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Article

Introgression of *qDTY1.1* governing reproductive stage drought tolerance into an elite 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 donor was sourced from an *aus* variety, Nagina 22 (N22). A QTL linked microsatellite (SSR) marker 'RM 431' 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 genome recovery. After three backcrosses followed by five generations of selfing, eighteen near isogenic lines (NILs) were developed, through combinatory selection for agro-morphological, grain and cooking superiority 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 NIL3, NIL5, NIL6, NIL7, NIL12, NIL15 and NIL17 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 310255.6 million (US$ 4,330.6 million) during 2019–20 from an annual milled rice production of more than 8 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 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 major factors 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 liters 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 8 million under upland ecology [8].

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 prevailing areas. Additionally, breeding of futuristic cultivars resilient to multiple stresses requires enhanced drought tolerance, especially that occurring at the reproductive stage. The 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 stress as the surrogate trait. Among these, *qDTY1.1* is a major QTL for grain yield under drought stress, mapped on chromosome 1 independently from two tolerant cultivars such as N22 [15] and Dhagaddeshi [16]. 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 [18,14].

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-smart cultivars [19,20], such as Sabitri (*qDTY3.2* and *qDTY12.1*) [21], IR 64 (*qDTY2.2* and *qDTY4.1*) and Vandana (*qDTY12.1*) [22]. In the past twelve years, there has been about sixty-six release of RSDS tolerant varieties 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 recurrent Basmati parent, while introgression [23]. However, marker assisted backcross breeding coupled with phenotypic selection, has been demonstrated to effectively address this problem [24, 25, 26].

Developed by the 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, having 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 in to 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. Results

2.1. Parental Polymorphism

The genome wide polymorphism survey using 651 SSR markers, revealed a diversity of 17.4% between the parents, identifying 113 polymorphic markers 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.

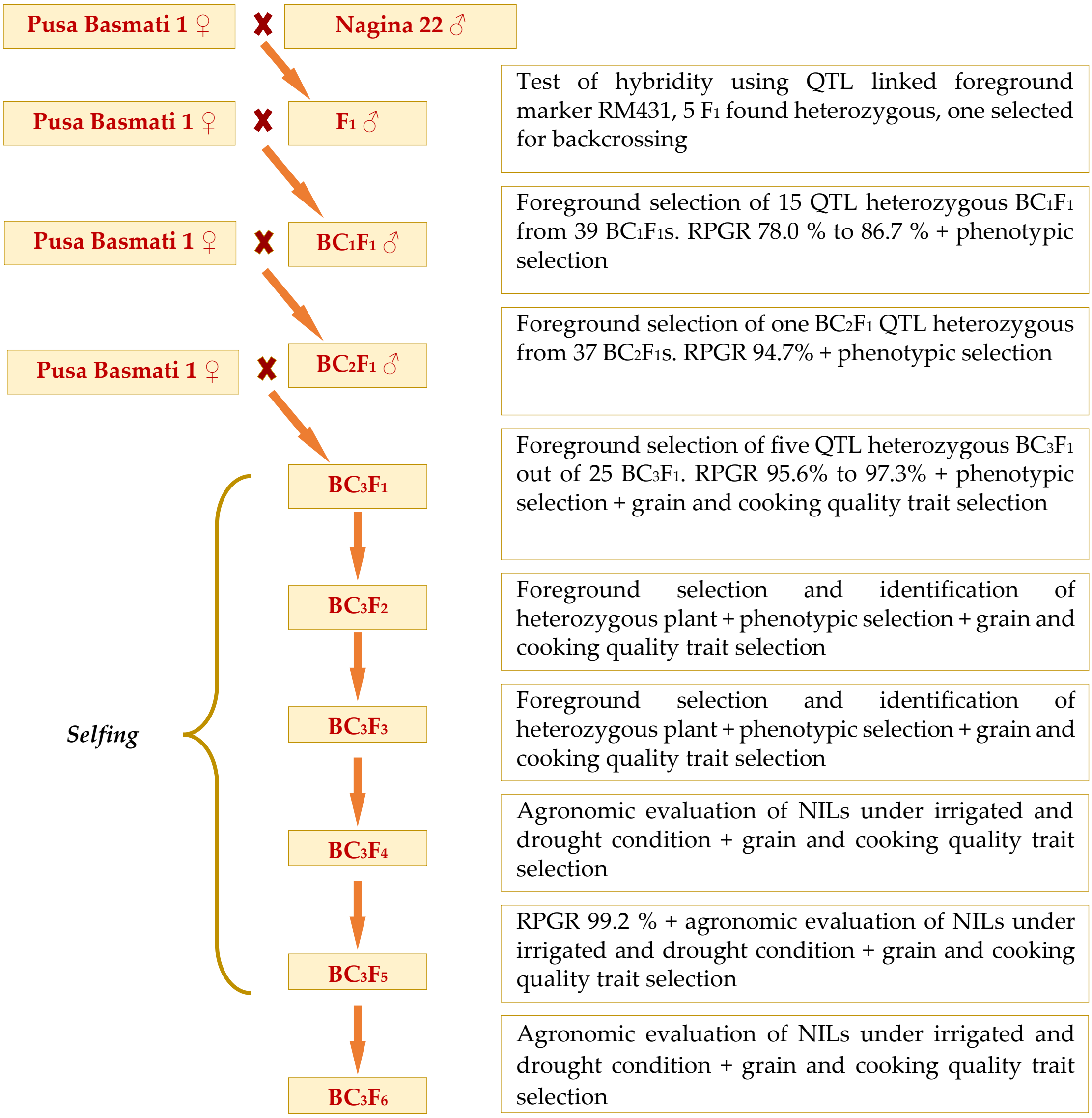
Table 1. Genome wide marker polymorphism between the recurrent parent Pusa Basmati 1 (PB1) and donor parent Nagina 22 (N22)

