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Development of early maturing, high yielding, drought tolerant rice variety with superior grain quality through molecular breeding and its performance evaluation

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Abstract
Drought is one of the major constraints limiting rice production and most of popular mega varieties of Tamil Nadu are highly susceptible to drought. Developing drought resilient rice cultivars will help in sustaining rice productivity under marginal environments. With the view to develop early maturing, drought tolerant rice lines possessing superior grain quality, a RIL population was developed between popular drought susceptible rice genotype Improved White Ponni and drought tolerant rice genotype *Apo*. Genotyping in F_2 population using SSR markers linked to mega effect QTLs of *Apo* resulted in the identification of progenies harboring various combinations of drought tolerant QTLs. Two early maturing progenies namely #76 and #110 possessing high yield potential and superior grain quality were found to harbor 2-3 mega effect QTLs of *Apo*. QTL positive lines were further advanced up to F_6 generation. About 159 F_6 progenies were evaluated during January'2015 under field conditions for earliness, yield and grain quality traits which resulted in the identification of superior single plants of # 76 (3 QTLs) # 110 (2 QTLs) possessing early duration (< 110 days), high yield and good grain quality. These progenies were named as CBMAS14076 and CBMAS14110. CBMAS14076 and CBMAS14110 were evaluated for their drought responses under both greenhouse and field conditions along with IWP and Apo. Both these lines were found to be early (100 – 110 days) and exhibited improved drought tolerance when compared to IWP. IWP showed 23.3% reduction in single plant yield under drought; whereas CBMAS14076 and CBMAS14110 showed only around 16% reduction in single plant yield.

Keywords: drought tolerance, rice, Apo, QTL, molecular breeding

Introduction
Rice is one of the major staple foods of Asia which is cultivated under diverse ecosystems. In India, rice is cultivated in nearly 60% of the total 140 million hectares of cultivated area under rainfed conditions (Barah, 2012). Rainfed rice is primarily dependent on the rains received during south west and north east monsoon seasons. Rice productivity under rainfed system is frequently affected by unpredictable vagaries of monsoons due to changing climatic scenario during recent years (Krishnakumar, 2016) [7]. There is an estimate that on an average one drought episode hits Tamil Nadu once in every 2.5 years (GOTN, 2013) [6]. Based on the projected population growth in India, rice production needs to be doubled by 2050. Achieving this target needs overcoming challenges *viz.*, yield plateau, declining land, water and labor resources, increasing cost of fertilizers, sustaining environmental health and predicted effects of climate change. Out of the major stresses limiting rice productivity, drought/water deficit remains No. 1 by causing serious yield losses under rainfed upland/irrigated lowlands. Hence, developing drought tolerant rice genotypes has been one of the greatest challenges in the field of rice breeding.
Despite concerted efforts through conventional breeding no promising high yielding varieties possessing improved drought tolerance have been developed. Recent advancements made in the field of genomics offer new opportunities to dissect the QTLs for drought tolerance. In rice numerous QTLs associated with drought tolerance have been identified and mapped (Ye *et al*., 2010) [25]. The identification of major QTLs for grain yield under drought—$qDTY_{12.1}$(Bernier *et al*., 2007) [2], $qDTY_{2.1}$ and $qDTY_{3.1}$(Venuprasad *et al*., 2009) [17], and $qDTY_{1.1}$ (Vikram *et al*., 2011) [24]; in rice using modern molecular biology tools has provided new opportunities to breeders to develop varieties tolerant against drought. This has opened the way for the development of drought-tolerant versions of susceptible high-yielding varieties through marker-assisted back cross breeding.

To expedite the breeding cycle and hasten the hybridization and selection, molecular markers have provided leads in rice molecular breeding (Ye *et al*., 2009) [16]. At IRRI, Phillipines, initiatives have been made towards introducing drought tolerance into popular high-yielding rice varieties such as IR64, Swarna, and Vandna. Earlier, marker assisted back cross breeding have been adapted to develop superior drought tolerant improved version of rice variety Improved White Ponni through introgression of QTLs from *Apo* (Muthukumar *et al*., 2017) [12]. Similar efforts have been made in the present study for introgressing QTLs controlling grain yield under stress from drought tolerant *Apo* into an elite rice genotype, Improved White Ponni towards development of early maturing, drought tolerant rice lines with good grain quality.

Materials and methods

The present study was undertaken with an aim of enhancing drought tolerance ability of popular rice varieties through marker assisted breeding. All the hybridization experiments, genotyping and green house experiments were conducted at Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore. All the field experiments were conducted at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India.

