	0.12	0.13	0.04	0.05	0.13	0.17	0.4	0.36	0.03	0.03	

KLBC, kernel length before cooking in mm; KBBC, kernel breadth before cooking in mm; LBR, length by breadth ratio; KLAC, kernel length after cooking in mm; KBAC, kernel breadth after cooking in mm; KER, kernel elongation ratio; ASV, alkali spreading value; SE_m, standard error of mean; S, stressed; NS, unstressed. Means followed by same letters are statistically at par based on the least significant difference test at 95% confidence level.

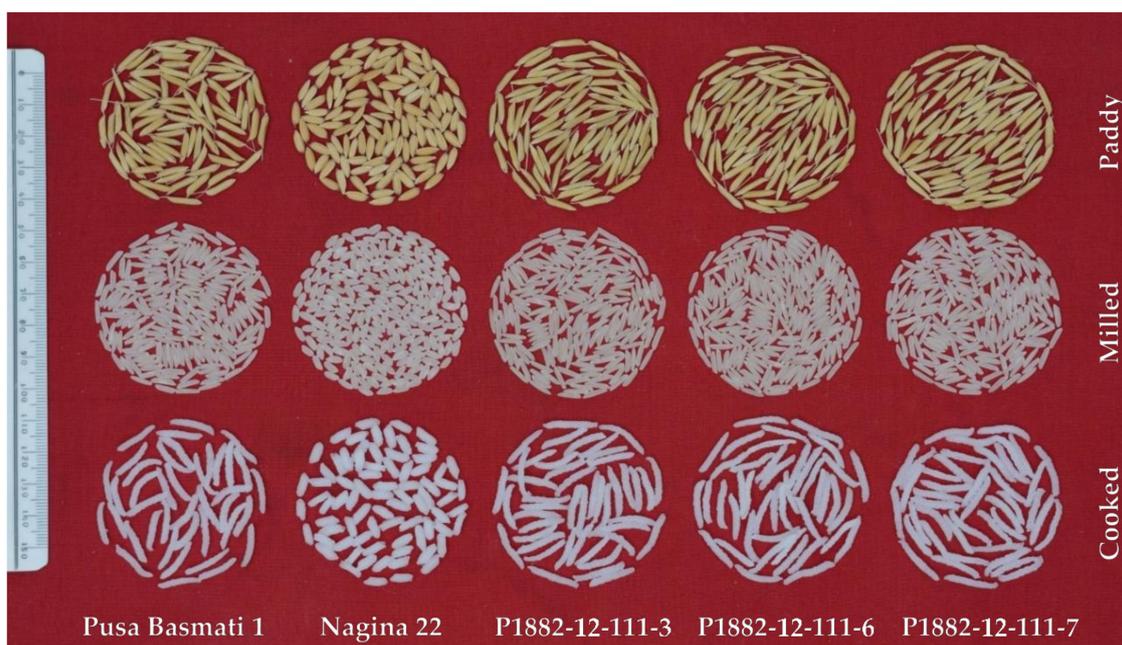


Figure 4. Grain and cooking quality of the representative NILs of Pusa Basmati 1 carrying *qDTY 1.1*.

4. Discussion

Climate change is predicted to affect rice cultivation worldwide adversely, through various impacts, such as drought, excess rainfall, temperature fluctuations and also predisposing it to several biotic stresses. Any adverse effect on rice production would threaten world food security, because more than half of the world population is rice dependent. This is particularly important to rice consuming countries like India, where about 90% of the total rice production is internally consumed [40]. Among these, biotic threats due to diseases and pests, as well as abiotic stresses such as drought, submergence and salinity are particularly relevant under climate change scenario. Predominantly in the rainfed environment of South and Southeast Asia, drought and submergence are more frequently encountered during the crop growing season [41]. Most of the prominent and popular rice varieties are vulnerable to these abiotic stresses [20]. Since climatic vagaries occur spontaneously and intermittently, growing tolerant rice varieties for wide range of stresses is the only economically viable option to manage abiotic stresses. This option is particularly important in the case of drought, because it remains as the most frequently occurring stress that bears the potential to fail a rice crop. Judging drought tolerance of genotypes and its transfer into elite backgrounds is quite laborious due to complex nature of tolerance. It is this complexity that renders the improvement for drought resilience through conventional breeding tardy. However, efforts can be remarkably hastened by leveraging molecular markers that are linked to various traits associated with drought tolerance. Moreover, use of markers can aid a relatively cost-effective and environment neutral selection, while improving the accuracy and reducing the turnover time. Molecular marker assisted introgression of traits provides various advantages such as easy recovery of an otherwise difficult phenotypic trait, easy selection, increased accuracy and shortening the breeding time. Therefore, marker assisted backcross breeding (MABB) was proven to be an efficient strategy for incorporation of desired trait associated genes/QTLs in numerous prominent rice varieties [21,42,43]. MABB along with stringent phenotypic selection was successfully employed for Basmati rice improvement for different biotic stresses [26,30,44–48]. MABB is also proven to a superior method for QTLs transfer into desired cultivar/variety for different abiotic stresses as well, such as drought. Out of the several QTLs reported for