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 x 100/ Total number of markers. ¶Foreground marker not included

2.2. 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_1s 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 RPG recovery 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 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 RPG recovery (86.7%) was used to backcross with PB1 to generate 37 BC_2F_1 seeds. However, only one BC_2F_1 plant was found to be heterozygous for RM431. Background analysis on this plant using 30 markers heterozygous from the previous backcross generation, showed the RPG recovery of 94.7%. Subsequent backcrossing of this plant to PB1 yielded 25 seeds. On raising the BC_3F_1 generation from these seeds, five plants were found to be heterozygous for *qDTY1.1* linked marker, RM431.



Test of hybridity using QTL linked foreground marker RM431, 5 F_1 found heterozygous, one selected for backcrossing

Foreground selection of 15 QTL heterozygous BC_1F_1 from 39 BC_1F_1s. RPGR 78.0 % to 86.7 % + phenotypic selection

Foreground selection of one BC_2F_1 QTL heterozygous from 37 BC_2F_1s. RPGR 94.7% + phenotypic selection

Foreground selection of five QTL heterozygous BC_3F_1 out of 25 BC_3F_1. RPGR 95.6% to 97.3% + phenotypic selection + grain and cooking quality trait selection

Foreground selection and identification of heterozygous plant + phenotypic selection + grain and cooking quality trait selection

Foreground selection and identification of heterozygous plant + phenotypic selection + grain and cooking quality trait selection

Agronomic evaluation of NILs under irrigated and drought condition + grain and cooking quality trait selection

RPGR 99.2 % + agronomic evaluation of NILs under irrigated and drought condition + grain and cooking quality trait selection

Agronomic evaluation of NILs under irrigated and drought condition + grain and cooking quality trait selection

Figure 1. Breeding scheme used in the marker-assisted backcross programme for the transfer of *qDTY1.1* QTL in the background of the elite rice variety, Pusa Basmati 1. RPGR denotes recurrent parent genome recovery.

Table 2. Progressive selection statistics for the development of PB1 near isogenic lines introgressed with the RSDS tolerance QTL, *qDTY1.1*

Generation	No. of seedlings/families tested			RPG recovery (%)		
	Total	QTL positive	Selected	Range	Mean	Average gain
F_1	23	5	1	50.0	50.0	0.0
BC_1F_1	39	15	1	78.0-86.7	73.4	23.4
BC_2F_1	37	1	1	94.7	94.7	21.3
BC_3F_1	25	5	1	95.6-97.3	96.5	1.8
BC_3F_2	229	95	55	97.8-98.7	98.2	1.7
BC_3F_3	55	55	24	*	-	-
BC_3F_4	24	24	18	*	-	-
BC_3F_5	18	18	18	98.2-99.1	99.0	0.8