Genetic materials used and breeding scheme for development of recombinant inbred lines in rice

Improved White Ponni (IWP) is a medium duration, *indica* rice popularly cultivated in Tamil Nadu due to its high yield potential and superior grain and cooking qualities. However, it is highly susceptible to most of the biotic/abiotic stresses in addition to its lodging character. Apo is a drought tolerant and upland *indica* genotype from Phillipines which harbors major effect QTLs *viz*., *qDTY1.1*, *qDTY2.1*, *qDTY3.1* and *qDTY6.1* controlling grain yield under stress (Venu Prasad *et al*., 2009; 2012a and 2012b) [17, 20, 21]. A cross was made between IWP and Apo and True F_1 plants (IWP x Apo) were selected through SSR genotyping, advanced to F_2 generation and subjected to foreground selection (FGS) using polymorphic SSR markers *viz*., RM472, RM240, RM520 and RM3414 linked to the 4 target QTLs from Apo, *viz*., *qDTY1.1*, *qDTY2.1*, *qDTY3.1* and *qDTY6.1*, respectively (Table-1). The superior lines with target 2-3 QTLs were advanced upto F_2 generation by making FGS in each generation using the linked SSR markers (Fig. 1).

Table 1: Description of QTLs controlling yield under drought stress from Apo and linked markers used for foreground selection

S. No	QTL	Chr. No	Marker interval (QTL region)	Linked marker	Position (Mb)	Reference
1	*qDTY1.1*	1	RM486-RM472 (34.95 - 37.88 Mb)	RM472	37.89	Venu Prasad *et al*. (2012a) [20]
2	*qDTY2.1*	2	RM327-RM262 (28.68 - 35.72 Mb)	RM240	31.50	Venu Prasad *et al.* (2009) [17]
3	*qDTY3.1*	3	RM520-RM16030 (30.91 - 32.5 Mb)	RM520	30.91	Venu Prasad *et al.* (2009) [17]
4	*qDTY6.1*	6	RM589-RM204 (01.39 - 03.16 Mb)	RM3414	02.88	Venu Prasad *et al.* (2012b) [21]

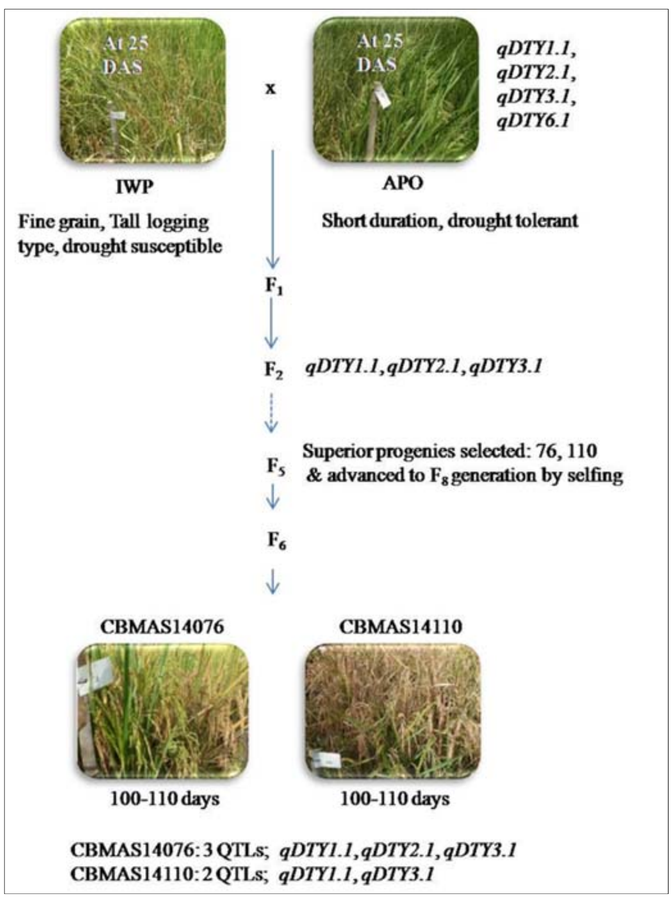

Fig 1: Breeding scheme representing development and evaluation of Recombinant Inbred lines (RILs) of IWP x Apo

DNA isolation, SSR genotyping and analysis

Fresh leaf samples were collected from all 152 progenies and parents and used for extracting DNA by modified CTAB protocol (Doyle and Doyle, 1990). Isolated genomics DNA was used for SSR genotyping. SSR genotyping was done by PCR (50 ng of template DNA, 1 X PCR buffer, 1.5mM $MgCl_2$, 0.4mM dNTPs, 10µM primers and 1.5U of *Taq* DNA polymerase) by using following thermal cycler program: one cycle of 95ºC for 5 min, 35 cycles of 95 ºC for 30sec, 55 ºC for 30 sec and 72 ºC for 30 sec followed by final extension of one cycle at 72 ºC for 10 min. PCR products were resolved by3 % agarose gel electrophoresis, stained with Ethidium Bromide and visualized with UV trans-illuminator. Allelic pattern of each SSRs among the progenies was scored in comparison with the parents.

