



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 2399-2408
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www.thepharmajournal.com
Received: 08-04-2022
Accepted: 13-05-2022

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Marker assisted backcross breeding to develop the drought tolerant version of IR58025B, a popular maintainer line of hybrid rice

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Abstract

Drought stress has become more important abiotic factor affecting rice yield under present changing climate conditions. IR58025B is a maintainer line of widely used WA CMS line IR58025A in commercial rice hybrids production in India. Most of the rice hybrids released for the diverse ecosystem conditions using three-line system. Consequently, these derived hybrids suffer drastic yield decline under moisture stress conditions. To facilitate the efficient use of complex polygenic traits in hybrid rice molecular breeding research, we undertook development of introgression lines in background of IR58025B with tolerance to moisture stress by introgression of major QTL *qDTY_{3.1}* controlling grain yield under moisture stress. The present study reports the development and evaluation of 230 introgression lines (BC₂F₂) lines in IR58025B background for improvement of grain yield under moisture stress condition. Among 230 ILs, 24 introgression lines were found positive for *qDTY_{3.1}* and negative for *rf3*. Promising BILs exhibited earliness of 7 days under moisture stress in comparison to the recurrent parent. Grain yield advantage of 8.54g to 10.78g was recorded among selected BILs compared to IR58025B under moisture stress condition. This suggested that the efficacy of introgressed drought tolerant QTL *qDTY_{3.1}* in enhancing grain yield under moisture stress conditions. Further, breeding lines of IR58025B possessing *qDTY_{3.1}* could also serve as good donors for development of tolerant rice hybrids under drought stress.

Keywords: Rice, ir58025b, drought, yield, BILs and marker assisted backcross breeding

Introduction

Rice is an important food crop for more than 50% of the world's population. More than 90% of the world's rice is grown in Asia, accounting for 20% of the total calorie intake on any given day (Dwivedi *et al.* 2021) [3]. The revolutionary changes occurred by the advent of green revolution in the past six decades and the rice production are equivalent to the growth of the population in India. Considering the annual average population growth rate of 1.5% and current per capita consumption of about 250g of rice per day, rice demand is expected to be at least 140 M T by 2050. India needs to increase the production of rice by 2.5 M T per year to meet its population demand by 2050 (<http://www.fao.org/rice2004/en/pdf/khush.pdf>). The global rice production is at present facing the problem of changing climate manifested in terms of shrinking resources such as land, water and nutrients etc. There is an urgent need to develop climate resilient rice cultivars (Sandhu *et al.* 2017) [17]. The present abiotic stresses are also hardship to increase rice production. Hence, within the existing agricultural lands, the genetic improvement of yield potential in rice by combating abiotic stresses could be the ideal way to increase yield. The Hybrid rice technology is indispensable to raise the productivity of rice in the country. A total of 127 rice hybrids were released so far for commercial cultivation in India (AICRIP progress report 2021). Hybrid rice is grown in 3 M ha with a production of 3.5 M t during *Kharif* 2017 (Hari Prasad *et al.*, 2018) [6]. Majority of the rice hybrids were developed for irrigated conditions. These were severely affected by biotic and abiotic stresses. Currently, three line CMS system was widely used for exploitation of hybrid vigour in rice. Therefore, developing hybrids tolerant to moisture stress will help in avoiding the quantum yield losses. Though conventional plant breeding made significant impact on increasing the yield coupled with resistance to various biotic and abiotic stresses for target environments, these efforts are slow, need expertise labour and resource intensive.

Genetically, drought tolerance in rice is a complex response. Several large-effect and small-effect QTLs and meta-QTLs have been reported for grain yield under reproductive stage drought stress (Sandhu *et al.* 2017) [17]. A large-effect QTL, *qDTY_{2.2}*, on chromosome 2 was identified in the MTU 1010/Kali Aus population under upland (Sandhu *et al.* 2014) [18] and lowland (Palong *et al.* 2014) [14] conditions. Mishra *et al.* (2013) [11] reported another QTL, *qDTY_{12.1}*, on chromosome 12 that was significantly associated with reproductive-stage drought stress. *qDTY_{12.1}* explains 42% of the genetic variation with an additive effect of 172 kg/ha for grain yield under drought with no significant penalty under unstressed conditions (Venuprasad *et al.* 2009) [22]. Another major-effect QTL, *qDTY_{1.1}* was identified on chromosome 1 between the marker intervals RM431 and RM12091 in the Swarna/IR 64 population (Ghimire *et al.* 2012) [4]. Venuprasad *et al.* (2009) [22] identified two major QTLs, *qDTY_{2.1}* and *qDTY_{3.1}*, on chromosome 2 and 3, respectively, from the population of Apo/2*Swarna, that explained 13–16% and 31% of the genetic variances, respectively. The subsequent use of these QTLs, particularly of *qDTY_{3.1}*, has been demonstrated to impart improved drought tolerance among several genetic backgrounds, such as improved Sabitri (Yadav *et al.* 2013) [24], IR64 and Vandana (Kumar *et al.* 2014) [8], MR219 (Shamsudin *et al.* 2016) [19], Samba Mahsuri (Sandhu *et al.* 2017) [17], White Ponni (Muthu *et al.* 2020) [13], and Pusa 44 (Dwivedi *et al.* 2021) [3].

Marker-assisted selection (MAS) approach to transferring these QTLs into drought sensitive genetic backgrounds of high-yielding varieties has been widely adopted in rice (Price *et al.* 1999) [15]. Jongdee *et al.* (2002) [7] advocated the use of molecular breeding to accelerate product development, especially for characters which are complex and have low heritability, or if the breeding process is time-consuming and expensive. The marker-assisted incorporation of major- and minor-effect QTLs for grain yield under drought stress has been recognized as time- and cost-effective, as well as a fast-track approach for breeding drought tolerant rice varieties (Bernier *et al.* 2007) [1]. Marker assisted breeding (MAB) is practiced to introgress genes from a donor into the background of an elite cultivar (recurrent parent) and to recover the recurrent parent genome as rapidly as possible, which may take a minimum five to six generations in conventional plant breeding. Recent experiments at International Institute of Rice Research lead to identification of major effect QTLs like *qDTY_{3.1}* (Venuprasad *et al.* 2009)

[22] regulating grain yield under drought stress. IR58025B is a maintainer line of widely used CMS line IR58025A in commercial rice hybrids production. IR58025B is commonly used in the production of superior hybrids in rice due to its desirable features like good combining ability, plant stature, high pollen load, good panicle exertion of 96%, excellent spikelet opening, stigma exertion, synchronized tillering, medium grain type, dark green thick leaves, long heavy panicles and excellent phenotypic acceptability. This study reports the introgression of *qDTY_{3.1}* into IR58025B to develop drought tolerant BILs with high grain yield under moisture stress condition using marker assisted backcross breeding. Apo is a drought tolerant upland indica genotype developed at IRRI, Philippines harboring major effect QTL *qDTY_{3.1}* controlling grain yield under moisture stress.