*Not tested

Background selection with the remaining unrecovered heterozygous markers among the BC_2F_1 plant, resulted in an RPG recovery ranging from 95.6% to 97.4%. These BC_3F_1 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 BC_3F_1, yielded 229 BC_3F_2 seeds. Through foreground followed by background selection of these BC_3F_2 plants, 55 plants homozygous for *qDTY1.1* with an RPG recovery of 97.8-98.7% were identified. All these 55 plants were advanced to BC_3F_3 families which underwent a rigorous phenotype selection for agro-morphological and grain quality traits to select 24 families for further advancement. Grown under both irrigated and stressed conditions, the selection among these 24 families in BC_3F_4 generation was carried out for agronomic performance, drought response and grain quality. This resulted in 18 families of near isogenic lines (NILs), 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 (320 bp) parent for *qDTY1.1* (Figure 2). The RPG recovery at BC_3F_5 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.

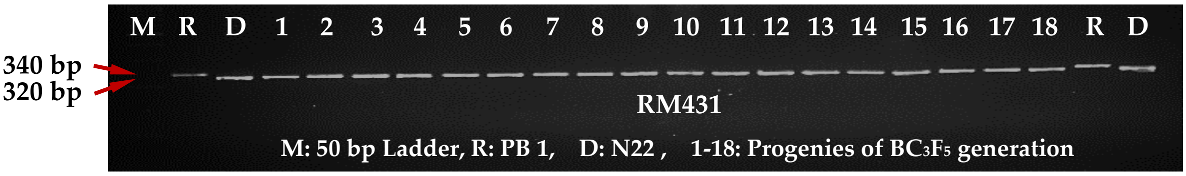


Figure 2. Foreground selection of PB1 NILs for *qDTY1.1* using RM 431 marker

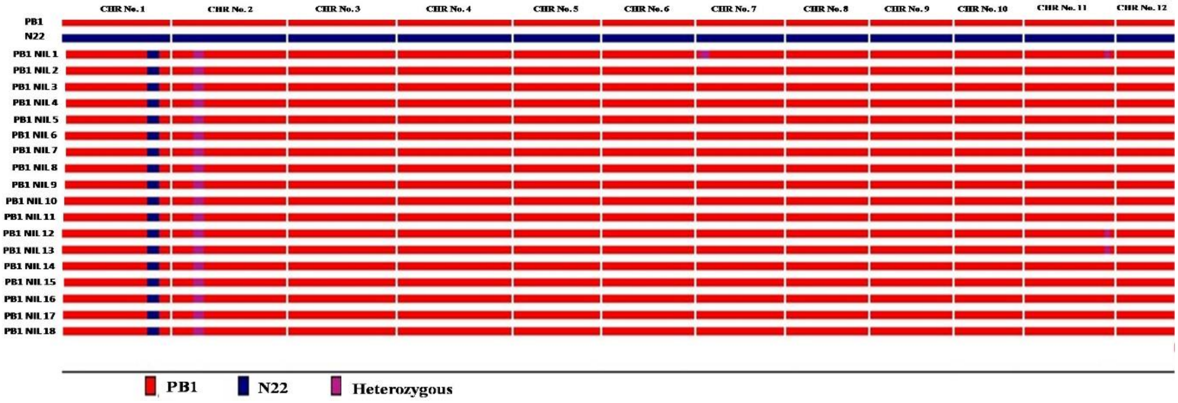


Figure 3. Graphical representation of the genotypes of 18 *qDTY1.1* introgressed NILs of PB1. All the NILs had maximum recovery on the carrier chromosome 1. CHR: chromosome.

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 x S	Y x G	Y x S x 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**
TN	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; TN, 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.

2.3. Recovery of RP Alleles in Carrier Chromosome

Recovery of the RPG in chromosome 1, the carrier chromosome of *qDTY1.1* was performed using 102 markers, of which 15 were polymorphic including the foreground marker, RM431. These markers, except for RM431 were also included in the whole genome background analysis. All the 14 polymorphic markers in chromosome 1 showed complete recovery of RP allele in the 18 NILs by BC_3F_5 generation.