Evaluation of F_2 population derived between IWP x Apo

With the view to developing early maturing and high yielding rice genotypes with acceptable grain quality, a segregating F_2 population developed between IWP and Apo was subjected to thorough genotyping and phenotyping. F_2 generation comprising of 152 progenies were subjected to foreground selection (FGS) using polymorphic SSR markers *viz*., RM472, RM240, and RM520 linked to the 3 target QTLs from Apo, *viz*., *qDTY1.1*, *qDTY2.1* and *qDTY3.1*, respectively.

Generation advancement of the selected F_2 progenies

Selected F_2 progenies were advanced upto F_6 generations and in each generation QTL positive lines/plants were selected based on SSR genotyping and among the QTL positive plants superior progenies were selected based on duration (100-110 days), grain yield per plant (> 25 g) and grain type. Selected lines were subjected to background selection using >50 genome wide polymorphic SSR markers to assess contribution from the superior parent IWP.

Greenhouse Evaluation of CBMAS14076 and CBMAS14110 for drought responses

CBMAS14076 and CBMAS14110 were evaluated for their responses against drought under green house conditions along with the parents during June'2015. Plants were grown in pots (42.5 cm height x 45 cm diameter) filled with 17 Kgs of pot mixture (2: 1 parts of coir pith: soil) containing required quantity of nutrients. Equal number of plants per pot was maintained in all genotypes with adequate replications. All the pots were watered regularly till 45th day and drought stress was imposed by withholding irrigation in one set of plants by maintaining corresponding controls. All the pots were saturated fully with water in the previous evening before imposing stress.

Progression of drought stress was monitored at regular intervals by measuring soil moisture content (SMC) and relative water content (RWC) of leaves. SMC was measured in both control and stressed pots by following gravimetric method (Black *et al*., 1965; De Angelis, 2007) [3, 4]. Fresh weight of the samples was recorded and the soil was dried at 65°C and the dry weight was recorded. Soil moisture was calculated and expressed in terms of percentage using the following formula,

$$\text{Soil moisture (\%)} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \text{X } 100$$

The RWC of the flag leaf samples collected from plants in stress and control pots were measured based on the method described by Turner (1981) [15]. The fresh weight of the sample leaves was recorded and the leaves were immersed in distilled water in a 25 ml falcon tube. After 8 hours, the leaves were removed, the surface water was blotted off, and the turgid weight was recorded. The samples were then dried in an oven at 70˚C to constant weight. RWC was calculated using the following formula given below and expressed in percentage.

$$\text{RWC (\%)} = \frac{\text{Fresh leaf weight} - \text{Dry leaf weight}}{\text{Turgid leaf weight} - \text{Dry leaf weight}} \text{X100}$$

Other observations *viz*., days to flowering, spikelet fertility percentage and grain yield per plant were also recorded in both well-watered and drought stressed plants. Physiological and gas exchange parameters such as Transpiration rate (E expressed in mmol H_2O m^{-2} s^{-1}), Stomatal conductance (g_s expressed in mol H_2O m^{-2} s^{-1}), Internal or Intercellular Co_2 Concentration (C_i in ppm), Assimilated Co_2 Concentration (C_a in ppm) and C_i/C_a were recorded using photosynthesis (Li-COR 6400 Photosynthetic system, USA).

Performance of CBMAS14076 and CBMAS14110 under field conditions

Two selected RILs (CBMAS14076 and CBMAS14110) were evaluated along with their parents during Summer' 2016 under both well-watered (open field) and drought (ROS; rain out shelter) conditions. A total of 91 plants / plot (5 x 4.8 ft) was maintained for each genotype. All recommended agronomic practices were followed (Crop Production Guide, 2015). Under ROS, normal irrigation was provided upto 64 days after sowing and drought stress was imposed from 65th day after sowing onwards by withholding watering draining the water completely. The soil moisture was allowed to deplete gradually and stress condition was maintained till harvest. Under well-watered conditions, irrigation was provided continuously till maturity. Observations on days to first flowering, days to 50% flowering, number of spikelets per panicle, spikelet fertility percentage and grain yield per plant (GYP) were recorded and analyzed statistically.

Results

Development of early maturing, drought tolerant rice lines possessing superior grain quality

With a view to develop early maturing, drought tolerant rice lines with fine grains (similar to BPT5204 and IWP), IWP was crossed with a drought tolerant genotype Apo, true F_1s selected were advanced by selfing to F_6 generations in order to develop superior recombinant inbred lines (Breeding scheme shown in Fig.1).

