

Development of drought tolerant rice breeding lines of an elite Indian rice (*Oryza sativa* L.) variety Improved White Ponni through molecular breeding

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ABSTRACT

Changes in climatic conditions especially unpredictable drought occurrences are challenging for food security. The present investigation was carried out to improve the grain yield in Improved White Ponni (IWP) rice under reproductive stage drought stress. This variety is well known for its grain quality. Physiological changes, grain yield and related traits under drought were evaluated in the advanced breeding lines of Improved White Ponni X Apo containing *qDTY 3.1*. The experiment was conducted at the Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during 2020. Two superior backcross inbred lines of IWP X Apo, donor parent and recipient parent were raised under water stress in rain out shelter as well as in well irrigated conditions in replications. Soil moisture content was reduced to 12.7% in drought field showing the stress severity. Physiological parameters such as photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content and relative water content, Yield and related traits such as days to fifty percent flowering, productive tillers, spikelet fertility, grain yield and grain weight were recorded in the BILs and parents during flowering stages. Reduction was observed in all these traits under water stress. However, in comparison with IWP (94.6%) which lacks *qDTY 3.1*, BILs with *qDTY 3.1* showed less reduction in grain yield (63%) and other traits. Earliness was also observed in *qDTY 3.1* containing BILs under drought stress (BILs - 106 days, IWP-117 days) and controlled conditions (BILs- 83 days, IWP-107 days) when compared to IWP. Grain quality estimates in the BILs showed similarity to IWP. These BILs need to be evaluated further for confirmation of drought tolerance and they are effective resources for utilisation in drought breeding programmes.

Keywords: Drought, *qDTY*, marker assisted, backcross

1. INTRODUCTION

Rice crop productivity must be improved under changing climatic circumstances to ensure global food security. Due to considerable increase in evapotranspiration, dryness is predicted to peak in the twenty-first century, and drought incidences in India are expected to be at their highest over the years 2071-2100 [1]. As a result, next-generation crops should be water-efficient and provide sustainable yields without consuming a lot of water, as well as adaptable to a variety of conditions [2]. Lowland rice is particularly sensitive to reproductive stage drought stress, and if the drought continues, it will result in complete yield loss. Drought resistance screening during the flowering stage will be effective in identifying drought tolerant genotypes [3]. By transferring major effect loci through marker assisted breeding into elite genotypes along with conventional plant breeding approaches, measurable progress in boosting productivity under unfavourable conditions can be made, leading to the production of more productive, stress-tolerant cultivars [4]. In rice, *qDTY3.1* is a Quantitative Trait Loci (QTL) with genes controlling drought tolerance in chromosome 3 with large phenotypic variation of 31% was discovered [5]. Apo (drought tolerant) and Swarna (drought sensitive) rice varieties recorded grain yields of 516 gm⁻² and 300 gm⁻² under non-stress lowland conditions, respectively and 73 gm⁻² and 8 gm⁻² under extreme lowland drought stress respectively (Venuprasad *et al.*, 2009). Similarly, in the Pusa 44 backcross inbred lines - Pusa 1823-12-31-12-12-12 containing *qDTY3.1*, 2-2.5 times improvement in yield over Pusa 44 was recorded [6]. In Tamil Nadu (India), rice is mostly grown under irrigated conditions. The high yielding lowland rice varieties were not selected for

drought tolerance during the green revolution. Hence, these varieties lack the genes for drought tolerance. Studies have shown that Tamil Nadu is the second largest drought prone area [7] in the country. To increase the rice productivity under drought stress, drought tolerant varieties were released in Tamil Nadu. But most of these drought tolerant varieties belong to the bold grain type and hence not preferred for cultivation due to low consumer preferences. Hence, there is a need to develop donors that produce good yield under drought with acceptable grain type and cooking qualities preferred in Tamil Nadu, which is one of the states with largest rice cropping area in India. With this aim, *qDTY3.1* containing rice lines of IWP, the popular variety in Tamil Nadu were developed through marker assisted backcross breeding. Drought tolerance was assessed in the Backcross Inbred Lines (BILs) under irrigated and water stress conditions.