2.4. 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). ANOVA over individual years revealed significant variation for genotypes, treatment and genotype × treatment components, for most of the agronomic characters studied. However, only two traits, SF and GY showed consistently significant variation for genotype × treatment component in all the seasons. For the remaining traits, during 2018, genotype effects for TN and genotype × treatment effects for DF, TN and PL were found non-significant, as that of the genotype × treatment effect of TN in 2017. Combined ANOVA, indicated a uniform pattern across the traits, particularly for GY and SF. TN 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 × treatment interaction was non-significant, while PL had non-significant year × genotype effect. Except for PL and TN, year × treatment × genotype interaction was significant for all the other traits. 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, NIL 9 and NIL 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 NIL1 under stress, that showed delayed flowering. On 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).

Significant reduction in plant height (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 plant height among NILs were on par with PB1, but during 2019 six NILs showed significantly low PH than PB1. Whereas, under normal condition all the NILs showed PH on par with PB1 during all the seasons, except for NIL1 and NIL 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 check was found under stress during all three years. Two NILs out of 18, NIL14 and NIL15, 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 QTL transferred (Supplementary table S3). The GY of NILs under stress during 2017 ranged from 463 kg/ha (NIL 10) to 1015 kg/ha (NIL 18) in comparison to PB1 (309 kg/ha). In the same season, under unstressed treatment, the yield ranged from 5541 kg/ha (NIL 9) to 6355 kg/ha (NIL 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 (NIL 1) to 1654 kg/ha (NIL 17) in comparison to PB1 (573 kg/ha), however, under unstressed field the yield was in the range of 4733 kg/ha (NIL 9) to 5955 kg/ha (NIL 3) as against the PB1 (5291 kg/ha). During the next season, 2019, the yield of NILs was in the range of 727 kg/ha (NIL1) to 2516 kg/ha (NIL 17) under stress as against the yield of 692 kg/ha for PB1. But, 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.

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
NIL1	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}
NIL2	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}
NIL3	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}
NIL4	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}
NIL5	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}
NIL6	102.2^{c}	101.2^{bcd}	80.0^{c}	105.9^{c}	11.6^{a}	14.7^{a}	25.5^{ab}	28.5^{a}	57.7^{b}	86.7^{ab}
NIL7	102.7^{bc}	101.2^{bcd}	81.8^{c}	105.6^{c}	13.5^{a}	15.7^{a}	26.4^{a}	29.1^{a}	57.8^{b}	84.8^{abc}
NIL8	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}
NIL9	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}
NIL10	105.5^{b}	103.3^{abc}	78.7^{cd}	103.3^{cd}	10.9^{a}	14.1^{a}	24.9^{ab}	28.4^{a}	63.3^{ab}	83.1^{abc}
NIL11	104.5^{bc}	103.2^{abc}	79.0^{c}	104.9^{cd}	10.6^{a}	15.5^{a}	25.3^{ab}	29.5^{a}	57.2^{b}	81.4^{abc}
NIL12	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}
NIL13	103.7^{bc}	101.5^{bcd}	81.3^{c}	104.4^{cd}	10.6^{a}	13.4^{a}	26.3^{a}	29.0^{a}	57.0^{b}	80.1^{bc}
NIL14	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}
NIL15	102.3^{c}	101.3^{bcd}	81.5^{c}	104.2^{cd}	11.2^{a}	13.1^{a}	25.1^{ab}	28.6^{a}	65.5^{a}	80.5^{bc}
NIL16	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}
NIL17	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}
NIL18	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; TN, number of tillers; PL, panicle length in cm; SF, spikelet fertility in %. Means followed by same letters are statistically at par based on the least significant difference test at 95% confidence level.