Materials and Methods

This study was carried out from Kharif 2016 to Rabi 2018 at Indian Institute of Rice Research, Rajendranagar, and Hyderabad with the intention of developing backcross inbred lines of IR58025B with *qDTY_{3.1}* using Apo as drought tolerant QTL donor and IR58025B as recurrent parent.

Molecular and hybridization works were conducted in the MAS laboratory and greenhouse facility available at Department of Hybrid Rice, Indian Institute of Rice Research, Rajendranagar, and Hyderabad respectively. Phenotypic evaluation of backcross inbred lines under control and reproductive stage moisture stress was carried out during Rabi 2018 at the farm of Indian Institute of Rice Research, Hyderabad.

QTL introgression

Introgressed backcross inbred lines of IR58025B with drought tolerance QTL *qDTY_{3.1}* were developed by crossing IR58025B (female parent) with Apo (male parent) deploying marker assisted backcross breeding. Parental polymorphism was done between IR58025B and Apo using reported SSR markers linked to drought tolerance QTL *qDTY_{3.1}* (Venuprasad *et al.*, 2009) [22] (Table 1). Among them, RM520 was identified as foreground selection marker for QTL *qDTY_{3.1}*. It is a nondominant marker. Parental polymorphism between IR58025B and Apo was carried out with 300 SSR markers covering the entire genome. Out of 300 markers, 88 markers were found to be polymorphic between IR58025B and Apo. These 88 markers were used for background selection.

Table 1: Details of polymorphic primer used for foreground selection of *qDTY_{3.1}*

QTL	Chromosome	Position	Primer	Sequence
<i>qDTY_{3.1}</i>	3	247 bp	RM520	F:AGGAGCAAGAAAAGTCCCC R:GCCAATGTGTGACGCAATAG

Table 2: Details of the fertility restorer lines used for identification of IR58025B (maintainer line)

Fertility restorer gene	Chromosome	Position	Primer	Sequence
<i>Rf3</i>	1	188 bp	RM10313	ACTTACACAAGGCCGGGAAAAGG TGGTAGTGGTAACTCTACCGATGG
<i>Rf4</i>	10	185 bp	RM6100	TTCCCTGCAAGATTCTAGCTACACC TGTTTCGTCGACCAAGAAGACTCAGG

Genotyping

Fresh leaves from 25 days old young seedlings were collected freeze-dried and the DNA was extracted using the CTAB method (Murray and Thompson, 1980) [12]. Amplification was carried out using Polymerase Chain Reaction (PCR).

The cocktail for PCR amplification was prepared in a total reaction volume of 10 µl consisting of 2 µl DNA, 0.3 µl forward primer, 0.3 µl reverse primer, 6.0 µl distilled water, 0.3 µl dNTP's, 1 µl 10X Taq buffer and 0.1 µl Taq polymerase. The reaction mixture was given a momentary

spin for thorough mixing of the cocktail components. Then 0.20 ml PCR tubes were loaded in a thermal cycler. PCR amplifications were performed in thermal cycler (Eppendorf Mastercycler®). The cycling conditions included initial denaturation at 94°C for 5 minutes, followed by 30 seconds at 94°C, 1 minute at 55°C and 1 minute at 72°C for 35 cycles, followed by final extension at 72°C for 10 minutes. The PCR products were kept in storage at 4°C until further use. The PCR amplified products (10 µl) along with 100 bp ladder were subjected to electrophoresis in a 3.0 percent agarose gel in 1X TBE buffer at 120 Volts for 2 hours. The ethidium bromide stained gels were documented in UV gel documentation system (Syngene) and later banding pattern was scored. Stepwise selection involving phenotyping and genotyping was used to select and advance the desirable plants in every generation.

Graphical representation of the genome

Graphical representation of molecular marker data was performed using the software Graphical Genotype (GGT2.0).

Field experiment for evaluation of backcross inbred lines of IR58025B introgressed with drought tolerance QTL *qDTY*_{3.1}

The experiment was carried out during *Rabi* 2018 at farm of Indian Institute of Rice Research, Hyderabad. The experiment was laid out in randomized block design with two replications for both reproductive stage moisture stress and control experiment.

The seeds of BC₂F₂ of IR58025B x Apo were sown in nursery bed along with parents and seedlings were raised. 17 days old seedlings were transplanted in field with a row length of 2.5m. Inter and intra row spacing was maintained as 30cm and 10cm respectively in transplanted field. Recommended practices except water management were followed to raise a good crop. Under controlled conditions, irrigation and flooded conditions was maintained until maturity of the crop. The crop was harvested when the grains reached at physiological maturity. In the moisture stress experiment, at panicle initiation stage irrigation was withheld for 3 weeks. After 3 weeks, the plants were irrigated and stringent selection was phenotypically and the tolerant genotypes that recovered from stress were harvested when the grains reached at physiological maturity stage.

The rainfall data during crop growing season of *Rabi* 2018 was recorded from the Meteorological Observatory located at Agricultural Research Institute, Rajendranagar, and Hyderabad and prescribed in fig 2. A perusal of weekly mean rainfall data of the experimental site indicated the prevalence of moisture stress at reproductive crop growth stage.

Observations recorded

Plant height, days to 50% flowering, number of panicles per plant, panicle length, 1000 grain weight, spikelet fertility and grain yield per plant were recorded for five competitive BIL progenies.