2. MATERIAL AND METHODS

All the hybridization experiments and genotyping of backcrossed progenies were carried out at the Department of Plant Biotechnology, Centre for plant molecular biology and biotechnology, TNAU, Coimbatore, Tamil Nadu, India. Field trials were conducted at the Department of rice, Centre for Plant Breeding and Genetics, TNAU, Coimbatore, Tamil Nadu, India.

2.1 GENETIC MATERIALS USED

Improved White Ponni is a popular rice widely cultivated but susceptible to drought during reproductive stage. Apo is a drought tolerant rice harbouring the major drought *qDTY 3.1* which is linked to the SSR marker RM520 (Marker interval – RM520-RM16030, Position – 30.91 Mb) [5]. It was used as the donor for enhancing drought tolerance in IWP. True F₁s were backcrossed with IWP and the obtained progenies were again backcrossed and advanced further to obtain superior progenies with *qDTY3.1* along with similarity to IWP in yield performance and grain quality.

2.2 DNA ISOLATION AND GENOTYPING

The modified CTAB technique was used for DNA extraction [8]. RM520 was used for foreground selection (FS) of *qDTY 3.1*. A total of 69 SSR markers showing polymorphism between IWP and Apo were used for background selection. SSR genotyping was carried out using the following thermal cycler programme: one cycle at 95°C for 5 minutes, 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes [9]. 3 percent agarose gel electrophoresis was used to resolve PCR results and observed with a UV trans-illuminator (Bio rad laboratories, India). In comparison to the parents, the allelic pattern of each SSR among the progenies was assessed.

2.3 PHENOTYPIC SCREENING UNDER WATER DEFICIT CONDITIONS

Lowland trials were conducted in puddled transplanting conditions with the water level in the fields maintained throughout the crop season until harvesting. Seeds were directly sowed on non-flooded, puddled soil for the drought trial. The plants were kept in well-irrigated conditions until the 61st day after seedling emergence. Drought stress was induced on the 62nd day by fully ceasing irrigation till harvest. Soil sample(500g) was taken to estimate the final Soil Moisture Content (SMC) during grain filling stages.

$$\text{Soil moisture content(\%)} = \frac{\text{Initial soil weight} - \text{Final soil weight}}{\text{Initial soil weight}} \times 100 \quad [10-11]$$

2.3.1 PERFORMANCE OF THE LINES IN FIELD TRIALS

Days to flowering (DTF) was recorded when 50% of the plants started flowering. Number of productive tillers per plant (PT), spikelet fertility, grain yield (GY), and thousand grain weight (TGW) were measured on ten plants, and the average was used for analysis.

$$\text{Spikelet fertility \%} = \frac{\text{Number of fertile spikelets}}{\text{Total number of spikelets}} \times 100$$

Photosynthetic indicators viz., Photosynthetic rate (Pn), Transpiration rate (E), Stomatal conductance (gS) were recorded on fully expanded flag leaves using Portable photosynthetic system, ADC Bioscientific Ltd. Relative water content was measured by collecting leaf samples and sealed to minimise transpirational losses. Fresh weight was taken and then the tubes with the leaf samples were filled with water. Turgid weight was taken after 24 hours. Before taking turgid weight, the surface water in the leaves were blotted off. The samples were then dried in an oven at 70°C to constant weight [12]. RWC was calculated using the following formula [13]

$$\text{Relative water content \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

2.4 GRAIN QUALITY ANALYSIS

Grains of the BILs and IWP were evaluated for quality traits. The length and breadth of milled rice grains were measured using vernier calliper. By soaking 25 whole milled kernels in 20ml of water and boiling them in a water bath maintained at 98°C for 10 minutes, the kernel length and breadth after cooking (KLAC and KBAC) were measured in 10 grains [14]. The kernel length and breadth elongation ratios (KER and BER) were calculated by dividing the average length and breadth of cooked kernels to that of uncooked kernels [14].