Evaluation of F_2 population derived between IWP x Apo

True F_1 plants (IWP x Apo) were identified through SSR genotyping and forwarded to F2 generation. About 152 F_2 progenies were evaluated under field conditions and genotyped using polymorphic SSR markers *viz*., RM472 (*qDTY1.1*), RM240 (*qDTY2.1*), RM520 (*qDTY3.1*) and RM3414 (*qDTY6.1*) linked to mega effect QTLs controlling yield under drought in Apo. Observations on major agronomic traits *viz*., days to flowering, days to maturity, yield and grain quality were recorded in all the progenies. Superior lines possessing 2-3 DTY-QTLs of Apo combined with early duration and fine grain quality were identified and forwarded to further generation's upto F_6. Two early maturing F_2 progenies namely # 76 possessing 3 DTY QTLs (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*) and # 110 harboring 2 DTY QTLs (*qDTY1.1* and *qDTY3.1*) were found to possess good yield potential and fine grains (Fig.2).

Generation advancement of the selected F_2 progenies

F_2 progenies possessing 2-3 major effect QTLs of Apo were advanced upto F_6 generation. Superior lines were selected based on duration (100-110 days) and grain yield (> 25 g/plant). In F_6 generation, several single plant progenies of # 76 and # 110 were raised along with other progenies during 2014-15 and individual plants (159 progenies) were genotyped using SSR markers RM472, RM240 and RM520 linked with the 3 target QTLs namely *qDTY1.1*, *qDTY2.1* and *qDTY3.1*, respectively. Genotyping and phenotyping for duration, yield and grain type revealed in the identification of 4 single plants of # 76 (76-1-6-7,76-1-8-1, 76-1-9-2, 76-1-10-4) and possessing early duration (< 110 days), high yield, fine grains and 3 DTY-QTLs under homozygous condition (Fig.3A). Similarly, 3 progenies of # 110 (110-4-11-4, 110-4-11-3, 110-4-11-1) were also found to possess early duration (<110 days), high yield, fine grains and 2 DTY-QTLs under homozygous condition (Fig.3B). These progenies were named as CBMAS 14076 and CBMAS 14110, multiplied and used for further evaluation. These 2 superior rice lines CBMAS14076 and CBMAS14110 possessed the following desirable characteristics *viz*., short duration (100-110 days), high yield potential, fine grain quality and 2-3 drought tolerant QTLs of Apo. Genotyping of these two lines using genome wide polymorphic SSR markers revealed that they possess around 72 – 78% of IWP genome (Table-2).

Table 2: Description of drought tolerant QTLs and recipient parent genome recovery in the selected lines

S. No	Culture	Foreground selection (FGS)				Recipient parent genome (RPG) recovery (%)
		qDTY1.1	*qDTY2.1*	*qDTY3.1*	No. of QTLs	
1	CBMAS14076	√	√	√	3	70
2	CBMAS14110	√	-	√	2	68

Greenhouse Evaluation of CBMAS14076 and CBMAS14110 for drought responses

CBMAS 14076 and CBMAS 14110 were evaluated for their improved drought tolerance ability under greenhouse conditions during Rabi'2016. Plants were grown in large sized pots (42.5 cm height and 45 cm width) upto 45 days along with both the parents and one set of plants were subjected to drought stress by withholding watering. Progression of drought stress was monitored in drought stressed plants by recording relative water content (RWC) in leaves and soil moisture content at 14 DAS and 21 DAS (Table-3). At 21 DAS, soil moisture content was found to be around 20% in IWP, 21.3% in CBMAS 14110 and 12.4% in CBMAS 14076. RWC measurement revealed that IWP was found to retain only 50% of RWC whereas CBMAS14076 retained 75.2% RWC and CBMAS 14110 retained 73.9% RWC. The drought tolerant parent Apo was found to retain 84.1% of RWC even at 21 DAS (Table 4). Transpiration rate, stomatal conductance, internal Co_2 concentration and C_i/C_a ratio were reduced significantly during stress in CBMAS14110. These lines also exhibited early flowering which was synchronized with flowering stage of Apo (Fig.4).

Table 3: Profile of RWC (%) & SMC (%) in drought stress experiment

S. No.	Parents/Pyramided Lines	Condition	14 Days after stress		21 Days after stress	
			RWC (%)	SMC (%)	RWC (%)	SMC (%)
1	IWP (Female parent)	NS	93.4	51.8	97.8	48.9
		S	74.4	28.0	49.5	20.2
2	APO (Male parent)	NS	96.5	53.2	95.7	42.8
		S	90.3	32.4	84.1	27.9
3	CBMAS14076	NS	94.0	57.0	96.9	48.6
		S	92.2	26.9	75.2	21.3
4	CBMAS14110	NS	96.9	51.5	94.8	48.8
		S	88.4	15.9	73.9	12.4

RWC: Relative water content in percentage; SMC: Soil moisture content in percentage

Table 4: Physiological performance of early duration, during drought stress (Rabi' 2016) under greenhouse conditions

