Original Research Article

Molecular breeding for enhancing grain yield in elite Indian rice (*Oryza sativa* L.) variety Improved White Ponni through introgression of major effect *qDTY 3.1*

ABSTRACT

~~Frequent~~ 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 rice under reproductive stage drought stress. This variety is ~~very~~ 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 (IWP) X Apo introgressed with *qDTY 3.1.* The experiment was conducted at the ~~in~~ 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*

Formatted: Font: Bold

1. INTRODUCTION

Rice crop productivity must be improved under changing climatic circumstances to ensure global food security. Due to a 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. (Gupta *et al.,* 2020). 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 (Pareek *et al.,* 2020). 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 for greater drought screening (Seeraj *et al.,* 2009 NO REF). 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 (Thomson *et al.,* 2009). The major effect loci *qDTY3.1* with large phenotypic variation of 31% was discovered by Venuprasad *et al.,* 2009. Apo (drought tolerant) and Swarna (drought sensitive) had 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. Similarly, in the Pusa 44 backcross inbred lines - Pusa 1823-12-31-12-12-12 introgressed with *qDTY3.1*, Oo *et al.,* 2021 found a 2-2.5 times improvement in yield. Improved white ponni was introgressed with *qDTY3.1* through marker assisted backcross breeding with the goal of increasing its production under drought. 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 Department of Plant Biotechnology, Centre for plant molecular biology and biotechnology, TNAU, Coimbatore. Field trials were conducted at the Department of Rice, Centre for Plant Breeding and Genetics, Coimbatore.

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) as identified by Venuprasad *et al.,* 2009.It was used as the donor for enhancing drought tolerance in IWP. True F1s were backcrossed with IWP and the progenies were backcrossed 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 (Doyle and Doyle, 1990). RM520 was used for foreground selection 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. 3 percent agarose gel electrophoresis was used to resolve PCR results and observed with a UV trans-illuminator. 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 and continuing till harvest. Soil sample(500g) was taken to estimate the final Soil Moisture Content (SMC) during grain filling stages.

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

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.

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

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 and was calculated according to the formula by (Pieczynski *et al.* 2013),

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

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 (Azeez and Shafi, 1966). 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 (Azeez and Shafi, 1966). The volume expansion ratio (VER) was estimatedas the ratio of the volume of cooked rice to that of uncooked milled rice (Sidhu *et al.,* 1975).

The gel consistency was measured (Cagampang *et al.,* 1973) 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 petriplates and kept for 23 hours. The alkali spreading value of kernels were classified as low, Intermediate or High (Little *et al.,* 1958). For calculating amylose content, 1 ml ethanol (95%) and 9 ml of sodium hydroxide (1N) were added to 100 mg rice powder and heated on a boiling water bath followed by cooling for 1 h and then distilled water was added to make the final volume of 100 ml. 1 ml of acetic acid (1 N) and 2 ml of freshly prepared iodine solution were added to 5 ml of the stock sample solution. Absorbance of the solution was measured at 620 nm after 20 min (Juliano *et al.,* 1971).

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 given in Fig 1. True F_1s of IWP X Apo which amplified heterozygous alleles were identified using SSR marker associated with the target loci (*qDTY3.1*-RM520). BC_1F_1 plants were developed by backcrossing true F1 hybrids with IWP. *qDTY3.1* positive progenies were identified through FS. Among them, progenies with parent genome recovery (RPG) of above 60% were backcrossed with IWP to generate BC_2F_1. In BC_2F_1, progenies with grain type similarity to IWP along with maximum RPG above 80% were forwarded further for backcrossing. The BC_3F_1 progenies were selfed three times to obtain BC_3F_4. In BC_3F_3, 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.