2.5. Drought Tolerance Level of NILs Judged Through Per cent Reduction of Yield and Stress Indices

In order to assess the true tolerance of NILs, % reduction (%R) and stress indices were calculated (Table 5). 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 NIL 18 and 93% in NIL 8 during year 2017. The donor parent, N22 maintained the least %R of 64% and minimum and maximum % reduction among

NILs was for NIL 17 (72%) and NIL 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 59% of NIL 17 to 86% of NIL 1. The drought yield index (DYI) ranged from 0.98 (NIL 18) to 3.14 (PB1) in year 2017; DYI was between 0.49 (N22) to 2.79 (NIL 1) and in year 2019 DYI range was from 0.35 (N22) to 1.37 (PB1). Highest DYI was found for NIL17 followed by NIL 12, NIL 3 and NIL 6. The stress tolerance index (STI) was in the range 0.05 (PB1) to 0.19 (NIL 17) during 2017; during 2018 STI was ranged from 0.05 (NIL 1) to 0.31 (NIL 17) and during 2019 its range was from 0.12 (PB1) to 0.47 (NIL 17). NIL 17 had the highest STI in all the experimental seasons. SSI values are in range of 1.04 (NIL 17) to 1.18 (PB1) during 2017; while its range in 2018 was 0.90 (NIL 17) to 1.17 (NIL 1) and during 2019 it was 0.75 (NIL 17) to 1.09 (PB1). The SSI values have shown same pattern during 2018 and 2019, and on average NIL 17 showed the lowest SSI value of 0.90 followed by NIL 6 (0.94) and NIL 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})								Spikelet fertility (%)	
	Stressed	Unstressed	%R	DYI	STI	SSI	AST	YSI	Mean	AST
NIL1	572.3^{h}	5545.0^{abc}	89.8	1.9	0.1	1.1	45.8	42.0	52.6	6.1
NIL2	1090.6^{d-g}	5757.1^{abc}	80.9	1.1	0.2	1.0	9.7	19.0	62.3	6.2
NIL3	1295.8^{a-f}	5943.7^{abc}	78.2	0.9	0.2	1.0	9.1	12.0	60.2	3.0
NIL4	901.6^{d-g}	5542.2^{abc}	83.4	1.3	0.2	1.0	19.4	27.0	64.1	8.6
NIL5	1236.6^{b-g}	5839.8^{abc}	78.3	1.0	0.2	1.0	11.0	17.0	65.1	2.3
NIL6	1408.8^{a-d}	5841.0^{abc}	75.7	0.9	0.3	0.9	29.2	18.0	57.7	1.8
NIL7	1393.3^{a-e}	5871.8^{abc}	76.4	1.0	0.3	1.0	34.9	23.0	57.8	5.4
NIL8	879.6^{efg}	5656.5^{abc}	84.0	1.4	0.1	1.0	3.1	19.0	59.9	3.3
NIL9	806.9^{fg}	5324.4^{bc}	84.8	1.3	0.1	1.1	16.7	28.0	59.5	4.4
NIL10	725.9^{g}	5765.5^{abc}	87.3	1.6	0.1	1.1	17.5	30.0	63.3	5.9
NIL11	1122.3^{d-g}	5678.1^{abc}	80.1	1.0	0.2	1.0	2.8	12.0	57.2	2.4
NIL12	1254.6^{a-f}	5722.2^{abc}	78.0	0.9	0.2	1.0	6.5	12.0	62.5	0.6
NIL13	866.2^{fg}	5582.3^{abc}	84.4	1.3	0.2	1.1	13.1	26.0	57.0	0.9
NIL14	1050.0^{d-g}	5547.9^{abc}	81.0	0.9	0.2	1.0	33.4	30.0	60.4	7.5
NIL15	1238.8^{b-g}	5879.9^{abc}	78.9	0.9	0.2	1.0	5.1	12.0	65.5	3.8
NIL16	955.8^{d-g}	5938.4^{abc}	83.8	1.1	0.2	1.0	34.7	32.0	57.4	5.3
NIL17	1715.8^{ab}	6122.9^{a}	72.0	0.7	0.3	0.9	27.8	15.0	63.5	5.9
NIL18	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, AMMI stability value; YSI, yield stability index

2.6. 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 AMMI stability analysis across the season under drought condition, particularly for plot yield and spikelet fertility and stability indices, AST and YSI was generated based on the AMMI model formulated (Table 5). For plot yield, the AST (AMMI stability value) ranged was 2.80 (NIL 11) to 69.10 (IR86918-B-B-305) and the YSI (yield stability Index) range was 12 (NIL 3,11,12,15) to 42 (NIL 1). Considering AST and YSI values with above average plot yields, NIL 11, 8, 15 and 12 are the most stable among all NILs. For Spikelet fertility, the AST range was 0.61 (NIL 12) to 8.61 (NIL 4) and the YSI range was from 7 (NIL 15) to 40 (NIL 12). Considering AST, YSI and above average spikelet fertility values, NILs 12, 18, 13 and 21 were found most stable. Keeping in perspective above two traits, it is apparent that the NIL 12 could maintain superior plot yield coupled with spikelet fertility across seasons under drought situation.

