

## RESEARCH ARTICLE ||

# Genetic Improvement of Drought Tolerance in North Eastern Chakhao Amubi rice Through Marker Assisted Selection

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## ABSTRACT

Drought ranks the top among the abiotic stresses affecting rice production and sustaining rice production warrants the development of drought-tolerant rice cultivars. This study aimed to develop recombinant inbred lines of Chakhao Amubi possessing drought-tolerant QTLs of Apo. A RIL population developed between Chakhao amubi (popular rice in Manipur) and Apo (a drought-tolerant cultivar) was genotyped using SSR markers linked to major drought-tolerant QTLs, the positive lines were screened for grain characteristics and aroma. Foreground analysis of  $F_2$  (117 progenies) population using SSR markers RM11885 (*qDTY1.1*), RM7426 (*qDTY2.1*), RM16030 (*qDTY3.1*) and BADEX7-5 (OsBADH2 loci controlling aroma) resulted in the identification of 25 progenies harboring all the three drought-tolerant QTLs (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*) and aroma, 11 progenies were found to harbor two target QTLs and aroma and two progenies were found to harbor any one of the drought QTL and aroma. Agronomic evaluation of RILs ( $F_4$ ) identified 11 superior RILs possessing reduced duration, high yield, and all four QTLs for further evaluation and breeding applications.

Received : 17<sup>th</sup> January,2022Revised : 31<sup>st</sup> January,2022Revised: 03<sup>rd</sup> February,2022Accepted : 24<sup>th</sup> February,2022**Keywords:** Traditional rice; Genetic improvement; Drought tolerance; Marker assisted selection; QTLs

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple food crops serving as the primary source of nutrition for more than half of the world's population. Rice production is limited by abiotic stresses such as drought, salinity, submergence, and temperature (Vikram *et al.*, 2011; 2016). Among them, water deficit is a major concern for all agricultural crops, and it affects 23 million hectares of rainfed rice (Serraj *et al.*, 2011; Pandey *et al.*, 2015). The development of rice cultivars with enhanced ability to withstand drought will help increase rice production in rainfed environments (Salunkhe *et al.*, 2011) and thereby alleviate poverty (Pandey and Bhandari, 2007).

Breeding for improving drought tolerance in rice has been a focus of rice improvement for decades, but progress in developing drought-tolerant rice has been slow. Drought resistance/adaptation is a multifaceted trait including diverse mechanisms viz., drought escape, drought avoidance, drought tolerance, and drought recovery (Levitt, 1972; O'Toole and Chang, 1979, Gowda *et al.*, 2011).

Several physiological mechanisms contributing to drought QTLs (*qDTY2.1* and *qDTY4.1*) for higher root hydraulic conductivity and, in some cases, greater root growth at depth were identified (Henrey *et al.*, 2015). The complexity of tolerance mechanisms and lack of reliable phenotyping procedures impede the development of drought-tolerant rice varieties. Advancements in molecular genetics identified major QTLs for grain yield under drought, namely, *qDTY1.1* (Vikram *et al.*, 2011); *qDTY2.1* and *qDTY3.1* (Venuprasad *et al.*, 2009) and *qDTY12.1* (Bernier *et al.*, 2007) which provided newer opportunities to the breeders for developing rice varieties tolerant to drought. The simplest method is to develop segregating population(s) involving popular varieties and drought-tolerant donors and select elite progenies combining yield and drought-tolerant QTLs through MAS. Identified progenies are screened for the desirable attributes of the popular parent. In this study, attempts were made to enhance the drought tolerant ability of Chakhao amubi, a traditional black rice variety popular in North East India. Segregating population developed between Chakhao amubi and

a drought-tolerant parent Apo was genotyped by using SSR markers linked to three QTLs governing yield under drought (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*) and elite progenies were evaluated (Fig. 1). The study enabled the accelerated identification of elite progenies possessing Chakhao amubi like grains and three droughts tolerant QTLs.