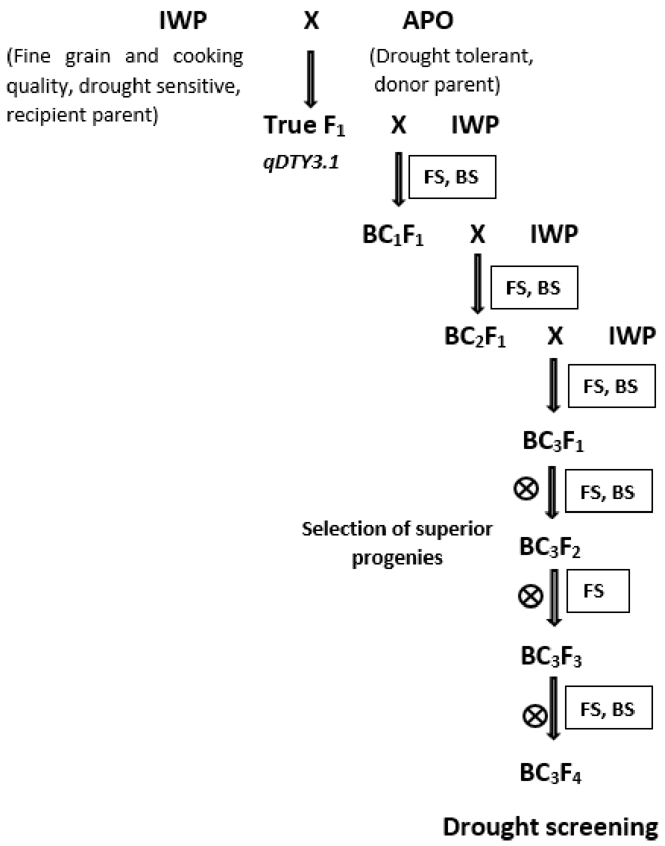


Figure 1. Backcross breeding scheme representing development of BILs of IWP X APO

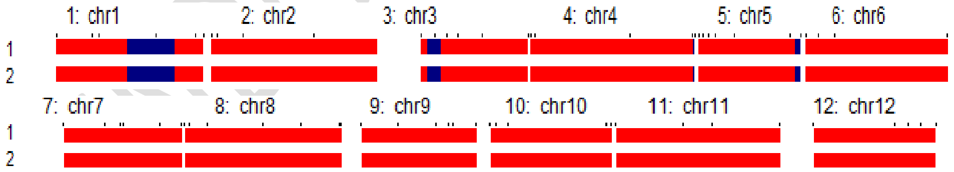


Figure 2. Graphical genotyping of the selected superior BILs with maximum RPG. 1) 4-7-4-24-4-15, 2) 4-7-4-24-4-16. Red color and blue color denotes IWP and APO respectively. *Graphical genotyping software V2*

Priyadharshini *et al.,* 2014 obtained best performing lines introgressed with drought QTLs in ADT45 rice background with RPG of 90% through MABC. Similarly, Ab Jalil *et al.,* 2018 introgressed root trait genes for drought tolerance into MRQ74 rice and obtained good phenotypic performance in BC_2F_1 with RPG upto 89.6% through MABC. 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%.

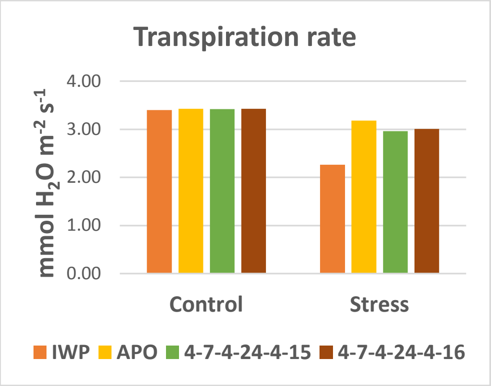
3.2 PERFORMANCE OF IWP BILs UNDER DROUGHT CONDITIONS