The gel consistency was measured [15] in the BILs and IWP. 95 percent ethanol containing 0.025 percent thymol blue and 2ml of 0.2N KOH were added to hundred mg of rice powder in 13 x 100mm culture tubes and heated for 10 minutes in a boiling water bath and then cooled for 20 minutes in an ice water bath. After 30 minutes, the length of cold gel in test tubes held horizontally on graph paper were measured. Time required for cooking is determined by the gelatinization temperature of starch. The gelatinization temperature of rice varieties, may be classified as low (55 to 69°C), intermediate (70 to 74°C), and high (>74°C) An estimate of the gelatinization temperature is estimated by the alkali digestibility test. Whole polished rice grains were immersed with 10 ml of 1.7% KOH in petri plates and kept for 23 hours. The alkali spreading value of kernels were classified as low, Intermediate or High [16].

All the recorded data were statistically analysed using SPSS software V22.

3. RESULTS AND DISCUSSION

3.1 DEVELOPMENT OF BILS HARBORING *qDTY3.1*

The backcross breeding scheme followed in this study is presented in Fig 1. True F₁s of IWP X Apo which amplified heterozygous alleles were identified using SSR marker associated with the target loci (*qDTY3.1*-RM520). BC₁F₁ plants were developed by backcrossing true F₁ hybrids with IWP. *qDTY3.1* positive progenies were identified through FS. Among them, progenies with recurrent parent genome recovery (RPG) of above 60% were backcrossed with IWP to generate BC₂F₁. In BC₂F₁, progenies with grain type similarity to IWP along with maximum RPG above 80% were forwarded further for backcrossing. The BC₃F₁ progenies were self-pollinated three times to obtain BC₃F₄. In BC₃F₃, a total of 125 individuals were raised, and the homozygous plants for the target QTL were identified using foreground genotyping. Background genotyping revealed 94.2% recovery of recipient parent genome (Fig 2). Positive progenies for the target loci with grain type of IWP and yielding similar to or above than IWP were raised as individual families and subjected to drought screening.

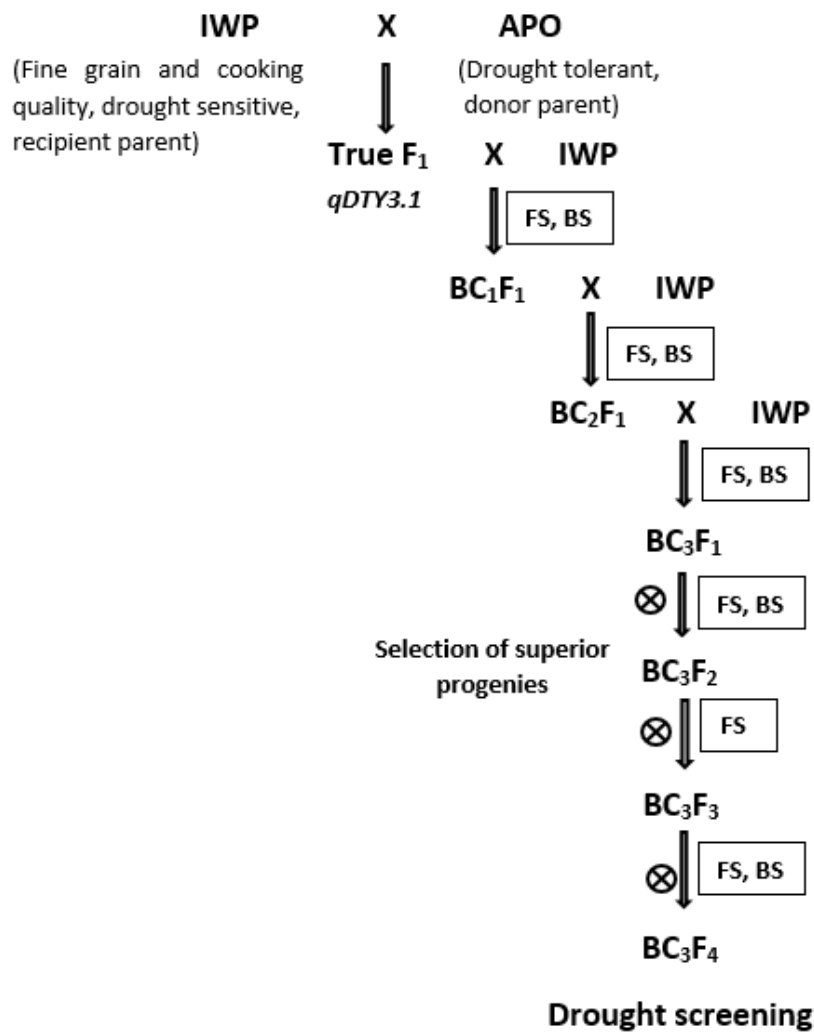


Figure 1. Backcross breeding scheme representing development of BILs of IWP X APO