## MATERIAL AND METHODS

### Genetic materials used and development of recombinant inbred lines (RILs)

Two rice genotypes, Chakhao amubi, a popular aromatic rice of North East India possessing dark purple grains and Apo, an upland cultivar harboring three major drought tolerance QTLs *viz.*, *qDTY1.1*, *qDTY1.2*, *qDTY 2.1*, *qDTY3.1* (Venuprasad et al., 2009; 2012a and 2012b) were selected (Table 1). The field studies were conducted at Paddy Breeding Station (PBS), and all the laboratory experiments were conducted at Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore.

### Evaluating the RILs of Chakhao amubi and Apo

True  $F_1$  progenies derived between Chakhao amubi and Apo were confirmed by the SSR markers, RM11885 (36.68 Mb, linked to *qDTY1.1* on chromosome 1); RM 7426 (16.67 Mb linked to *qDTY2.1* on chromosome 2) and RM 16030 (32.7 Mb linked to *qDTY3.1* on chromosome 3) and forwarded to  $F_2$  generation (Fig 2). Leaf samples were collected from 117,  $F_2$  progenies and used to isolate genomic DNA using a modified CTAB protocol (Murray and Thompson, 1980). The genomic DNA was quantified and diluted to a final concentration of 50 ng/ $\mu$ L. Polymorphic SSR markers RM 11885, RM 7426 and RM 16030 were used for genotyping of  $F_2$  progenies and the parents. PCR reactions were carried out using DNA as template (50 ng/ $\mu$ L), PCR buffer consisting of 1.5 mM MgCl<sub>2</sub>, 0.4 mM dNTPs, 10  $\mu$ M primers and 1.5 U of Taq DNA polymerase were utilized. PCR reactions were performed using thermal cyclers (M/s. BioRad Laboratories, USA) programmed as initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec and 72 °C for 30 sec followed by final extension of one cycle at 72 °C for 10 min. PCR amplified products were subjected to agarose gel electrophoresis, stained with Ethidium Bromide and visualized with UV trans-illuminator. Lines harboring Apo alleles of the three SSR markers either under homozygous

or heterozygous conditions were considered as positives and forwarded to  $F_3$  and  $F_4$  generation.

### Evaluation of RILs for agronomic performance and aroma

Sensory evaluation test (Sood and Siddiq 1978) was carried out using the leaves of  $F_2$  progenies for assessing their ability to produce aroma. Leaf was cut into fine bits in a Petri-dish followed by addition of 10 mL of 1.7 % KOH and left closed for about 10 minutes. After 10 minutes, Petri-plates were opened to assess for the presence/absence of aroma. Samples were scored on a scale of 1 to 4 where 4 indicate a strong aroma. Agronomic parameters, namely, days to flowering, number of productive tillers, plant height (PH), and grain yield per plant (GY/P) were recorded from all the selected RILs. At physiological maturity, ten single plants were selected and panicles were harvested individually, sun-dried, and weighed to determine grain yield per plant.

## RESULTS AND DISCUSSION

An increase in rice production is threatened by an increased occurrence of several biotic and abiotic stresses and among the various abiotic stresses drought stands at the top in affecting rice yields (Prince et al., 2015). In India, monsoon-dependent rice crop(s) is often subjected to terminal drought and, most of the existing varieties are highly sensitive to water deficit. Rapid progress in the molecular genetics dissection of drought tolerance traits in rice identified major loci governing performance under drought in a drought-tolerant variety Apo (Venuprasad et al., 2009), Nagina 22 (Vikram et al., 2011) and Vandana (Dixit et al., 2014). This paved the way for genetic manipulation of drought tolerance in rice through molecular breeding. Out of >1000 QTLs reported to be associated with various drought tolerance traits, only a few of them, including *qDTY1.1*, *qDTY2.1*, and *qDTY3.1* (Venuprasad et al., 2007; 2009 and 2012) have been put into breeding applications (Kumar et al., Valarmathi et al., 2019). Earlier, attempts have been made by the deployment of QTLs (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*) into breeding applications has resulted in the development of improved rice lines in the genetic background of IR72, IR64, and Swarna (Venuprasad et al., 2007; 2008; 2009) and Improved White Ponni (Muthukumar et al., 2017, Valarmathi et al., 2019). This study aims to improve drought tolerance in a traditional variety Chakhao amubi by introgressing