Lines	Condition	Transpiration rate(E) (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (g_s) (mol H_2O m^{-2} s^{-1})	Internal CO_2 concentration (C_i) (ppm)	Internal CO_2/ CO_2 assimilated ratio (C_i/C_a)
IWP	NS	4.376 ± 0.053	0.262 ± 0.003	282.497 ± 2.091	0.740 ± 0.004
	S	3.788 ± 0.064	0.133 ± 0.002	187.167 ± 1.230	0.490 ± 0.003
Apo	NS	10.359 ± 0.013	0.704 ± 0.003	322.249 ± 0.989	0.847 ± 0.002
	S	6.791 ± 0.019	0.308 ± 0.002	251.573 ± 3.382	0.671 ± 0.009
CBMAS14076	NS	9.782 ± 0.052	0.698 ± 0.007	299.592 ± 1.198	0.811 ± 0.003
	S	8.184 ± 0.076	0.349 ± 0.003	224.120 ± 2.550	0.598 ± 0.006
CBMAS14110	NS	8.701 ± 0.040	0.545 ± 0.005	309.422 ± 1.863	0.817 ± 0.004
	S	3.833 ± 0.093	0.151 ± 0.005	166.054 ± 6.836	0.439 ± 0.018

NS = No stress, S = Stress, All the values presented in the table represents mean of 3-5 observations recorded along with the standard error values. The observations were recorded at 14DAS.

Field Performance of CBMAS14076 and CBMAS14110

CBMAS14076 (harboring 3 QTLs) and CBMAS14110 (2 QTLs) were evaluated for their agronomic performance under field conditions during Summer’ 2017 along with the parents IWP and Apo (Fig.5). One set of plants were subjected to drought on 60th day by withholding irrigation. IWP was found to possess around 19% increased spikelet sterility under drought when compared to control plants. Both the RILs, *i.e*., CBMAS 14076 and CBMAS 14110, were found to exhibit lesser reduction in their spikelet sterility under drought when compared to their control plants (Table 5). Both the RILs were found to possess on par single plant yield when compared to IWP under control conditions. IWP showed 23.3% reduction in single plant yield under drought; whereas CBMAS 14076 and CBMAS14110 showed only around 16% reduction in single plant yield. Grain type of both CBMAS 14076 and CBMAS14110 were slender like that of IWP (Fig. 6).

Table 5: Means of agronomically important morphological traits in selected cultures under drought stress during Summer’ 2016

S. No.	Genotypes	Condition	Days to Flowering		Plant height (cm)	Spikelet Fertility (%)	Increase in Spikelet Sterility (%) during drought	GYP (g)	Reduction in yield (%)	Grain Type
			1st	50%						
1	IWP (RP)	NS	116 ± 0.73	124 ± 0.97	118.00 ± 1.22	80.71 ± 3.82	18.9	27.23 ± 0.82	23.3	S
		S	113 ± 0.70	122 ± 2.12	116.33 ± 1.81	65.41 ± 0.48		20.87 ± 1.01		S
2	Apo (DP)	NS	66 ± 1.05	72 ± 0.97	101.33 ± 1.29	83.98 ± 0.64	3.51	23.63 ± 1.09	5.92	B
		S	64 ± 2.79	68 ± 4.91	98.00 ± 0.83	81.03 ± 1.26		22.23 ± 1.09		B
3	CBMAS14076	NS	69 ± 0.89	79 ± 1.50	71.33 ± 2.60	83.48 ± 1.47	11.3	26.98 ± 3.26	16.0	S
		S	66 ± 3.11	73 ± 2.65	69.71 ± 2.64	74.00 ± 1.11		22.65 ± 4.27		S
4	CBMAS14110	NS	70 ± 0.89	78 ± 0.80	72.33 ± 2.60	80.47 ± 0.71	9.94	29.29 ± 2.12	16.5	S
		S	68 ± 0.30	75 ± 1.50	68.71 ± 2.64	72.47 ± 1.27		24.47 ± 2.43		S

NS= No stress (Control), S=Stress (Rain out shelter, GYP= grain yield per plant (g), DTF= Days to flowering, PH= Plant height (cm), GT= Grain type, S: Slender, B: bold. The values prefixed represent the means of 5 plants per genotype and the value after ± symbol indicates standard error (SE)