2.7. 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

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 %

2.8. 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 (Table 7). Visually, the milled grains of the NILs were appeared similar to that of PB1. However, NIL1, NIL2, NIL3, NIL5, NIL6 and NIL13 showed higher KLBC, while L/B ratio was found increased in NIL13. After cooking, NIL2, NIL3, NIL6, NIL7 and NIL18 showed better KLAC than PB1 (Figure 4). Interestingly, the elongation ratio was found significantly good in all NILs except in NIL9, NIL10 and NIL12. Furthermore, the alkali spreading value (AS) 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 recurrent parent, PB1 along with checks during *Kharif* 2019 under stressed (S) and unstressed (NS) conditions

Entry	KLBC		KBBC		L/B Ratio		KLAC		KER	
	NS	S	NS	S	NS	S	NS	S	NS	S
NIL1	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}
NIL2	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}
NIL3	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}
NIL4	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}
NIL5	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}
NIL6	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}
NIL7	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}
NIL8	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}
NIL9	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}
NIL10	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}
NIL11	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}
NIL12	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}
NIL13	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}
NIL14	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}
NIL15	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}
NIL16	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}
NIL17	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}
NIL18	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}
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}
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}
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}
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}
$S.E_m$	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; L/R Ratio, length by breadth ratio; KLAC, kernel length after cooking in mm; KBAC, kernel breadth after cooking in mm; KER, kernel elongation ratio; SEm, standard error of mean.

Means followed by same letters are statistically at par based on the least significant difference test at 95% confidence level.

3. 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 [39]. 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 [40]. 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

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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 traits, 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 [21,41,42]. MABB along with stringent phenotypic selection was successfully employed for Basmati rice improvement for different biotic stresses [43,44,45,25,46,29,47]. 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* [32], *qDTY3.1* [48,32] and *qDTY12.1* [49,50], showed consistent grain yield under drought across different genetic backgrounds and has been used in breeding applications. 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 aromatic 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 (HSB19), that was later released as Taraori Basmati [51]. 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 [52]. 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. Also, 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 pureline selection from a landrace, Rajbhog [53] that possesses deeper root system, shorter duration and tolerant to heat and drought [54]. This variety has short bold non-aromatic grains with very less elongation on cooking.

QTLs from a particular genetic background usually show minor effects or may remain completely silent in diverse genetic backgrounds [55]. In the case of drought, interaction between QTL × genetic background has been a major bottleneck limiting the use of QTLs for MABB in rice [56,57,49]. Nevertheless, N22 had a major effective QTL, *qDTY1.1*, governing grain yield under reproductive stage water stress which reportedly showed consistent enhancement in GY under stressed in diverse genetic backgrounds [15]. This qualifies *qDTY1.1* as a most desirable candidate for use in MABB to improve prominent high-yielding varieties with augmented drought tolerance.

Major reduction was noticed for yield under drought which was found significantly influenced by spikelet fertility. In comparative assessment, there was no yield penalty noticed under unstressed condition among the NILs as a consequence of introgressing the *qDTY1.1*, in PBI NILs. In an earlier report on introgression of another QTL, *qDTY3.1*, a negative effect on grain yield was accounted by Venuprasad et al. [32] under unstressed treatment. The NILs evolved under this program, showed significant higher gain for grain yield over PB1, with NIL2, NIL3, NIL5, NIL6, NIL7, NIL10, NIL12, NIL15, and NIL17 having clear advantage under both stressed and unstressed treatments. This also indicated that negative effect of QTL x background interaction was not observed under PB1 background. NIL 17 had the maximum grain yield among the NILs followed by NIL3, NIL5, NIL6, NIL7 and NIL12 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 [58], 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 [17,59,60]. Meta QTLs have also been documented for maximum root length and GY within the *qDTY1.1* region [61, 62]. In the PB1 background, *qDTY1.1* seems to enhance the yield under drought indirectly by modulating spikelet fertility as inferred from step wise 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, NIL12 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 by [16,63,64], 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 shorter 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. But 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 [65]. In the present study, we surmise that linkage between the *qDTY1.1* and sd1 gene 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 × stress level interaction across different environments [36]. STI sorts out the entries which perform well under non stress and fairly well under stress condition [38]. Lower the SSI value lesser the yield difference between stress and non-stress condition and hence greater is the tolerance of the genotype [66]. Thus, DYI, STI and SSI favored NIL17 as best genotype for drought situation due to its highest grain yield under both stressed and unstressed conditions followed by NIL3, NIL6, NIL7, NIL12, NIL15 and NIL18. Further, NIL17 outyielded majority of the remaining NILs.