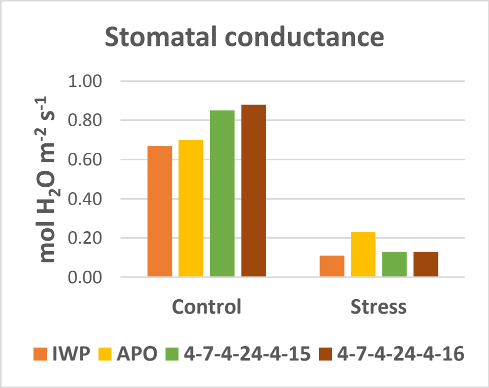
Drought stress during reproductive stage reduced tillering, spikelet fertility and ultimately yield of all the genotypes (Table 1). However, the *qDTY3.1* introgressed BILs showed improved performance in all the traits than IWP. Sahoo *et al.,* 2020 identified drought tolerant rice genotypes under severe and moderate stress conditions wherein the soil moisture ranged between 13.6% - 17.8% respectively. Soil moisture declined to 11.4%, 12.2%, 13.2%, and 14.1% at 15 cm, 30 cm, 45 cm and 60 cm depts respectively showing the severity of drought stress. In stress conditions where the drought sensitive parent shows yield reduction of atleast 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 (Venuprasad *et al.,* 2007, Swain *et al.,* 2017). 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. But the 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 identified by Shamini *et al.,* 2019. 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. Varshney and Tuberosa, 2013 also reported that *qDTY3.1* promotes early flowering to overcome drought stress.

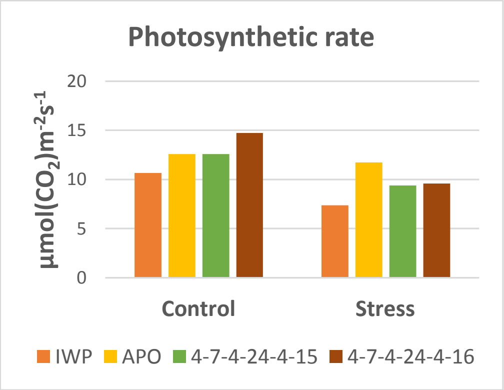
Table 1. Mean performance of the BILs under drought stress and irrigated conditions

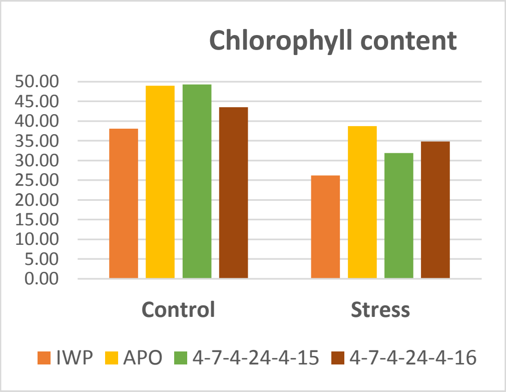
Parents/ BILs	*qDTY3.1*	Duration	Days to fifty percent flowering		Number of productive tillers		Spikelet fertility (%)		Grain yield (g)		Yield reduction (%)
			Control	Stress	Control	Stress	Control	Stress	Control	Stress	
IWP	-	Medium	117.0	107.0	22.0	7.0	90.5	25.2	24.2	1.3	94.6
APO	+	Medium	98.0	80.0	12.3	11.3	86.3	76.9	20.0	15.0	25.0
4-7-4-24-4-15	+	Medium	106.0	82.0	15.0	8.9	92.4	56.6	28.2	9.8	65.2
4-7-4-24-4-16	+	Medium	105.0	84.0	17.6	10.2	93.1	60.1	26.5	10.4	60.8

In this study 41.7% and 74.8% sterility in spikelets were recorded in the BILs and IWP respectively. Similar results were obtained by Kumar *et al.,* 2015. They obtained lower spikelet sterility in drought-tolerant genotypes than susceptible cultivars. Under drought, high sterility occurs due to the impairment of pollen development (Higuchi *et al.,*2020 NO REF), defects in the formation of basal and apical pores in anthers by endothecium and problems in pollen swelling thus affecting dehiscence (Liu *et al.,* 2006) and pollination. Selamat *et al.,* 2021 fine mapped MQTL 3.1 where they identified that the MQTL3.1 contained genes for detoxification *(DTX/MATE)* which can maintain the cell membrane stability. In this study, *qDTY3.1* introgressed BILs showed 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. Selamat *et al.,* 2021 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.