Statistical Analysis

WINDOWSTAT software version 9.1 was used to analyze the data of the present experiment for the computation of means and standard error of difference (SED).

Results

Marker assisted backcross breeding and BILs Development

Figure 1 represents the marker assisted backcross breeding

scheme used for the development of *qDTY*_{3.1} positive BILs of IR58025B. A total of 30 F₁ plants were obtained by crossing IR58025B with Apo. Among these, eight true F₁ plants were identified and forwarded to next backcross generation. True F₁s plants were backcrossed with the recurrent parent IR58025B and 140 BC₁F₁ seeds were produced. Individual plant hills of BC₁F₁ seeds were sown in field and evaluated during *rabi* 2017. The foreground selection was carried out for 140 BC₁F₁ plants and 40 true BC₁F₁ were obtained as positive for *qDTY*_{3.1}. Among 40 plants, 11 BILs were chosen based on vigorous phenotype and genotypic selection. The introgressed BILs exhibited a maximum recurrent parent genome recovery ranged between 76 to 86%. The selected 11 plants were advanced to further backcross generation *i.e.*, BC₂F₁. 11 BILs from BC₁F₁ were individually backcrossed with IR58025B to produce 200 BC₂F₁ seeds. All 200 BC₂F₁ plants were evaluated in the field by growing in individual plant hills during *kharif* 2017.

Foreground selection was carried out in 200 BC₂F₁ plants with RM520 marker linked to drought tolerance QTL *qDTY*_{3.1}. On scoring, 50 plants were positive for *qDTY*_{3.1} (heterozygous condition) and were subjected to background selection by using 88 SSR markers. A total of 15 genotypes with target QTL were selected. Recurrent parent genome recovery ranged from 88% to 90% among the best BILs. Fifteen positive plants of BC₂F₁ generation were selfed to produce BC₂F₂ generation. Seeds of BC₂F₂ were raised as single plant hills during *rabi* 2018 under field conditions. A total of 230 BC₂F₂ plants were screened for target QTL linked SSR marker *i.e.*, using RM 520. On scoring, 24 BC₂F₂ plants were found positive for target QTL *qDTY*_{3.1}.

Validation of marker assisted breeding for drought tolerance by phenotyping

The overall mean performance of the IR58025B introgressed BILs and their parents (Apo, IR58025B) is shown in Table 3. Plant height ranged from 78.66 cm to 128.88 cm under control and 62.66 cm to 112.88 cm under moisture stress conditions. Moisture stress reduced plant height by 16 cm. The mean days to 50 % flowering in the IR58025 BILs varied from 80 to 96 days under control and 78 to 94 days under moisture stress conditions. Early flowering of BILs by 2 days was observed in moisture stress conditions. Number of panicles per plant of BILs ranged from 10 to 20 and 8 to 15 for under control and moisture stress conditions. The 20 to 25% reduction in panicles per plant in BILs was recorded under moisture stress condition. Panicle length ranged from 18.24 cm to 25.35 cm under control and 16.76 cm to 23.87 cm under moisture stress condition. Moisture stress reduced panicle length by 1.48 cm. Mean 1000 grain weight in the IR58025B BILs varied from 16.84 g to 22.87 g under control and 15.22 g to 21.25 g under moisture stress conditions. A reduction of 1.62 g in 1000 grain weight was recorded under moisture stress conditions. Spikelet fertility ranged from 60.65 % to 83.43 % under control and 53.38 % to 78.61 % under moisture stress conditions. Moisture stress reduced spikelet fertility by 4.28%. The mean grain yield of BILs ranged from 15.14 g to 23.68 g under control and 11.90 g to 20.44 g under moisture stress conditions. The 13 to 20 % reduction in yielding BILs was recorded under moisture stress conditions. The analysis of variance revealed significant difference among 24 BILs (BC₂F₂ progenies) for all the traits except panicle length in moisture stress and control situations showing the presence of sufficient genetic variability in 24 BILs (Table 4).

Performance of the promising BILs of IR58025B with target QTL $qDTY_{3.1}$ for grain yield and its component traits

In the present study, seven BILs of IR58025B *viz.*, GSD-2-1-8, GSD-4-6-2, GSD-12-6-4, GSD-1-4-46, GSD-20-4-2, GSD-21-8-3 and GSD-23-8-2 were selected based on their higher grain yield under moisture stress conditions (Table 5). All the BILs out-yielded IR58025B under reproductive stage moisture stress condition. Yield advantage over IR58025B was observed for all the selected $qDTY_{3.1}$ positive BILs of IR58025B under reproductive stage moisture stress as all them yielded higher than IR58025B. Among the selected BILs, GSD-4-6-2 produced highest grain yield followed by GSD-20-4-2, GSD-2-1-8, GSD-12-6-4, GSD-23-8-2, GSD-16-4-4 and GSD-21-8-3 under reproductive stage moisture stress condition. Grain yield advantage of 8.54 g to 10.78 g was recorded among selected BILs compared to IR58025B under moisture stress condition. The promising lines with $qDTY_{3.1}$ exhibited earliness of 6 days under moisture stress in comparison to recurrent parent. This suggested that the effectiveness of introgressed drought tolerance QTL $qDTY_{3.1}$ in increasing grain yield under moisture stress conditions. Among the selected BILs, GSD-4-6-2 showed higher values for number of panicles per plant and grain yield per plant. GSD-21-8-3 showed higher values for number of panicles per plant, panicle length, and 1000 grain weight and spikelet fertility. Among the BILs, seven BILs of IR58025B *viz.*, GSD-2-1-8, GSD-4-6-2, GSD-12-6-4, GSD-16-4-4, GSD-20-4-2, GSD-21-8-3 and GSD-23-8-2 were selected based on their higher grain yield under control conditions (Table 5). All the BILs out-yielded IR58025B under control condition. Among the selected BILs, GSD-20 produced highest grain yield followed by GSD-2-1-8, GSD-4-6-2, GSD-21-8-3, GSD-23-8-2, GSD-16-4-4 and GSD-12-6-4 under control condition. Grain yield advantage of 0.70 g to 3.43 g was recorded among selected BILs compared to IR58025B under control condition and is accordance with the results of Priyadarshini (2013) [16], Yadav *et al.* (2013) [24], Kumar *et al.* (2014) [8], Shamsudin *et al.* (2016) [19], Bhagyalakshmi *et al.* (2018) [2], Sandhu *et al.* (2017) [17], Muthu *et al.* (2020) [13] and Dwivedi *et al.* (2021) [3]. The promising lines with $qDTY_{3.1}$ exhibited earliness of 3 days under moisture stress in comparison to recurrent parent. Similar results were obtained earlier for days to flowering under moisture stress conditions by Jongdee *et al.* (2002) [7], Bernier *et al.* (2007) [1], Venuprasad *et al.* (2009) [22] and Ghimire *et al.* (2012) [4]. Among the selected BILs, GSD-23-8-2 showed higher values for plant height and number of panicles per plant. GSD-12-6-4 showed higher values for 1000 grain weight. GSD-16-4-4 showed higher values for panicle length. GSD-2-1-8 showed higher values for spikelet fertility. The BILs of IR58025B with $qDTY_{3.1}$ has to be further validated under preliminary and multi-location trials for confirming their stability of performance under target environments and finally could be released as improved version of IR58025B tolerance to reproductive stage moisture stress condition. Further, breeding lines of IR58025B possessing $qDTY_{3.1}$ could also serve as good donors for development of rice hybrids with tolerance to drought stress.