qDTY QTLs from adrought-tolerant genotype Apo. Parental polymorphism survey by using more than 30 markers within the QTL regions identified four polymorphic markers, namely BADEX7-5 (OsBADH2 controlling aroma in rice) RM11885 (*qDTY1.1*), RM 7426 (*qDTY2.1*) and RM16030 (*qDTY3.1*) for breeding applications. Evaluation of >30 F1 progenies using the above three markers identified three true F1s and forwarded to F2 by selfing. F2 populations derived from the cross between Chakhao amubi and Apo were subjected to precision genotyping using tightly linked polymorphic SSR markers.

A total of 117 F2 progenies were genotyped using the linked SSR markers viz., BADEX7-5, RM 11885; RM 7426 and RM 16030. Thirty-eight progenies were found to possess Chakhao allele of OsBADH2 producing aroma. These 38 progenies were then genotyped using RM 11885; RM 7426 and RM 16030 which resulted in the identification of 25 progenies harboring all the three qDTY target loci (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*), 11 progenies harbouring various combinations of two qDTY QTLs and two progenies harbouring single qDTY QTL. The sensory evaluation test identified five progenies (# 3, # 104 and # 17 harboring all three QTLs; # 91 possessed two qDTY QTLs and # 37 possessed one qDTY QTL) stronger aroma. Progenies harboring aroma and three drought QTLs (*qDTY1.1*, *qDTY2.1* and *qDTY3.1*) were forwarded to F3 generation. Foreground analysis F3 progenies identified thirty progenies harboring aroma and the target QTLs and forwarded to F4 generation.

F4 RILs were evaluated for their agronomic performance under field conditions. Parental genotypes Chakhao amubi (149 cm) and Apo (108 cm) differed in their plant height. Plant height (PH) among the selected RILs ranged between 113 and 142 cm. All the selected RILs were found to have reduced (10 – 30 cm) plant height than the parent Chakhao amubi (Fig 3). The parental lines differed widely in days to fifty percent flowering, Chakhao amubi (145 days) and Apo (94 days). RILs were found to be intermediate (107 - 117 days) between the parents thus indicating the chances for selection. RILs # 3-1, # 17-19, and # 42-3 were found to flower approximately 40 days earlier than the female parent Chakhao amubi. The yield performance of RILs is provided in Table 2. Chakhao amubi recorded a mean grain yield of 23.46 ± 0.82 g which was

higher than the male parent Apo (18.38 ± 1.07 g). RILs recorded on par or higher grain yield per plant compared to the parents. Except for two RILs (# 77-3 and # 17-19), all other RILs were found to have higher yield potential (more than 25 g/plant). Three RILs, namely # 53-1, # 54-3, and # 66-21 recorded the highest yield of 30.62 g, 30.31 g, and 30.65 g per plant, respectively (Table 2).

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

Trait	Targeted QTLs and Position	Chromosome	Markers used for foreground	Annealing temperature °C
Drought	<i>qDTY1.1</i> (34.9–37.8 Mb)	1	RM 11885	55
	<i>qDTY2.1</i> (11.4–20.7 Mb)	2	RM 7426	55
	<i>qDTY3.1</i> (30.91 - 32.5 Mb)	3	RM 16030	55

Table 2. Performance of RILs of Chakhao amubi under field conditions

Parents/RILs	DTF (50%)	Grain Yield/Plant (g)
Chakhao amubi	145	23.46 ± 0.82
Apo	94	18.38 ± 1.07
RIL # 3-1	107	27.57 ± 1.18
RIL # 17-10	110	27.14 ± 1.94
RIL # 17-17	108	28.53 ± 2.71
RIL # 17-19	102	23.66 ± 1.11
RIL # 42-3	102	25.14 ± 0.89
RIL # 42-4	117	28.14 ± 2.16
RIL # 53-1	110	30.62 ± 2.62
RIL # 54-3	112	30.31 ± 2.21
RIL # 66-21	108	30.65 ± 2.47
RIL # 77-3	115	23.91 ± 4.18
RIL # 77-8	104	29.45 ± 3.10

\*All the values represent mean of 10 plants and ± value indicates standard error.