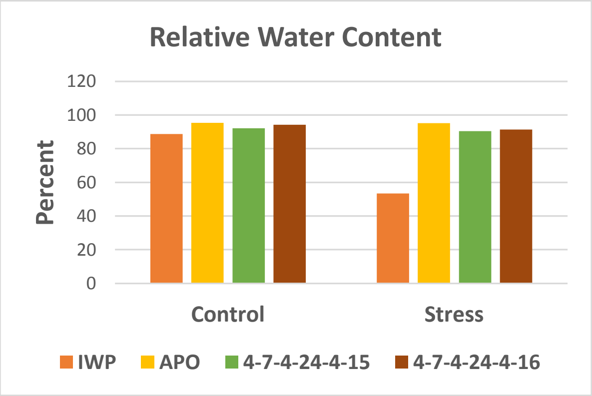


Figure 3. Physiological performance of the BILs and parents under drought stress and irrigated conditions.

Similar results were reported by Mishra *et al.,* 2018. They studied the drought tolerance in indigenous rice landraces and identified that when compared to the tolerant control variety, three landraces (Kalajeera, Machhakanta, and Haldichudi) demonstrated the highest level of drought resistance (N22) which was due to the improved photosynthetic rate and maintenance of higher leaf relative water content under drought conditions. Plants can optimise CO_2 uptake for photosynthesis while reducing water loss by adjusting stomatal pore apertures but still maintaining their stomatal conductance to do photosynthesis (Bertolino *et al.,* 2019, Caine *et al.,* 2019). Barik *et al.,* 2020 NO REF recorded higher chlorophyll content in the drought tolerant rice CR 143-2-2 (36.06) than the susceptible variety Krishnahamsa (27.07 under drought stress. In this study, both BILs and IWP reduced 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 PERFROMANCE 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 by Kumar *et al.,* 2018 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. Major effect QTLs identified for drought tolerance can be introgressed into high yielding varieties through molecular breeding. The aim of this research was to generate high-yielding, drought-tolerant rice lines with acceptable grain quality. 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.

REFERENCES

1. Ab Jalil M, Juraimi AS, Yusop MR, Uddin MK, Hakim MA. Introgression of root trait genes for drought tolerance to a Malaysian rice variety by marker-assisted backcross breeding. International Journal of Agriculture and Biology. 2018;20(1):119-26.
2. Azeez MA, Shafi M. Quality in rice. Dept. Agri.(Pakistan). Tech. Bull. 1966:13-23.