Background screening was done with 88 polymorphic SSR markers for the promising BILs of IR58025B using GGT 2.0 software (fig 3). The background genome recovery of the selected BILs ranged from 88.63% to 95.45%. Maximum recovery of 95.48% was observed in the lines GSD-12-6-4

and GSD-23-8-2 (Table 6) and could be further utilized in breeding programme as donor since it had desirable agronomic characters in addition to introgressed $qDTY_{3.1}$ in it. Based on over all mean performance of BILs under both reproductive stage moisture stress and control conditions, GSD-12-6-4, GSD-23-8-2 and GSD-16-4-4 were selected due to their superior grain yield and higher recurrent parent genome recovery. These lines could be further utilized in development of hybrids tolerant to reproductive stage moisture stress condition.

Discussion

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

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

Selection of parents

The most important step in hybridization programme is selection of parents. The present study was undertaken with the intention of developing backcross inbred lines of IR58025B with $qDTY_{3.1}$ by introgression drought QTL $qDTY_{3.1}$ from Apo into IR58025B. Apo is a drought tolerant upland indica genotype developed at IRRI, Philippines harboring major effect QTL $qDTY_{3.1}$ controlling grain yield under moisture stress. IR58025B is a maintainer line of widely used CMS line IR58025A in commercial rice hybrids development. This line is commonly used in the production of

superior hybrids in rice due to its desirable features like good combining ability, plant stature, high pollen load, synchronized tillering, and dark green and thick leaves with long and heavy panicles. However, decrease in its agronomic performance was reported under moisture stress conditions (Gouda *et al.*, 2012) [5]. Hence it obviates genetic enhancement of parental lines for moisture stress in order to develop superior rice hybrids.

Marker assisted backcross breeding for drought tolerance in rice

Marker-assisted breeding, particularly using the backcross method, has been extensively used for introgression genes governing resistance/tolerance biotic as well as abiotic stresses in rice (Muthu *et al.* 2020) [13]. In rice, drought can affect crop growth in various stages, ultimately resulting in different outcomes. In the seedling stage, drought hinders population establishment, while that at the vegetative stage affects crop proliferation and tillering. Although drought at early growth stages may indirectly affect the crop yield, drought stress at the reproductive stage is more crucial because it directly affects grain development and ultimately the yield. Therefore, to breed rice varieties with drought endurance, reproductive stage drought tolerance is the most important trait to be harnessed.

There are many QTLs governing tolerance to drought stress that have been mapped in rice (Venuprasad *et al.* 2009., Ghimire *et al.* 2012., Mishra *et al.* 2013., Sandhu *et al.* 2014 and Palong *et al.* 2014) [22, 4, 11, 18, 14] and many of them have been transferred into the well-known varieties (Singh *et al.* 2016) [20]. Among these, *qDTY_{3.1}* is one of the large-effect QTLs governing tolerance under severe lowland drought stress (Venuprasad *et al.* 2009) [22]. *qDTY_{3.1}* was identified on chromosome 3 from Apo and found to explain 31% of genetic variance for grain yield. During the recent years, progress has been made in introgression of identified grain yield QTLs under drought stress in different genetic backgrounds of rice using marker assisted backcrossing with an yield advantage of 300 to 500 kg ha⁻¹ under reproductive stage drought stress (Venuprasad *et al.* 2009., Swamy *et al.* 2013., Shamsudin *et al.* 2016 and Sandhu *et al.* 2017 Muthu *et al.* 2020 and Dwivedi *et al.* 2021) [22, 21, 19, 17, 13, 3]. However, the introgression of yield QTLs into elite Indian cultivars not yet accomplished. Hence, there is a dire need to further introgressed the identified grain yield QTLs into the genetic background of elite cultivars of rice to further improve its genetic level. Hence the present investigation was undertaken to develop drought tolerant version of IR58025B using Apo as *qDTY_{3.1}* through marker assisted backcrossing.

Development of drought tolerant IR58025B BILs using stepwise marker assisted backcross breeding approach

In the present study peak marker *i.e.*, RM520 tightly linked to drought tolerant QTL *qDTY_{3.1}* was taken for foreground selection (fig 3). 88 background selection markers were used for fast recovery of the recurrent parent. A total of 30, 140, 200 and 230 plants were genotyped with RM520 SSR marker linked to drought tolerance QTL *qDTY_{3.1}* in F₁ (*kharif*, 2016), BC₁F₁ (*rabi*, 2017), BC₂F₁ (*kharif*, 2017) and BC₂F₂ (*rabi*, 2018) generations respectively resulted in the development of 24 *qDTY_{3.1}* positive lines BILs (BC₂F₂) of IR58025B.