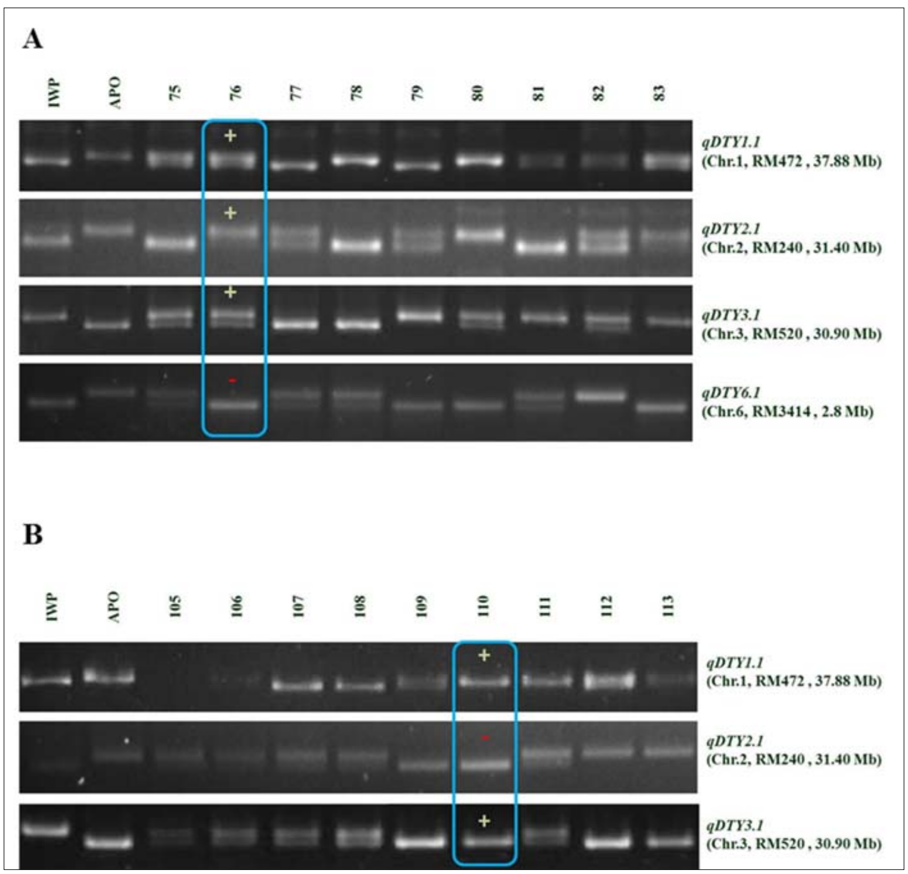

Fig 2: Foreground selection of F_2 progenies (IWP x Apo) using SSRs linked to QTLs controlling yield under drought stress in Apo

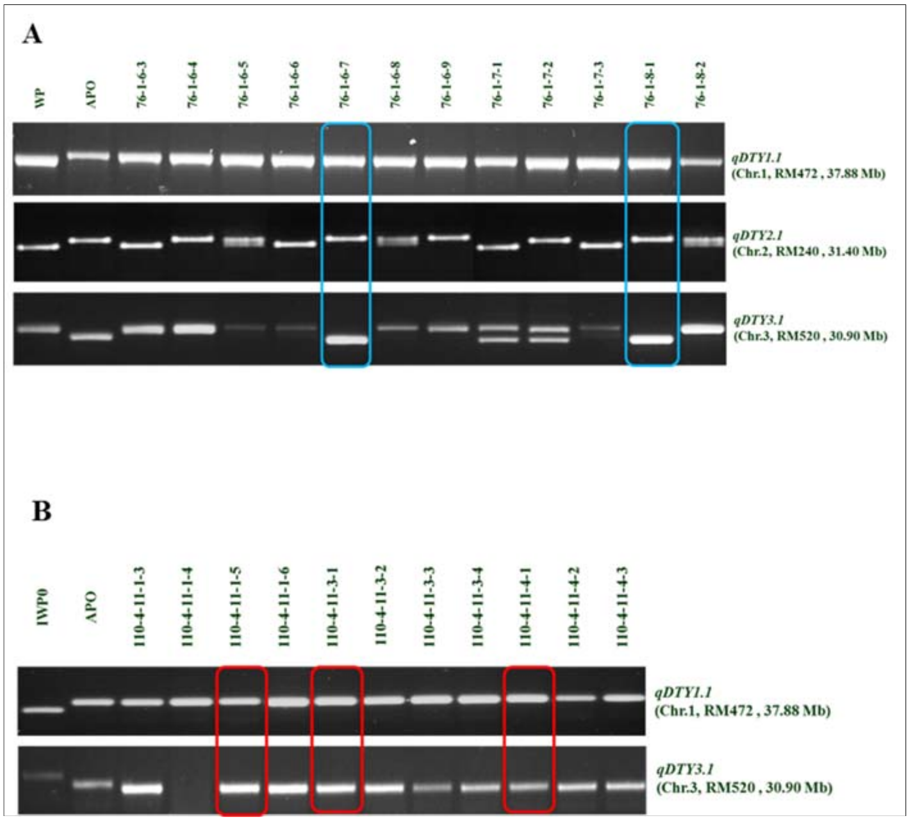

Fig 3: Foreground selection of F_6 progenies of 76 (A) and 110 (B) for target QTLs of Apo using SSR markers linked with the QTLs