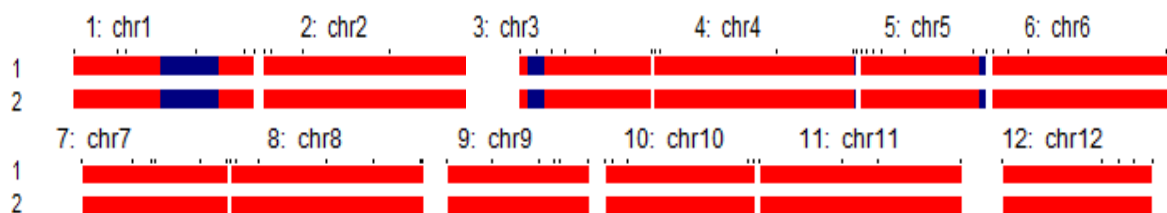


Figure 2. Graphical genotyping of the selected superior BILs with maximum RPG. 1) 4-7-4-24-4-15, 2) 4-7-4-24-4-16. Red colour and blue colour denote IWP and APO respectively. Graphical genotyping software V2

Best performing lines containing drought QTLs in ADT45 rice background with RPG of 90% through MABC and similarly, MRQ74 rice lines with root trait genes for drought tolerance with good phenotypic performance in BC₂F₁ with RPG up to 89.6% through MABC were developed in drought studies [17-18]. These studies indicate the efficiency of MABC in accelerating the breeding for stress tolerance in rice. In the current study, the selected BILs possessed maximum RPG of 94.2%.

3.2 PERFORMANCE OF IWP BILs UNDER DROUGHT CONDITIONS

Drought stress during reproductive stage reduced tillering, spikelet fertility and ultimately yield of all the genotypes (Table 1). However, the *qDTY3.1* containing IWP BILs showed improved performance in all the traits than IWP. Drought tolerant rice genotypes under severe and moderate stress conditions were identified in drought field trials where the soil moisture ranged between 13.6% - 17.8% respectively [19]. Soil moisture declined to 11.4%, 12.2%, 13.2%, and 14.1% at 15 cm, 30 cm, 45 cm and 60 cm depths respectively showing the severity of drought stress. In stress conditions where the drought sensitive parent shows yield reduction of at least 60% and the mean yield reduction of the stress trial is 50-60% is considered as an effective method of drought screening to amplify the genetic differences between the drought sensitive and tolerant lines [20-21]. In this study, drought stress caused yield reduction of 94.6% in the susceptible parent IWP and the mean yield reduction of the stress trial was 58.2%. This indicates the drought intensity during reproductive stage of the stress trial. When compared with IWP, the BILs recorded less reduction in all these traits. Delay in flowering is caused by drought conditions. However, the IWP BILs with *qDTY3.1* in this study showed earliness of 11-12 days than IWP under irrigated and 23-24 days earliness than IWP under drought conditions. Similar results were observed in studies where they reported that *qDTY3.1* is linked with earliness and also found in further analysis that this region is co-localized near *Hd-6*, a major heading date locus [22]. *qDTY3.1* was also reported to promote early flowering to overcome drought stress [23]

Table 1. Mean performance of the BILs under drought stress and irrigated conditions