Earlier, Valarmathi *et al.* (2020) developed dehydration tolerant rice lines in the genetic background of the Improved White Ponni (IWP) by

introgressing major effect QTLs (*qDTY1.1*, *qDTY2.1*) of Apo conferring tolerance against drought. This study identified and forwarded to further generations in elite lines with 2 - 3 *qDTY* QTLs of Apo combined with early duration and superior grain quality. Earlier, similar attempts have been made by introgressing major effect QTLs of Apo viz., *qDTY1.1*, *qDTY2.1*, and *qDTY3.1* controlling yield under drought conditions into popular varieties like IR 64 and Swarna (Venuprasad *et al.*, 2007; 2008; 2009) and Improved White Ponni (Muthukumar *et al.*, 2017, Valarmathi *et al.*, 2019, Muthu *et al.*, 2020).



Fig 1. Evaluation of F4 RILs at vegetative stage in field

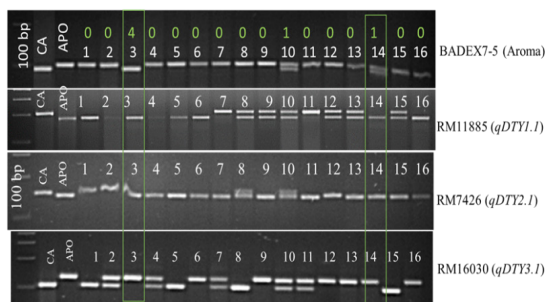


Fig 2. Foreground selection of F2 progenies of Chakhao amubi x Apo for *qDTY1.1*, *qDTY2.1*, *qDTY3.1* and *OsBADH2*.

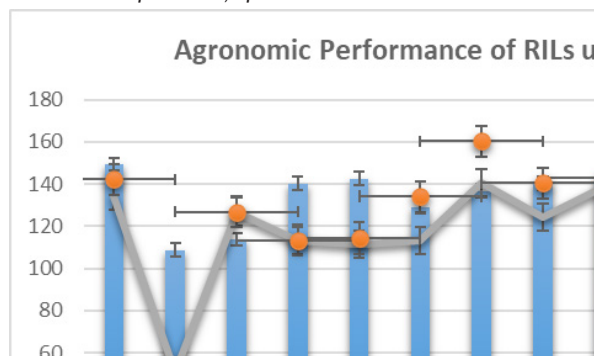


Fig 3. Agronomic performance of RILs (F4) harboring drought QTLs

## Conclusion

The present investigation indicated the accelerated development of drought-tolerant genetic stocks through MAS. Further, validation of selected RILs against drought and biochemical profiling will identify elite genetic stocks of Chakhao amubi with enhanced drought tolerance.

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## Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

## Consent for publication

All the authors agreed to publish the content.

## Competing interests

The Author(s) declare(s) that there is no conflict of interest.