reproductive stage drought tolerance in rice, *qDTY1.1* [15,16], *qDTY2.1* [33], *qDTY3.1* [33,49] and *qDTY12.1* [50,51], showed consistent grain yield under drought across different genetic backgrounds and has been used in breeding applications. Recently, Dwivedi et al. [23] reported successful pyramiding of two QTLs, *qDTY2.1* and *qDTY3.1* into the megavariety, Pusa 44 significantly enhancing yield under drought. Despite the proven advantage, however, only few success stories in improving rice varieties for drought tolerance using MABB has been reported rice.

In the present study, MABB was utilized to introgress a RSDS tolerance QTL *qDTY1.1* from N22 into PB1, one of the popular Basmati rice varieties of India. Popular for its high yield, better-quality grain, and excellent cooking quality and pleasing aroma, PB1 is the first ever semi-dwarf Basmati rice variety developed. Bred through conventional convergent breeding procedures, PB1 was derived from a cross between Pusa 150/Karnal local. Pusa 150 has Basmati 370 in its lineage, while Karnal local was a landrace with better aroma and cooking quality. Karnal local was a selection from Haryana Basmati collection 19 (HBC19), that was later released as Taraori Basmati [52]. Despite its excellent yield gain over the conventional Basmati cultivars, PB1 was semi-dwarf with excellent plant architecture, photo insensitivity, high yielding and with unparalleled grain quality [53]. However, like its congeners, PB1 is also sensitive to drought as well as to many biotic stresses. Released during 1989, PB1 remains popular even today among the Basmati farmers of India, despite above limitations. Furthermore, till date there is no report on improvement any Basmati rice variety for drought tolerance. Therefore, the present was carried out with the objective to incorporate drought tolerance in PB1. The donor parent, N22 is a tall upland rice variety belonging to *aus* group. N22 is a pure line selection from a landrace, Rajbhog [54] that possesses deeper root system, shorter duration and tolerant to heat and drought [55]. This variety has short bold non-aromatic grains with very less elongation on cooking.

The genome wide average polymorphism between the parents, PB1 and N22 was 17.35%, which was more than that of the carrier chromosome of *qDTY1.1*. The molecular analyses on the background recovery has revealed maximum recovery of chromosome 1, thereby eliminating the chances for linkage drag. The fact that maximum background markers (101 markers) were surveyed on chromosome 1, substantiate our claim for the elimination of linkage drag. Furthermore, the agronomic recovery of the NILs in general suggests that no undesirable trait has been incorporated by chance from other donor fragments, that might have escaped marker-based background selection. In this study, *qDTY1.1* was found providing increased level of drought tolerance in the NILs vis-à-vis PB1. It is also known that QTLs from a particular genetic background usually show minor effects or may remain completely silent in diverse genetic backgrounds [56]. In the case of drought, interaction between QTL \times genetic background has been a major bottleneck limiting the use of QTLs for MABB in rice [50,57,58]. Nevertheless, *qDTY1.1* derived from N22 has been demonstrated effective in multiple non-Basmati backgrounds such as MTU1010, IR64 and Swarna, which showed its role as a major effective QTL governing grain yield under reproductive stage water stress [15]. This justifies the use of *qDTY1.1* as a most desirable candidate for MABB to improve prominent high-yielding varieties with augmented drought tolerance.

Notwithstanding, drought has a significant role in reducing the yield under drought relative to irrigated cultivation. Under drought, yield reduction was found majorly influenced by spikelet fertility. In comparative assessment, there was no significant yield reduction noticed under unstressed condition among the NILs as a consequence of introgressing the *qDTY1.1*. In an earlier report on introgression of another QTL, *qDTY3.1*, a negative effect on grain yield was encountered by Venuprasad et al. [33] under unstressed treatment. The NILs evolved under this program, showed significant higher gain for grain yield over PB1, with P1882-12-111-2, P1882-12-111-3, P1882-12-111-5, P1882-12-111-6, P1882-12-111-7, P1882-12-111-10, P1882-12-111-12, P1882-12-111-15 and P1882-12-111-17 having clear advantage under both stressed and unstressed treatments. This also indicated that negative effect of QTL \times background interaction was not observed under PB1 background.