Parents/ BILs	<i>qDTY3.1</i>	Duration	Days to fifty percent flowering		Number of productive tillers		Spikelet fertility (%)		Grain yield (g)		Yield reduction (%)
			Control	Stress	Control	Stress	Control	Stress	Control	Stress	
IWP	-	Medium	117 ± 0.4	107 ± 0.7	22 ± 1.0	7 ± 0.2	90.5 ± 1.4	25.2 ± 1.8	24.2 ± 0.7	1.3 ± 0.4	94.6
APO	+	Medium	98 ± 0.7	80 ± 0.9	12.3 ± 0.7	11.3 ± 1.1	86.3 ± 1.8	76.9 ± 1.9	20 ± 0.9	15 ± 0.1	25.0
4-7-4-24-4-15	+	Medium	106 ± 0.3	82 ± 0.5	15 ± 0.2	8.9 ± 0.1	92.4 ± 0.2	56.7 ± 0.9	28.2 ± 0.6	9.8 ± 0.4	65.2
4-7-4-24-4-16	+	Medium	105 ± 0.6	84 ± 0.2	17.6 ± 0.4	10.2 ± 0.2	93.1 ± 0.7	60.1 ± 0.7	26.5 ± 0.5	10.4 ± 0.5	60.8
General CV (%)			0.53		4.44		0.90		3.81		
CD (P = 05) (Genotypes)			0.72		0.84		0.97		1.01		
CD (P = 05)(Genotypes x stress)			1.01		1.18		1.37		1.42		

In this study 41.7% and 74.8% sterility in spikelets were recorded in the BILs and IWP respectively. Similar results were obtained in studies where they obtained lower spikelet sterility in drought-tolerant genotypes than susceptible cultivars [24]. Under drought, high sterility occurs due to the impairment of pollen development [25], defects in the formation of basal and apical pores in anthers by endothecium and problems in pollen swelling thus affecting dehiscence [26] and pollination. In a study [27], fine mapping of MQTL 3.1 revealed that the MQTL3.1 contained genes for detoxification (*DTX/MATE*) which can maintain the cell membrane stability. In this study, *qDTY3.1* containing IWP BILs recorded high spikelet fertility in comparison with IWP lacking this region probably due to the effect of *qDTY3.1* in avoiding the injuries to pollen development by maintaining the cell membrane stability under water stress. They also reported genes in MQTL3.1 that participate in phytic acid biosynthesis in developing seeds since it is the primary storage form of phosphorus in cereal grains. In the present study, IWP produced mean yield of 1.3 g per plant while the BILs

produced mean yield of 9.8 g (4-7-4-24-4-15) and 10.4 g (4-7-4-24-4-16) per plant. The two BILs also showed less reduction in physiological parameters (Fig 3.) whereas IWP was severely affected.