## References

- Bernier, J., A. Kumar, V. Ramaiah, D. Spaner and G. Atlin. 2007. A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci.*, 47(2): 507-516.
- Dixit, S., B.E. Huang, M.T. Sta Cruz, P.T. Maturan, J.C.E. Ontoy, and Kumar, A. 2014. QTLs for tolerance of drought and breeding for tolerance of abiotic and biotic stress: An Integrated Approach. *PLoS One*,9(10): e109574.
- Gowda, V.R., A. Henry, A. Yamauchi, H.E. Shashidhar and R. Serraj. 2011. Root biology and genetic improvement for drought avoidance in rice. *Field Crops Res.*, 122: 1–13.
- Levitt, J. 1972. Responses of Plants to Environmental Stresses, Academic Press, New York. pp. 698.
- Muthu, V., Abbai, R., Nallathambi, J., Rahman, H., Ramasamy, S., Kambale, R., Thulasinathan, T., Ayyenar, B. and Muthurajan, R., 2020. Pyramiding QTLs controlling tolerance against drought, salinity, and submergence in rice through marker assisted breeding. *PLoS one*, 15(1), p.e0227421.
- Murray M G and Thompson W F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Res.*, 8(19): 4321 - 4325.

- O Toole, J.C. and T.T. Chang. 1979. Drought resistance in cereals-rice: a case study. In: Stress physiology in crop plants. Eds. H. Mussell and R. Staples, John Wiley and Sons, Inc, pp. 373-405.
- Pandey, S, and H. Bhandari. 2007. Drought: an overview. In: Economic costs of drought and rice farmers' coping mechanisms: a cross-country comparative analysis. Eds. S. Pandey, H. Bhandari and B.Hardy, Los Banos (Philippines): *International Rice Research Institute*. p. 11-30.
- Pandey, V. and A. Shukla. 2015. Acclimation and tolerance strategies of rice under drought stress. *Rice Sci.*, 22(4): 147-161.
- Prince, S. J., R. Beena, S. M. Gomez, S. Senthivel and R. C. Babu. 2015. Mapping consistent rice (*Oryza sativa* L.) yield QTLs under drought stress in target rainfed environments. *Rice*, 8(1): 25.
- Serraj, R., K.L. McNally, I. Slamet-Loedin, A. Kohli, S.M. Haefele, G. Atlin and A. Kumar. 2011. Drought resistance improvement in rice: an integrated genetic and resource management strategy. *Plant Prod. Sci.*, 14: 1-14.
- Salunkhe, A.S., R. Poornima, K.S.J. Prince, P. Kanagaraj, J. Annie Sheeba, K. Amudha, K.K. Suji, A. Senthiland and R. Chandra Babu. 2011. Fine mapping QTL for drought resistance traits in rice (*Oryza sativa* L.) using bulk segregant analysis. *Mol. Biotechnol.*, 49: 90-95.
- Venuprasad, R., C. Dalid, M. Del Valle, D. Zhao, M. Espiritu, M.S. Cruz, M. Amante, A. Kumar and G. Atlin. 2009. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor. Appl. Genet.*, 120(1): 177-190.
- Venuprasad, R., M. Bool, L. Quiatchon, and G.N Atlin. 2012a. A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds. *Theor. Appl. Genet.*, 124: 323-332.
- Venuprasad, R., M. Bool, L. Quiatchon, M.S. Cruz, M. Amante and G.N Atlin. 2012b. A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. *Mol. Breed.*, 30(1): 535-547.
- Venuprasad, R., C.M.T. Sta, M. Amante, R. Magbanua, A. Kumar, G. N. Atlin. 2008. Response to two cycles of divergent selection for grain yield under drought stress in four rice breeding populations. *Field Crops Res.*, 107: 232-244.
- Venuprasad, R., H. Lafitte and G. Atlin. 2007. Response to direct selection for grain yield under drought stress in rice. *Crop Sci.*, 47(1): 285-293.
- Vikram, P., M.B.P. Swamy, S. Dixit, H.U. Ahmed, M.T. StaCruz, A.K. Singh and Kumar, A. 2011. qDTY1.1, a major QTL for rice grain yield under reproductive stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genet.*, 12: 12-89.
- Vikram P., B.P.M. Swamy, S. Dixit, J. Trinidad, M.T. StaCruz, P.C. Maturan, Amante, M. and Kumar, A., 2016. Linkages and Interactions analysis of major effect drought grain yield QTLs in rice. *PLoS One*, 11(3): e0151532.