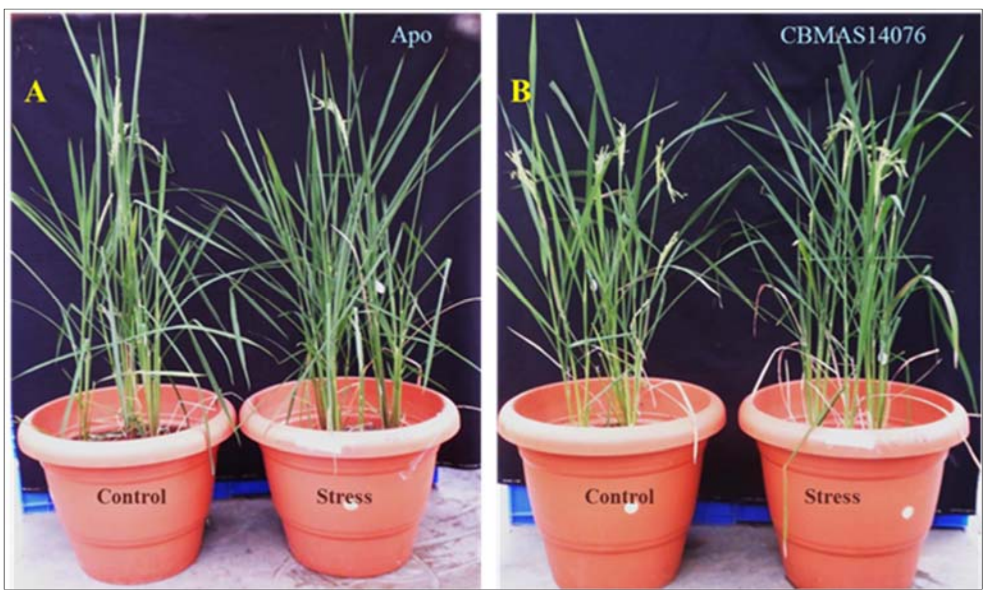

Fig 4: Early flowering in Apo (A) and CBMAS14076 (B) both under control and stress conditions during drought screening experiment under greenhouse conditions

Fig 5: Field view of CBMAS14076 & CBMAS14110 evaluated during Summer' 2017. A) Vegetative stage, B) Reproductive stage

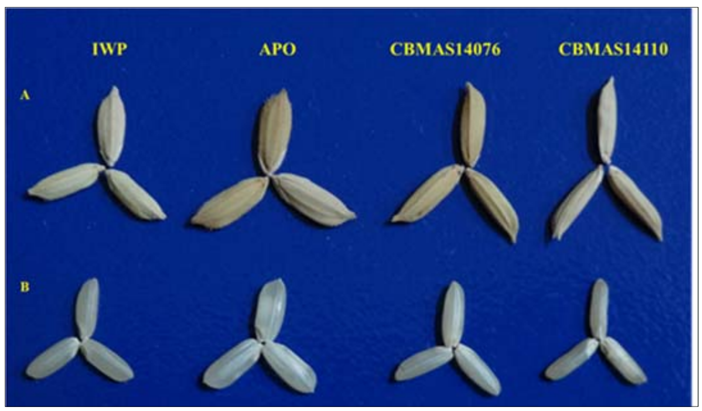

Fig 6: Grain type in CBMAS14076 and CBMAS14110 with the parents IWP and Apo. A) Whole grain, B) Dehusked grain

Discussion

In Tamil Nadu, rice cultivation during rabi season is frequently affected by all major abiotic (drought, submergence etc.,) and biotic (blast, BLB etc.,) stresses which results in substantial yield loss. Most of the medium duration varieties preferred for cultivation for this season like BPT5204, IWP and CR1009 are susceptible to all kinds of abiotic/biotic stresses and developing stress tolerant version(s) would help in sustaining rice production in Tamil Nadu. This situation warrants development of drought resilient rice varieties to sustain rice production in Tamil Nadu. Efforts through conventional breeding methods have met with little success in developing drought tolerant rice varieties (Fukai and Cooper, 1995) [5]. This is due to the polygenic nature of drought tolerance phenomenon with low heritability and high G × E interaction. Recent reports revealed the suitability of grain yield as selection criteria which showed moderate heritability under reproductive stage drought (Venuprasad *et al*., 2007, 2008; Kumar *et al.*, 2008, 2012) [18, 19, 10, 8]. Later, it has been reported that the correlation between high yield potential and good yield under drought was low but always positive (Kumar *et al.*, 2008) [10], suggesting the possibility to combine high yield potential and good yield under drought successfully. Using this as a criterion, a number of large-effect QTLs for grain yield under reproductive-stage drought for both upland and lowland conditions have been identified *viz*., *qDTY12.1* in Way Rarem (Bernier *et al*., 2007) [2]; *qDTY1.1*, *qDTY2.1* and *qDTY3.1* in *Apo* (Venuprasad *et al*., 2007, 2012) [19, 20]. Two QTLs (*qDTY2.1* and *qDTY3.1*) showed a very high effect under severe lowland reproductive-stage drought (R^2=16.3% and 30.7%). Advances in molecular biology have provided new opportunities to move those regions into drought-susceptible varieties, an opportunity that was not available a few years back to break the yield improvement barrier under drought. By deployment of these QTLs into breeding applications has resulted in the development of improved rice lines in the genetic background of Swarna, IR64, Vandana, Sabitri, TDK1, Anjali, and Sambha Mahsuri, exhibiting enhanced level of drought tolerance (Kumar *et al*., 2014) [9].