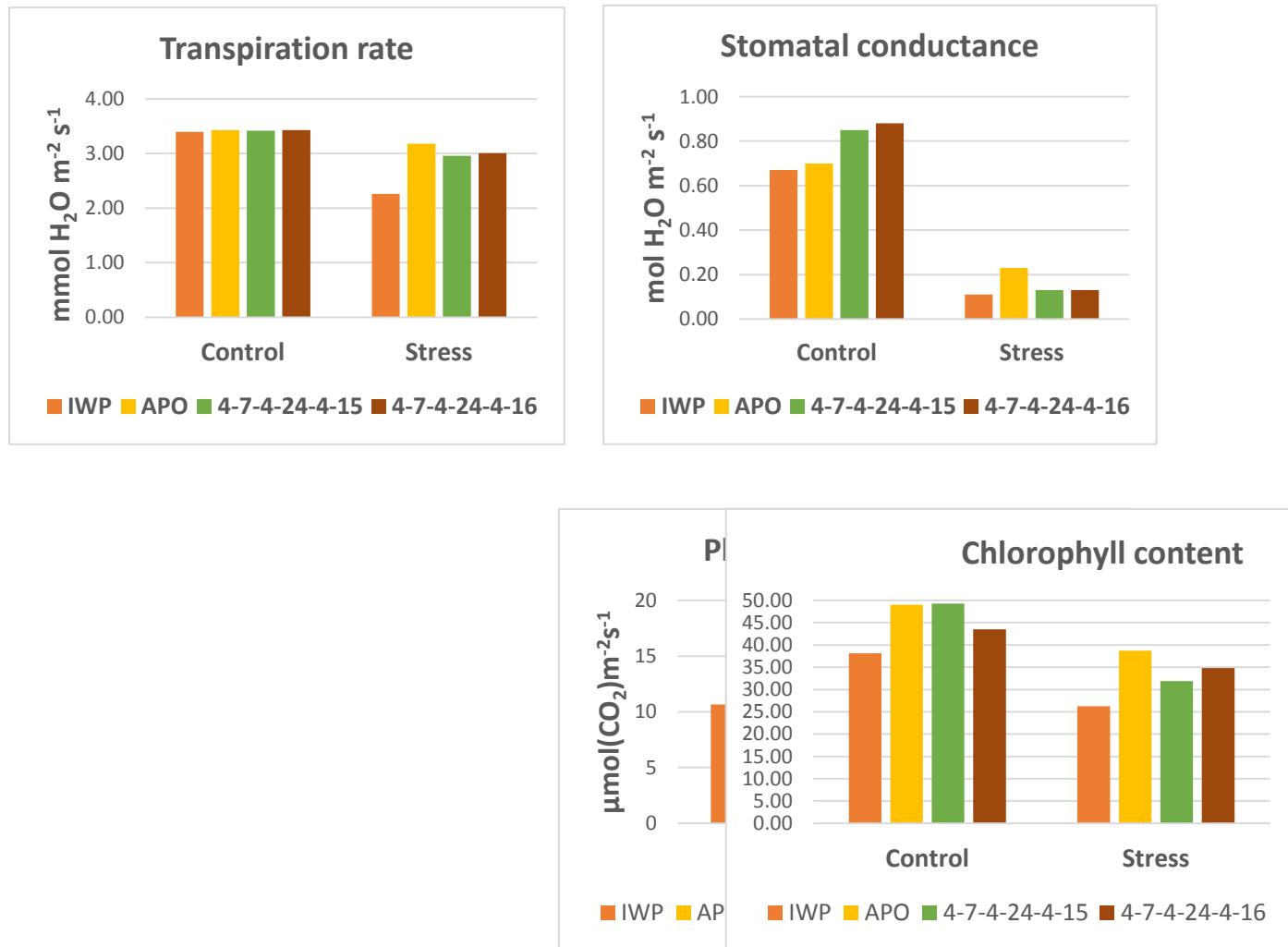


Figure 3.
performance of
parents under
and irrigated

Similar results were reported in a study [28] of landraces. They when compared to control variety, (Kalajeera, and Haldichudi) the highest level of resistance (N22) to the improved rate and maintenance of higher leaf relative water content under drought conditions. Plants can optimise CO₂ uptake for photosynthesis while reducing water loss by adjusting stomatal pore apertures but still maintaining their stomatal conductance to do photosynthesis [29-30]. Higher chlorophyll content was recorded in the drought tolerant rice CR 143-2-2 (36.06) than the susceptible variety Krishnahamsa (27.07) under drought stress [31]. In this study, both BILs and IWP reduced

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their stomatal conductance but the reduction in photosynthetic rate was lesser than IWP under drought stress (Fig 1) indicating the ability of the BILs to maintain physiological functions.

3.3 FIELD PERFORMANCE OF THE SELECTED BILs

The two superior BILs identified performed well under non-stress conditions similar to IWP. Earliness was observed in the BILs when compared to IWP (Table 1). But they obtained higher spikelet fertility and yield when compared to IWP under non-stress conditions. Similar results were obtained in a study [32], where they recorded grain yield advantage of 395.7–2376.3 kg ha⁻¹ under non-stress conditions in drought QTL pyramided lines. The BILs were compared for the grain quality traits with IWP (Table 2) and were found to be possessing superior grain quality traits.

Table 2. Physical and cooking quality of the selected BILs

	100 seed weight (g)	L/B ratio	Kernel elongation ratio	Breadth expansion ratio	Grain type	Gelatinization temperature	Gel consistency	
							mm	Category
IWP	1.66	2.73	1.65	1.46	Medium slender	Intermediate	80	Soft
4-7-4-24-4-15	1.92	2.54	1.68	1.11	Medium slender	Intermediate	65	Soft
4-7-4-24-4-16	1.91	2.69	1.64	1.26	Medium slender	Intermediate	67	Soft

4. CONCLUSION

Drought-tolerant genotypes ensure higher rice production under changing climatic conditions. Two superior BILs with high yield potential, superior grain, and improved drought tolerance were developed which can be used as valuable sources for drought breeding programmes. This study also proves the efficiency of molecular breeding to improve the drought tolerance trait in lowland rice cultivars.

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