P1882-12-111-17 had the maximum grain yield among the NILs followed by P1882-12-111-3, P1882-12-111-5, P1882-12-111-6, P1882-12-111-7 and P1882-12-111-12 under both stressed, and unstressed conditions during all the years. This signified the effectiveness of *qDTY1.1* in enhancing yield under drought stress. However, the effect of the introgressed QTL among the PB1 NILs was more conspicuous in the 2019 than in the previous years. In the recent report from World Meteorological Organization [59], it has been reported that climate change is accelerating by the last five-year period between 2015–2019. Although 2016, has been identified as the most erratic year, 2019 also showed significantly high incidence of drought than 2017 and 2018. Therefore, 2019 season data were more explicit to showcase the drought tolerance response of the NILs. This was evident from performance of NILs vis-à-vis PB1, which showed significant deviation for traits such as SF, GY, DF and PH particularly during 2019 and 2017 seasons. In all the seasons, however, the donor (N22) and the positive control (IR86918-B-B-305) remained the best performers under drought possibly indicating the necessity to transfer additional QTLs from multiple donors to further push the tolerance threshold of PB1. Similar observations were made by Vikram et al. [15] that *qDTY1.1* had a considerable effect on enhancing the GY under both stressed and unstressed conditions. Similar effect of *qDTY1.1* on improving the yield was earlier reported, conditioned through several adventitious drought-linked traits such as root characteristics, relative water content, biomass, and osmotic adjustment [17,60,61]. Meta QTLs have also been documented for maximum root length and GY within the *qDTY1.1* region [62,63]. In the PB1 background, *qDTY1.1* seems to enhance the yield under drought indirectly by modulating spikelet fertility as inferred from stepwise regression analysis. This implies on the importance of maintaining higher spikelet filling in achieving higher yields. Therefore, we hypothesize that grain filling could be the putative trait, *qDTY1.1* is associated with, that require further validation.

Stability of yield performance under stress situations across the seasons is also important for a NIL to be considered for varietal evaluation prior to release as a cultivar across different rice growing areas of the country. The AMMI stability analysis carried out towards assessing the stable yielding potential, revealed that the NILs showed differential stability pattern. Two concomitant stability indices AST and YSI were used for assessing the yield stability, while AST alone was used for SF. Considering the stability indices and mean performance, one of the NILs, P1882-12-111-12 was adjudged stable for both GY and SF. This line can be a potential candidate for varietal evaluation pipeline in the future.

Among the agronomic traits, it was found that there was a delay in DF found across the NILs under stressed conditions. Delay in the flowering time under drought was also reported earlier in rice [16,64,65], which is due to delay in flower development and slow rate of panicle elongation under stress. The delay was however conspicuous in IR64, the short duration high yielding check variety used in the study. In short duration varieties with little drought adaptation, flower development delay occurs more significantly than longer duration cultivars. Similarly, there was a general decrease in PH among all the lines under stress, the degree of decrease was slightly reduced among the NILs. However, there was no increase in height observed among the NILs, although they have all possessed *qDTY1.1*, attributable to the tight linkage of *qDTY1.1* with *SD1* gene, the gene responsible for tallness in N22 [66]. In the present study, we surmise that linkage between the *qDTY1.1* and *sd1* allele might have broken resulting in semi-dwarf NILs with height similar to that of PB1.

Tolerance indices are better yardsticks for judging the tolerance of genotypes than the yield *per se* under stress condition. Three popular stress indices were utilized in the present study to identify best performing NILs. DYI is based on the mixed model which accounts for the genotype \times stress level interaction across different environments. For drought prone areas, the combination of DYI with deviations in genotype performance under irrigated conditions may facilitate breeders to select genotypes with no yield reduction under favorable environment in comparison to currently cultivated varieties [37]. STI sorts out the entries which perform well under non-stress and fairly well under stress condition, high value of STI for a genotype infers higher tolerance level to the drought stress [39].