Agronomic performance of IR58025B BILs under reproductive stage moisture stress and control conditions

A significant disparity found in expression of traits by BILs of

qDTY_{3.1} under moisture stress and control environments. The BIL progenies as well as parents *viz.*, IR58025B and Apo grown under moisture stress condition, in general, produced significantly lower grain yield than control conditions. It might have happened due to the reduction in photosynthesis and assimilate translocation under moisture condition. Kumar *et al.* (2018) [9] also observed yield reduction of 45%, 77%, 79% and 97% in F₃, F₅, F₇ and F₉ generations of Swarna-Sub1 introgression lines under reproductive stage moisture stress. Early flowering was hastened in all the BILs grown under stress and corroborates with the findings of Venuprasad *et al.* (2002) [23], Bernier *et al.* (2007) [1], Venuprasad *et al.* (2009) [22], Priyadarshini (2013) [16], Ghimire *et al.* (2012) [4], Yadav *et al.* (2013) [24], Kumar *et al.* (2014) [8], Shamsudin *et al.* (2016) [19], Bhagyalakshmi *et al.* (2018) [2], Sandhu *et al.* (2017) [7], Muthu *et al.* (2020) [13] and Dwivedi *et al.* 2021 [3]. There is a significant reduction in plant height under moisture stress in all the BILs and is in consonance with the findings of Venuprasad *et al.* (2002) [23], Bernier *et al.* (2007) [1], Priyadarshini (2013) [16], Yadav *et al.* (2013) [24], Kumar *et al.* (2014) [8], Shamsudin *et al.* (2016) [19], Bhagyalakshmi *et al.* (2018) [2], Sandhu *et al.* (2017) [17], Muthu *et al.* (2020) [13] and Dwivedi *et al.* 2021 [3]. It could be due to the undesirable linkage between *qDTY_{3.1}* and plant height under moisture stress condition. The number of panicles per plant was significantly reduced under moisture stress condition and correlates with the findings of Dwivedi *et al.* 2021 [3]. Rice plants respond differently to drought by reducing number of panicles and leaves as adaptive features to cope with induced moisture stress. Moisture stress at reproductive stage had lowered 1000 grain weight and spikelet fertility than control as evident from the present study. This might be due to lack of moisture for pollen fertilization. Similar results were obtained for number of 1000 grain weight and spikelet fertility under moisture stress conditions by Dwivedi *et al.* 2021 [3].

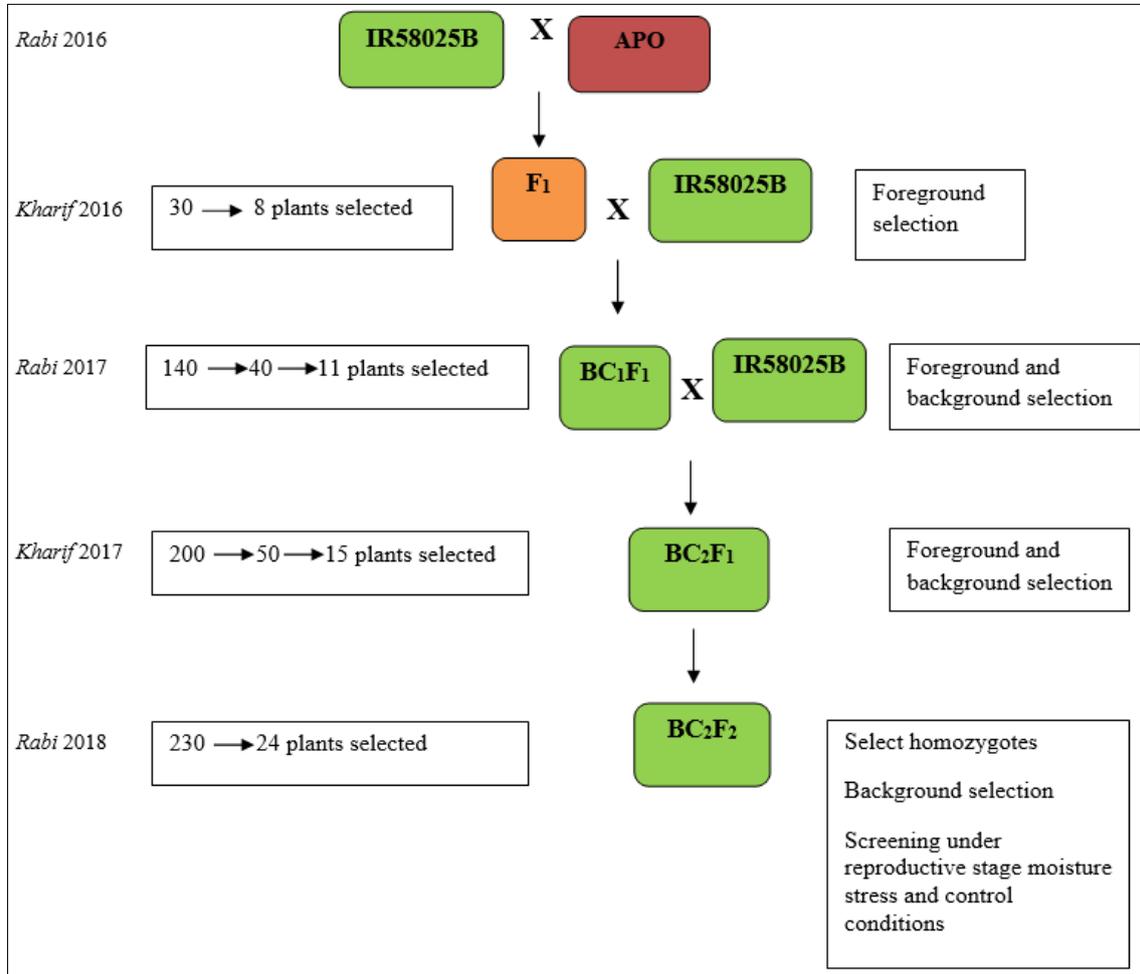
Performance of identified improved drought tolerant IR58025B BILs