PB1 being a basmati rice variety having specialized 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 quality attributes of PB1 along with drought tolerance (Figure 4).

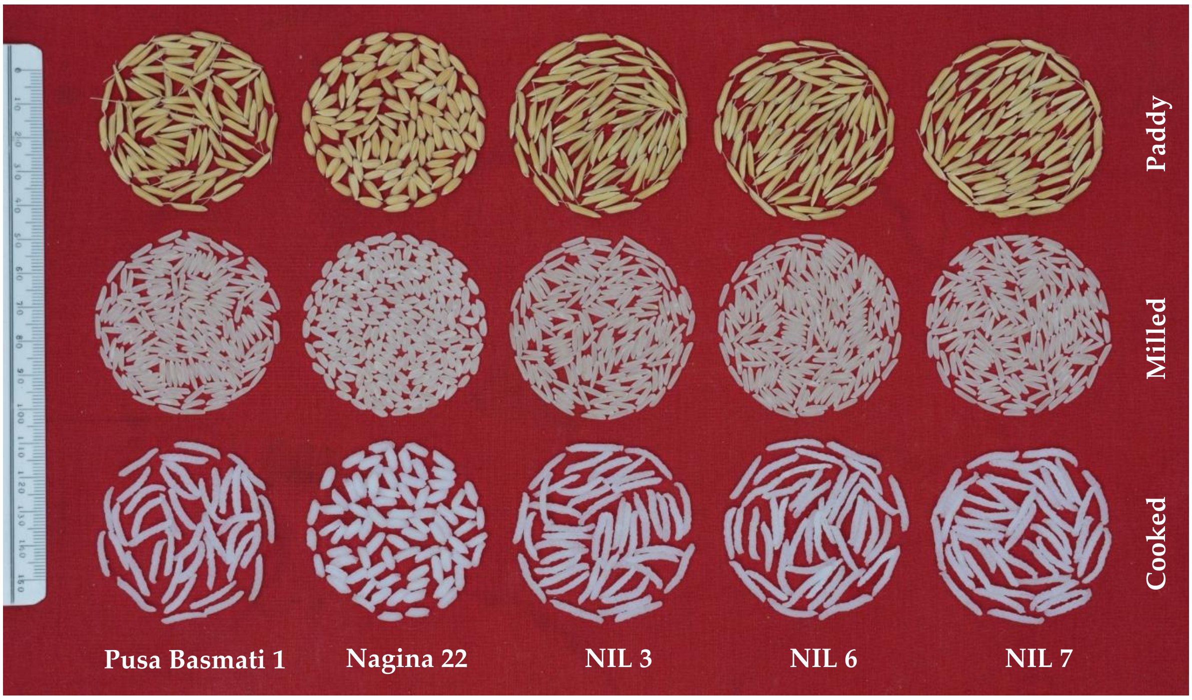

Figure 4. Grain and cooking quality of some of the NILs of Pusa Basmati 1 carrying *qDTY 1.1* QTL.

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. Although statistically insignificant, there was a marginal reduction in head rice recovery noticed under severe stress, along 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 [67]. In spite of the successful recovery of PB1 genome component governing grain quality among the NILs. 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 [26,29,68].

4. Materials and Methods

4.1. Plant Material and Experimental Sites

PB1, the elite Basmati cultivar was selected as the recurrent parent. An *aus* cultivar, Nagina 22 (N22), was used as donor parent for the QTL, *qDTY1.1*. N22 is a pureline selection from a landrace, Rajbhog. Well-known as a universal donor for high temperature tolerance, drought tolerance and grain dormancy [27], 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 though shuttle breeding mode. Final drought stress evaluation of NILs was done at New Delhi for three subsequent crop seasons.

