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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 was aimed at developing 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 harbour two target QTLs and aroma and two progenies were found to harbour 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.

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). 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 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 continue to 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 genotypes 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 accelerated identification of elite progenies possessing Chakhao amubi like grains and three drought 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, F2 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/µL. Polymorphic SSR markers RM 11885, RM 7426 and RM 16030 were used for genotyping of F_2 progenies along with the parents. PCR reactions were carried out using DNA as templet (50 ng/µL), PCR buffer consisting of 1.5 mM $MgCl_2$, 0.4 mM dNTPs, 10 µM 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 ºC for 5 min, 35 cycles of 94 ºC for 30 sec, 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 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 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 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 the improvement of drought tolerance in a traditional variety Chakhao amubi by introgressing qDTY QTLs from a drought 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 RM 16030 (*qDTY3.1*) for breeding applications. Evaluation of >30 F_1 progenies using the above three markers identified three true F_{1s} and forwarded to F_2 by selfing. F_2 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 F_2 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. Sensory evaluation test identified five progenies (# 3, # 104 and # 17 harboring all three QTLs; # 91 possessing two qDTY QTLs and # 37 possessing one qDTY QTL) possessing stronger aroma. Progenies harboring aroma and three drought QTLs (*qDTY1.1, qDTY2.1* and *qDTY3.1*) were forwarded to F_3 generation. Foreground analysis F_3 progenies resulted in the identification of thirty progenies harboring aroma and the target QTLs and forwarded to F_4 generation.

F_4 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. 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 in comparison with 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).

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. In this study, elite lines possessing 2 - 3 qDTY QTLs of Apo combined with early duration and superior grain quality were identified and forwarded to further generations. 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).

Conclusion

The present investigation clearly 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.

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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	***qDTY1.1*** (34.9–37.8 Mb)	1	RM 11885	55
	qDTY2.1 (11.4–20.7 Mb)	2	RM 7426	55
	qDTY3.1 (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.

Fig 1. Evaluation of F_4 RILs at vegetative stage in field

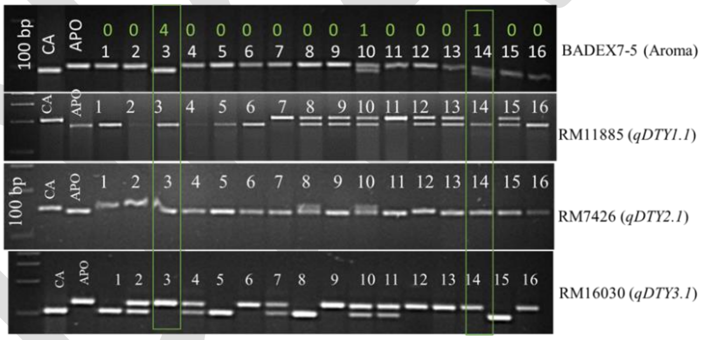

Fig 2. Foreground selection of F_2 progenies of Chakhao amubi x Apo for *qDTY1.1, qDTY2.1, qDTY3.1* and *OsBADH2*.

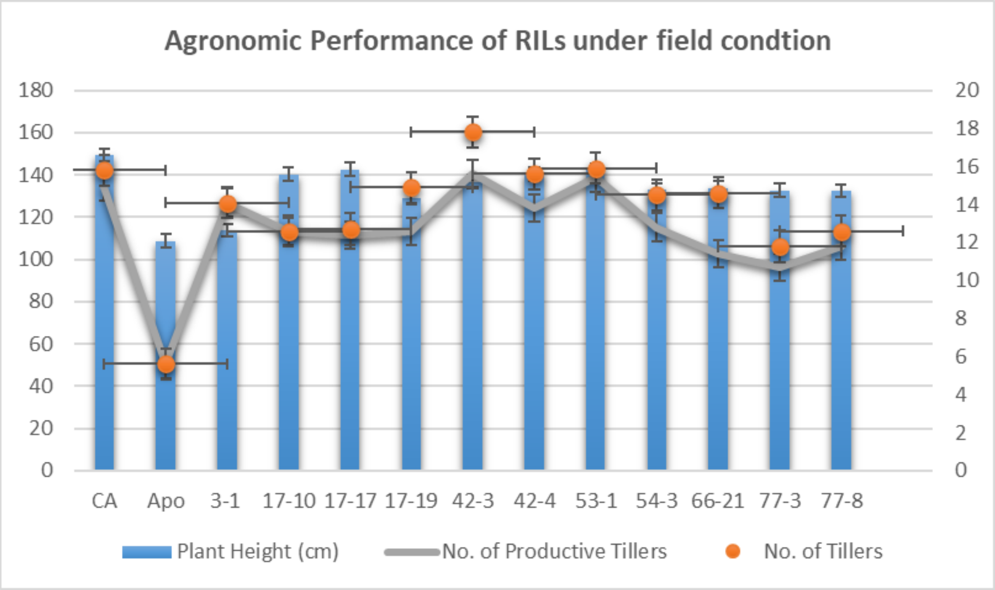

Fig 3. Agronomic performance of RILs (F_4) harboring drought QTLs