Grain yield advantage of 8.54 g to 10.78 g was recorded among selected BILs compared to IR58025B under moisture stress condition and is in accordance with the results of Priyadarshini (2013) [16], Yadav *et al.* (2013) [24], Kumar *et al.* (2014) [8], Shamsudin *et al.* (2016) [19], Bhagyalakshmi *et al.* (2018) [2], Sandhu *et al.* (2017) [17], Muthu *et al.* (2020) [13] and Dwivedi *et al.* 2021 [3]. The promising lines with *qDTY_{3.1}* exhibited earliness of 7 days under moisture stress in comparison to recurrent parent. This suggested that the effectiveness of introgressed drought tolerance QTL *qDTY_{3.1}* in increasing grain yield under moisture stress conditions. Grain yield advantage of 3.43g to 2.84g was recorded among selected BILs compared to IR58025B under control condition and is in accordance with the results of Priyadarshini (2013) [16], Yadav *et al.* (2013) [24], Kumar *et al.* (2014) [8], Shamsudin *et al.* (2016) [19], Bhagyalakshmi *et al.* (2018) [2], Sandhu *et al.* (2014) [18], Muthu *et al.* (2020) [13] and Dwivedi *et al.* 2021 [3]. The promising lines with *qDTY_{3.1}* exhibited earliness of 5 days under moisture stress in comparison to recurrent parent.

The BILs of IR58025B with *qDTY_{3.1}* has to be further validated under preliminary and multi-location trials for confirming their stability of performance under target environments and finally could be released as improved version of IR58025B tolerance to reproductive stage moisture stress condition. Further, breeding lines of IR58025B

possessing *qDTY_{3.1}* could also serve as good donors for development of rice hybrids with tolerance to drought stress. Background screening was done with 88 polymorphic SSR markers for the promising BILs of IR58025B using GGT 2.0 software (fig 4). The background genome recovery of the selected BILs ranged from 88.63% to 95.48%. Maximum recovery of 95.48% was observed in the line GSD-12-6-4 and could be further utilized in breeding programme as donor

since it had desirable agronomic characters in addition to introgressed *qDTY_{3.1}* in it. Further, the obtained best lines of IR58025B were screened for the presence of fertility restorer genes (fig 5 & 6) *i.e.*, *Rf3* and *Rf4* (Table 2). The best lines of IR58025B showed negative for *Rf3* and *Rf4* genes indicating that these best lines were good maintainers as that of IR58025B.



Details on the foreground, background selection and number of plants selected in every generation using marker assisted backcrossing breeding approach

Fig 1: A detailed scheme of marker assisted backcross breeding adopted for introgression of drought tolerance QTL *qDTY_{3.1}* from Apo into IR58025B

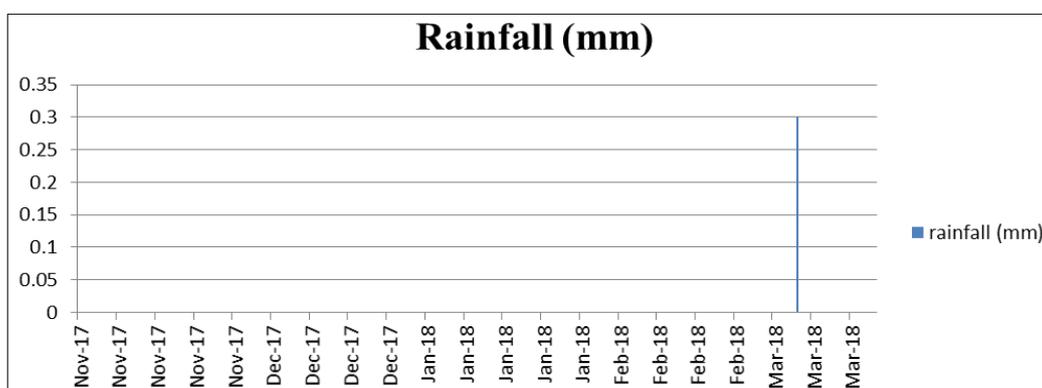
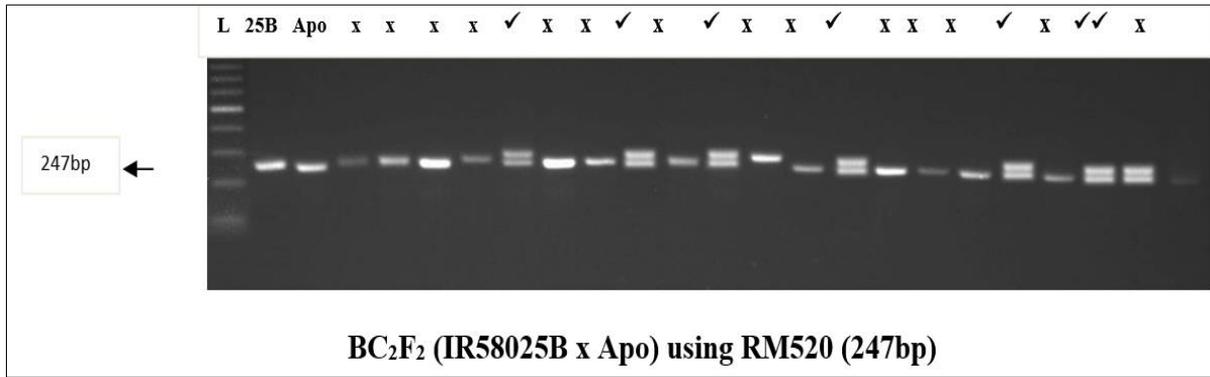


Fig 2: Daily rainfall during the experiment period from November 2017 to March 2018



✓ : Selected *qDTY_{3.1}* positive plants; L: 100 bp Ladder

Fig 3: Foreground selection for *qDTY_{3.1}* among BILs (BC₂F₂) of IR58025B using RM520 SSR marker