3. Bertolino LT, Caine RS, Gray JE. Impact of stomatal density and morphology on water-use efficiency in a changing world. Frontiers in Plant Science.2019;10:225.
4. Cagampang GB, Perez CM, Juliano BO. A gel consistency test for eating quality of rice. Journal of the Science of Food and Agriculture. 1973 Dec;24(12):1589-94.
5. Caine RS, Yin X, Sloan J, Harrison EL, Mohammed U, Fulton T, Biswal AK, Dionora J, Chater CC, Coe RA, Bandyopadhyay A. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. New Phytologist. 2019;221(1):371-84.
6. Doyl JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus.1990;12:13-5.
7. Gupta V, Kumar Jain M, Singh VP. Multivariate modeling of projected drought frequency and hazard over India. Journal of Hydrologic Engineering. 2020;25(4):04020003.
8. Iwata-Higuchi M, Sakagami JI, Maruyama S. Effect of soil moisture stress at booting and flowering stages on pollen development, pollination and fertilization in upland NERICA cultivars. Australian Journal of Crop Science. 2020;14(12):1935-41.
9. Juliano BO. A simplified assay for milled rice amylose. Cereal Sci. Today. 1971;16:334-60.
10. Kumar A, Sandhu N, Dixit S, Yadav S, Swamy BP, Shamsudin NA. Marker-assisted selection strategy to pyramid two or more QTLs for quantitative trait-grain yield under drought. Rice. 2018;11(1):1-6.
11. KUMAR S, Dwivedi SK, Haris AA, PRAKASH V, Mondal S, SINGH SK. Screening and identification of rice genotypes for drought tolerance at reproductive stage under rainfed lowland condition. Journal of AgriSearch. 2015;2(2):105-11.
12. Little RR. Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem.. 1958;35:111-26.
13. Liu JX, Liao DQ, Oane R, Estenor L, Yang XE, Li ZC, Bennett J. Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. Field Crops Research. 2006;97(1):87-100.
14. Mishra SS, Behera PK, Kumar V, Lenka SK, Panda D. Physiological characterization and allelic diversity of selected drought tolerant traditional rice (Oryza sativa L.) landraces of Koraput, India. Physiology and Molecular Biology of Plants. 2018;24(6):1035-46.
15. Oo KS, Krishnan SG, Vinod KK, Dhawan G, Dwivedi P, Kumar P, Bhowmick PK, Pal M, Chinnuswamy V, Nagarajan M, Bollinedi H. Molecular Breeding for Improving Productivity of *Oryza sativa* L. cv. Pusa 44 under Reproductive Stage Drought Stress through Introgression of a Major QTL, *qDTY12. 1*. Genes. 2021;12(7):967.
16. Pareek A, Dhankher OP, Foyer CH. Mitigating the impact of climate change on plant productivity and ecosystem sustainability. Journal of experimental botany. 2020;71(2):451-6.
17. Pieczynski M, Marczewski W, Hennig J, Dolata J, Bielewicz D, Piontek P, Wyrzykowska A, Krusiewicz D, Strzelczyk-Zyta D, Konopka-Postupolska D, Krzeslowska M. Down-regulation of CBP 80 gene expression as a strategy to engineer a drought-tolerant potato. Plant biotechnology journal. 2013;11(4):459-69.
18. Priyadarshini SK, Raveendran M, Manonmani S, Robin S. Effect of QTLs controlling grain yield under drought stress in the genetic background of ADT45 rice variety. Indian J. Genet. 2014;74(3):374-7.
19. Sahoo SK, Dash GK, Guhey A, Baig MJ, Barik M, Parida S, Swain P. Phenological, Physiological and yield markers as efficient tools to identify drought tolerant rice genotypes in Eastern India. bioRxiv. 2020.
20. Selamat N, Nadarajah KK. Meta-Analysis of Quantitative Traits Loci (QTL) Identified in Drought Response in Rice (Oryza sativa L.). Plants. 2021;10(4):716.
21. Serraj R, Bennett J, Hardy B, editors. Drought frontiers in rice: crop improvement for increased rainfed production. World Scientific; 2009.
22. Shamini K, Sudhagar R, Raveendran M, Joel AJ. qDTY3. 1, a major drought tolerant locus of APO promotes early flowering in the genetic back ground of a local cultivar improved white ponni. Electronic Journal of Plant Breeding. 2019;10(1):155-9.
23. Sidhu JS, Gill MS, Bains GS. Milling of paddy in relation to yield and quality of rice of different Indian varieties. Journal of Agricultural and Food Chemistry. 1975;23(6):1183-5.
24. Swain P, Raman A, Singh SP, Kumar A. Breeding drought tolerant rice for shallow rainfed ecosystem of eastern India. Field Crops Research. 2017; 209:168-78.

25. Thomson MJ, Ismail AM, McCouch SR, Mackill DJ. Marker assisted breeding. In Abiotic stress adaptation in plants 2009; 451-469. Springer, Dordrecht.
26. Varshney RK, Tuberosa R. Translational genomics for crop breeding: abiotic stress tolerance, yield, and quality, an introduction. Translational genomics for crop breeding. 2013:1-7.
27. Venuprasad R, Dalid CO, Del Valle M, Zhao D, Espiritu M, Cruz MS, Amante M, Kumar A, Atlin GN. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. Theoretical and Applied Genetics. 2009;120(1):177-90.
28. Venuprasad R, Lafitte HR, Atlin GN. Response to direct selection for grain yield under drought stress in rice. Crop Science. 2007 Jan;47(1):285-93.