Fisher and Maurer [39] proposed SSI that estimates the yield reduction due to unfavorable environment in comparison to favorable environment. Lower the SSI value lesser the yield difference between stress and non-stress condition and hence greater is the tolerance of the genotype [67]. Thus, DYI, STI and SSI favored P1882-12-111-17 as best genotype for drought situation due to its highest grain yield under both stressed and unstressed conditions followed by P1882-12-111-3, P1882-12-111-6, P1882-12-111-7, P1882-12-111-12, P1882-12-111-15 and P1882-12-111-20. Further, P1882-12-111-17 out yielded majority of the remaining NILs. Among various traits measured in the present study, SF stood out as the most significant trait influencing grain yield under drought stress. Successful reproduction and efficient grain filling are the important determinants of ultimate grain yield under stress which is reflected as higher spikelet fertility. Thus, the NILs showed superior yields due to the maintenance of spikelet fertility under drought stress.

PB1 being a Basmati rice variety having specialty grain characteristics, transfer of drought tolerance from a non-aromatic variety with totally different grain architecture was a great challenge. Since the QTL transfer was into a Basmati background from a non-Basmati donor, a critical comprehensive quality check was done under both non-stress and stress situations in 2019. The NILs generated, possessed all the grain and cooking quality attributes of PB1 along with drought tolerance (Figure 4).

The recovery of quality in the NILs was achieved by stringent phenotypic selection integrated to the marker-based selection for the target trait, RSDS. NILs under well-watered treatment has grain quality similar to that of PB1, while an inconspicuous quality variation was noticed under drought situation. In spite of the successful recovery of PB1 genome component governing grain quality among the NILs, there was a marginal reduction in head rice recovery noticed under severe stress, although statistically insignificant, together with a slight increase in chalkiness. It is well known that drought at grain filling interferes with starch packaging in the grains, leading to chalkiness. Besides, the grain quality under stressed treatment was not significantly different from that under unstressed, indicating that drought had little effect of grain quality in the NILs. Grain quality recovery using MABB with augmented phenotypic selection, particularly in Basmati cultivars, was earlier reported from several studies [27,30,68].

5. Conclusions

In the present investigation, we have developed NILs of the popular Basmati rice variety, PB1 carrying a major QTL, *qDTY1.1* for RSDS tolerance. This paper also forms the first ever report of successful transfer of a drought tolerance QTL into a Basmati rice cultivar. The improved drought tolerant NILs of PB1, were also combined with high yield and grain quality. These NILs could be a good alternative for the Basmati growing regions with limited soil moisture regimes. Additionally, these may help farmers to reduce number of irrigations without foregoing yield and grain quality potential of Basmati rice. These NILs are assessed for three consecutive seasons to assess their stability and drought response to identify potential candidates to be deployed for varietal testing and cultivar release. Moreover, these NILs can also serve as improved donor lines for imparting drought tolerance in future Basmati breeding programs.

Supplementary Materials: Supplementary materials can be found at <https://www.mdpi.com/2073-4395/11/2/202/s1>. Figure S1. Graphical genotypes of Pusa Basmati 1 near isogenic lines showing recovery of carrier chromosome of *qDTY1.1* (Chromosome 1); Table S1: List of markers used for foreground and background selection in the present study; Table S2: Agro-morphological parameters indicating the relative performance PB1 NILs and parents under stressed (S) and unstressed (NS) conditions during 2017, 2018 and 2019; Table S3: Drought tolerance indices and grain yield of PB1 NILs along with checks and parents under stressed and unstressed conditions during 2017, 2018 and 2019.

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

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Abbreviations

%R	Percent reduction in yield
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
AS	Alkali spreading value
AST	AMMI stability value
BLUP	Best linear unbiased predictor
DF	Days to 50% flowering
DTY	Yield under drought
DYI	Drought yield index
GY	Grain yield
ICAR	Indian Council of Agricultural Research
KER	Kernel elongation ratio
KLAC	Kernel length after cooking
KLBC	Kernel length before cooking
LBR	Length by breadth ratio
MABB	Marker assisted backcross breeding
N22	Nagina 22
NIL	Near isogenic line
NS	Unstressed
NT	Number of tillers
PB1	Pusa Basmati 1
PH	Plant height
PL	Panicle length
QTL	Quantitative trait loci
RP	Recurrent parent
RPGR	Recurrent parent genome recovery
RSDS	Reproductive stage drought stress
S	Stressed
SF	Spikelet fertility
SSI	Stress susceptibility index
SSR	Simple sequence repeats
STI	Stress tolerance index
YSI	Yield stability index

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