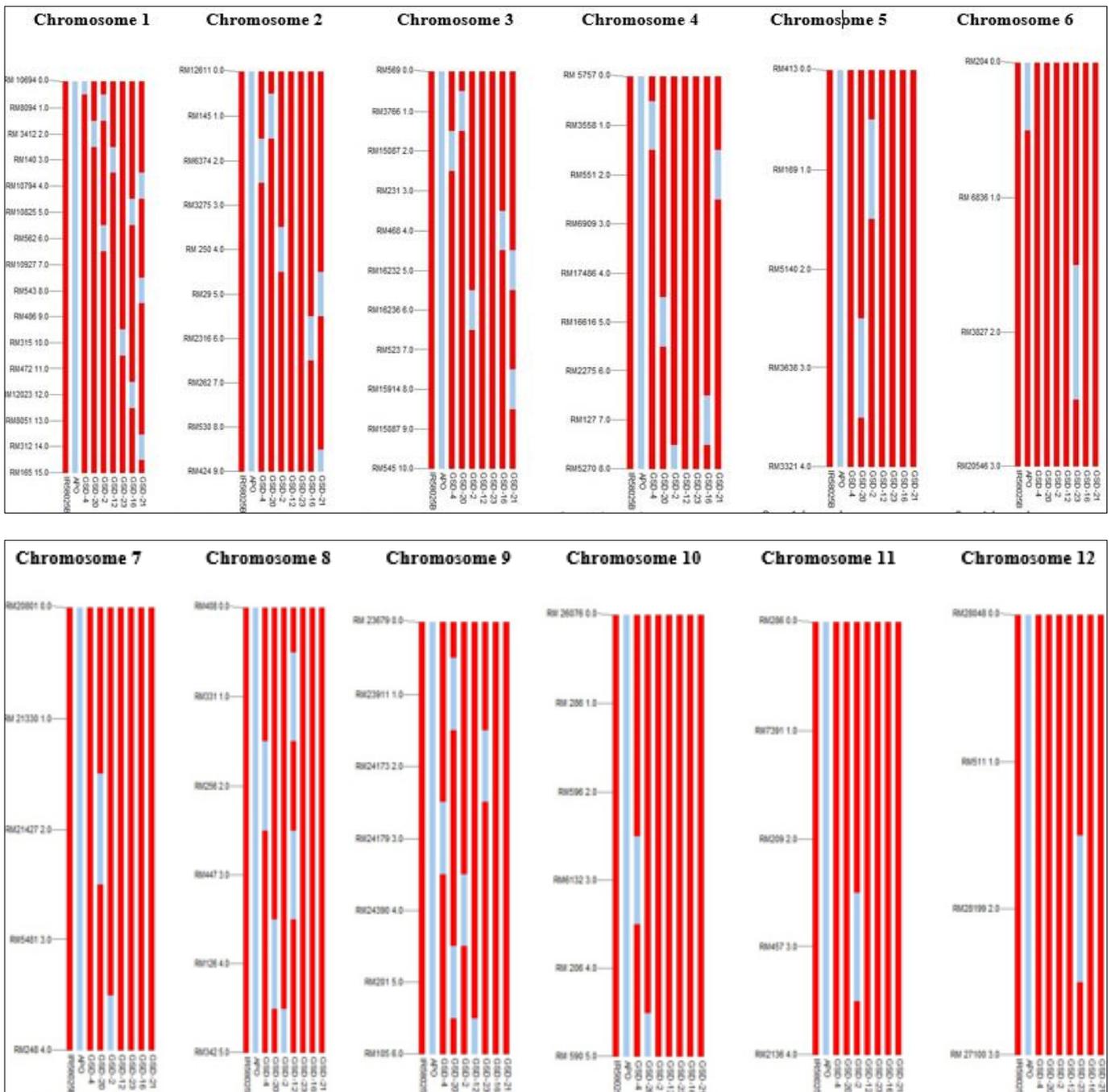


Fig 4: Graphical representation of background genome recovery in seven selected BC₂F₂ lines of IR58025B x Apo

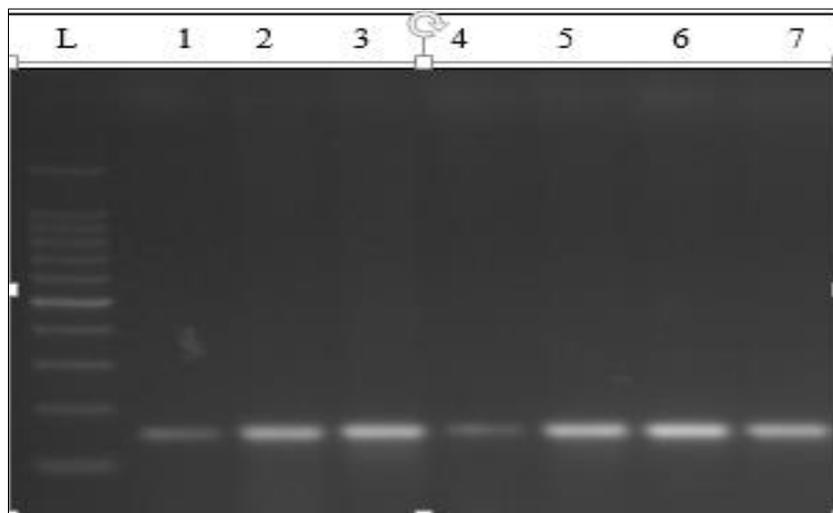


Fig 5: Screening for *Rf3* gene using RM 10313 SSR marker among the promising BILs of IR58025B

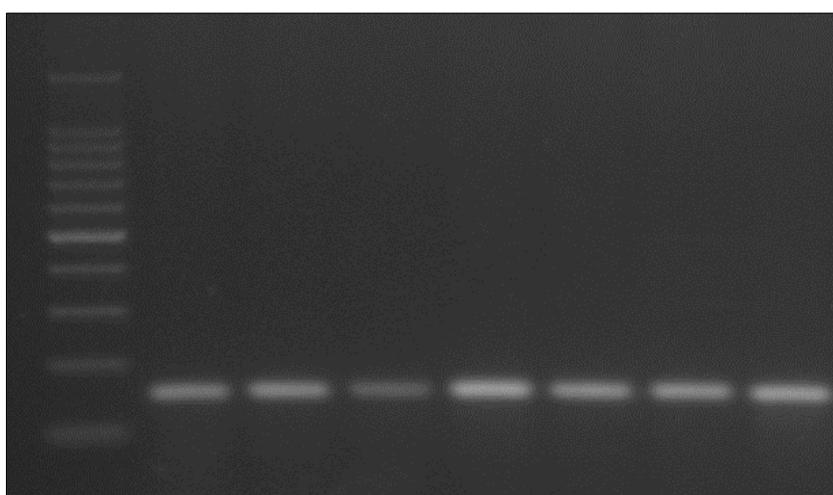


Fig 6: Screening for *Rf4* gene using RM 6100 SSR marker among the promising BILs of IR58025B