Development of early maturing, drought tolerant rice lines possessing superior grain quality

Developing early maturing and drought tolerant rice lines with grain quality is one of the promising strategies to sustain rice production under changing climatic scenario. IWP was crossed with a drought tolerant genotype Apo and the true F_1s were identified and forwarded to F_2 generation. About 152 progenies were genotyped using polymorphic SSR markers *viz*., RM472 (*qDTY1.1*), RM240 (*qDTY2.1*), RM520 (*qDTY3.1*) and RM3414 (*qDTY6.1*) linked to mega effect QTLs controlling yield under drought in Apo. Superior lines possessing 2-3 *DTY*-QTLs of Apo combined with early duration and fine grain quality were identified and forwarded to further generations' upto F_6. Two early maturing F_2 progenies namely # 76 possessing 3 *DTY*-QTLs (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*) and # 110 harboring 2 DTY-QTLs (*qDTY1.1* and *qDTY3.1*) were found to possess good yield potential and fine grains. Evaluation of F_6 population resulted in the identification of 4 single plants of # 76 possessing 3 DTY-QTLs under homozygous condition, early duration (< 110 days), high yield, fine grains and 3 progenies of # 110 possessing 2 DTY-QTLs under homozygous condition, early duration (<110 days), high yield, and fine grains. These two lines were named as CBMAS14076 and CBMAS14110 respectively (Fig.7). Both CBMAS14076 and CBMAS14110 were found to be semi-dwarf, early maturing, and high yielding with superior grain quality. Semi-dwarfness of these lines indicated the possible recombination between *qDTY1.1* and sd1 locus (Venuprasad *et al*., 2009; Vikram *et al*., 2016) [17, 23] and earliness may be due to the presence of *qDTY3.1* from *Apo* (Vikram *et al*., 2009).

Evaluation of CBMAS14076 and CBMAS14110 for drought responses under greenhouse conditions

CBMAS 14076 and CBMAS 14110 evaluated for their drought responses under greenhouse conditions along with IWP and Apo indicated that both these RILs maintained relatively higher level of internal water status than recurrent parent IWP which exhibited drought symptoms at 74% RWC (soil moisture = 28%) on 14th DAS. The changes in the gas exchange parameters were observed in response to drought conditions. Similar results have been reported in drought tolerant near isogenic lines of IWP (Muthukumar *et al*., 2016) [13]. This is because, plant response to drought stress is characterized by reduction in transpiration rate, increased stomatal closure and reduced loss of water through stomata operating water conservation strategy primarily with reduction in the photosynthetic rate (Rajiv *et al*. 2010) [14]. However, the reason behind stomatal conductance limiting photosynthetic rate is still unclear (Kusumi *et al*., 2012) [11].

Performance of CBMAS14076 and CBMAS14110 under field conditions

CBMAS14076 (3 QTLs) and CBMAS14110 (2 QTLs) were evaluated for the agronomic performance under field conditions during Summer' 2016 along with the parents IWP and Apo. Both the RILs, CBMAS 14076 and CBMAS, 14110 were found to exhibit lesser reduction in their spikelet sterility under drought when compared to their control plants. Spikelet sterility percentage has a negative correlation with grain yield (Usman *et al*., 2013) [16]. IWP showed 23.3% reduction in single plant yield under drought; whereas CBMAS 14076 and CBMAS14110 showed only around 16% reduction in single plant yield, thus proving that the RILs are superior in agronomic performance over the recurrent parent.

Conclusion and Future prospects

Rapid development of drought-tolerant versions of popular varieties can be one of the strategies to ensure rice production under reproductive-stage drought without compromising on yield potential and the preferences of farmers and consumers. In this context, molecular breeding using well-defined QTLs allows precise combining of high yield potential and good yield under reproductive-stage drought. Considering the prospects molecular breeding in improving drought tolerance ability of existing popular varieties, the present study was undertaken with an aim of developing high yielding and drought tolerant rice lines possessing superior grain quality. In the present study two superior RILs with early duration, high yield potential, superior grain and enhanced drought tolerance were selected and performance under field conditions were ascertained. Efforts has to be made in analysis of the quality parameters and phytochemicals present in these superior lines besides characterizing the genes underlying the target QTLs introgressed, which would not only help in authentically documenting the performance of the developed lines but also facilitate promotion for further varietal release.

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Conflict of interest

The authors express no conflict of interests.

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