4.2. 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_1s) were harvested and propagated in the late *Rabi* season of 2013-14 at Aduthurai, Tamil Nadu. F_1s 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 2014-15. In the BC_3 generation, from *Kharif* 2015, four successive selfings were carried out to generate BC_3F_5 NILs by 2016-17 late *Rabi* season at Aduthurai. All through during different generations, selections were carried out to recover maximum RPG among the progenies. The selected NILs, beginning from the BC_3F_5 generation, were subjected to three seasons of drought screening at New Delhi during 2017 to 2019.

For molecular marker analyses, the genomic DNA was isolated from fresh leaf samples using CTAB method with minor modifications [28]. Polymerase chain reaction was performed with the volume of 10 µl containing 20–30 ng template DNA, 5 pmol of each primer, 0.05 mM dNTPs (MBI, Fermentas, Lithuania, USA), 10× PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM MgCl2) and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India), running a amplification profile consisting of one cycle of initial denaturation at 94°C for 5 min; followed by 35 cycles containing denaturation with at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min; and a final extension at 72°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 pictographed using a BioRad® (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 SSR marker RM431 (F: tcctgcgaactgaagagttg; R: agagcaaaaccctggttcac) 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 [29]. The recovery of PB1 type alleles among the backcross progenies during each generation was used for computing the recurrent parental genome (RPG) recovery [30]. For the graphical comparison of the genomes of NILs and parents, graphical genotypes were drawn using GGT v.2.0 [31].

4.3. 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 and the sensitive check 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. 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 unirrigated until the soil moisture tension reached –70 kPa at 30 cm depth. Severe leaf rolling and firing were observed at this soil moisture level. At this severe stress, a flash life-saving irrigation was provided, and the water was drained out approximately after 24 hours. This cycle was constantly repeated until harvest [15, 32]. 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 608.1mm of rainfall was received in the years 2017, 2018 and 2019 respectively.

4.4. 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), plant height at maturity (PH), panicle length (PL), number of reproductive tillers (NT), spikelet fertility % (SF) and grain yield (GY). The NILs were also characterized for kernel length and breadth before and after cooking. From these measurements, length/breadth ratio (LB) and cooking quality characteristics, such as kernel length elongation ratio (KE) were computed. Further, alkali spreading value (AS) and aroma were determined using standard protocols [33].

4.5. 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 obtained from the model was saved as best linear unbiased predictors (BLUPs). The data were analyzed using STAR package version 2.0.1. 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) and yield stability index (YSI) were generated, as follows.

$$\text{AMMI stability value, } AST=\sqrt{\left[\frac{SS_{IPC1}}{SS_{IPC2}}\times IPC1\right]^2+(IPC2)^2} \qquad [34]$$

$$\text{Yield stability index, } YSI=R^{AST}+R^{Y} \qquad [35]$$

Where, SSIPC1 and SSIPC2 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, RAST and RY are the respective genotypes ranks based on AST and yield (Y).

To compare the effect of drought on different traits, a forward step-wise 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 were worked out as follows.

$$\text{Drought yield index, } DYI=\frac{(Y_i)^{NS}/(Y_i)^{S}}{(Y_G)^{NS}/(Y_G)^{S}} \qquad [36]$$

$$\text{Stress tolerance index, } STI=\frac{(Y_i)^{NS}\times(Y_i)^{S}}{(Y^{NS})^2} \qquad [37]$$

$$\text{Stress susceptibility index, } SSI=\frac{(Y_i)^{NS}-(Y_i)^{S}}{Y^{NS}-Y^{S}}\times\frac{Y^{NS}}{(Y_i)^{NS}} \qquad [38]$$

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

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.

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 programmes.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/xxx/s1.

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Abbreviations

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
KE	Kernel elongation ratio
MABB	Marker assisted backcross breeding
N22	Nagina 22
NIL	Near isogenic lines
NS	Unstressed
NT	Number of tillers
PB1	Pusa Basmati 1
PH	Plan 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 repeat

STI	Stress tolerance index
YSI	Yield stability index

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