Table 3: Trait mean values of parents and backcross inbred lines along with range and coefficient of variation for yield and its contributing traits under Control (C) and Reproductive Stage Moisture Stress (RSS) conditions

S. No.	Character	Environment	Parents		Mean	Range	C.V
			IR58025B	Apo			
1.	Plant height (cm)	C	100.25	113.75	97.54	78.66-128.88	15.06
		RSS	87.12	78.63	81.52	62.66-112.88	18.01
2.	Days to 50% flowering	C	97.00	90.00	84.98	80.00-96.00	5.80
		RSS	94.00	84.00	82.93	78.00-94.00	5.83
3.	Number of panicles per plant	C	16.00	18.00	13.38	10.00-20.00	14.79
		RSS	10.00	8.00	11.13	8.00-15.00	15.34
4.	Panicle length (cm)	C	24.32	26.40	20.37	18.25-25.35	9.73
		RSS	19.50	18.12	18.89	16.76-23.87	10.50
5.	1000 grain weight (g)	C	18.50	23.04	19.58	16.84-22.87	9.47
		RSS	12.80	22.23	17.96	15.22-21.25	10.32
6.	Spikelet fertility (%)	C	76.95	85.40	70.35	60.65-83.43	6.96
		RSS	64.70	76.41	64.37	53.38-78.61	8.50
7.	Grain yield per plant (g)	C	20.25	22.94	19.30	15.14-23.68	13.70
		RSS	9.81	18.28	16.01	11.90-20.44	16.50

Table 4: ANOVA of BILs for yield and its component traits under control (C) and Reproductive Stage moisture Stress conditions (RSS)

S. No.	Traits	Treatment mean sum of squares (df: 23)	
		Control	RSS
1.	Plant height (cm)	86.13**	43.26**
2.	Days to 50% flowering	7.73**	7.38**
3.	No. of panicles per plant	3.69**	9.26**
4.	Panicle length (cm)	2.60	4.06
5.	1000 grain weight (g)	3.52**	15.50**
6.	Spikelet fertility (%)	35.65**	55.54**
7.	Grain yield per plant (g)	15.00**	19.54**

Table 5 Agronomic performance of promising BILs of IR58025B with target QTL *qDTY_{3.1}* for grain yield and its component traits

Agronomic trait	Environment	GSD-4-6-2	GSD-20-4-2	GSD-2-1-8	GSD-12-6-4	GSD-23-8-2	GSD-16-4-4	GSD-21-8-3
Plant height	C	105.30	108.50	109.50	108.35	111.80	102.52	106.85
	RSS	85.35	82.08	84.75	78.50	80.90	88.60	76.65
Days to 50% flowering	C	91.00	94.00	92.00	90.00	91.00	93.00	92.00
	RSS	80.00	85.00	84.00	82.00	83.00	83.00	88.00
Number of panicles per plant	C	16.00	16.00	15.00	16.00	18.00	17.00	16.00
	RSS	11.00	9.00	9.00	10.00	11.00	9.00	11.00
Panicle length	C	23.25	24.05	23.82	22.65	23.48	24.51	22.08
	RSS	21.25	19.05	19.32	19.25	20.98	21.01	21.58
1000 grain weight	C	20.72	20.12	20.27	21.69	21.66	20.90	21.52
	RSS	17.94	18.27	19.35	21.50	18.30	19.47	19.60
Spikelet fertility	C	84.01	81.16	85.23	82.52	81.02	84.32	81.88
	RSS	75.76	76.68	73.63	74.09	72.97	71.94	77.53
Grain yield per plant	C	23.09	23.68	23.13	20.95	22.77	22.43	22.85
	RSS	20.59	20.35	19.63	19.45	19.27	18.96	18.35

Table 6: Grain yield advantage and recurrent parent genome recovery of the promising BILs of IR58025B x Apo under reproductive stage moisture stress condition

S. No.	Genotypes	<i>qDTY_{3.1}</i>	Grain yield per plant (g)	Grain yield advantage over IR58025B	Recurrent parent genome recovery
1	GSD-4-6-2	BB	20.59	10.78	% 92.04
2	GSD-20-4-2	BB	20.35	10.54	% 88.63
3	GSD-2-1-8	BB	19.63	9.82	% 88.63
4	GSD-12-6-4	BB	19.45	9.64	% 95.45
5	GSD-23-8-2	BB	19.27	9.46	% 95.45
6	GSD-16-4-4	BB	18.96	9.15	% 94.31
7	GSD-21-8-3	BB	18.35	8.54	% 90.90
8	IR58025B	AA	9.81	-	-

Conclusion

Drought stress is one of the major constraints in rainfed areas for rice productivity and has the greatest impact during flowering stage where the formation of grain is suppressed. This results in significant yield losses. The identification and introgression of QTL regions with a large and consistent effect on grain yield under reproductive stage moisture stress represents an opportunity to improve high yielding drought susceptible varieties through marker assisted backcross breeding. The Hybrid rice technology is indispensable to rise the productivity of rice in the country. Majority of the rice hybrids were developed for irrigated conditions. These were severely affected by both biotic and abiotic stresses. Currently, CMS system utilizing A line, B line and R line was widely used for exploitation of hybrid vigour in rice. Therefore, developing hybrids tolerant to moisture stresses will help in avoiding the quantum yield losses. This goal can be accomplished by genetic enhancement of parental lines of hybrid rice to these abiotic stresses. In our study we have developed *qDTY_{3.1}* positive BILs of IR58025B. The selected BILs conferred a grain yield advantage of 8.54g to 10.78g compared to IR58025B under moisture stress condition. This

suggested that the effectiveness of introgressed drought tolerant QTL *qDTY_{3.1}* in increasing grain yield under moisture stress conditions. Further, backcross inbred lines of IR58025B possessing *qDTY_{3.1}* could also serve as good donors for development of rice hybrids with tolerance to drought stress. This suggested that drought tolerance rice hybrids can be successfully developed through marker assisted backcross breeding by introgression of QTL regions with a large and consistent effect on grain yield under moisture stress into elite parental lines of hybrid rice.

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