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Marker Assisted Introgression of QTL (qDTY1.1) for Grain Yield under Drought at Reproductive Stage in *Oryza sativa* L. cv. Sita from Nagina -22

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ABSTRACT

Rice is the second major crop in the world in terms of area and production but due to change in environmental conditions, it is being affected by drought. Numerous rice varieties have been developed through conventional breeding method which takes a several years for its release and the varieties released Though this method may not be adequate to meet the future demand of growing populations. With the advancement of marker assisted breeding (MAB) along with the discovery of new QTLs, acted as a milestone in marker assisted introgression for development and release of variety. In the present research work emphasis has been given on introgression of QTL qDTY1.1 since it has been reported by researchers that it plays crucial role in drought tolerance which is located near to *sd-1* gene. So, in the present investment, introgression of qDTY1.1 has been carried out from Nagina-22 in the background of local rice cultivar Sita. SSR marker RM431 was used for foreground selection while recombinant selection was carried out through RM3825 and RM12091. Background selection was performed with 49 polymorphic SSR markers. Maximum recovery obtained for BC_2F_1 line was 79.59%. All positive BC_2F_1 lines developed were subjected to further breeding and field trial. The results showed that Sita could be efficiently converted into a drought tolerant variety through backcross followed by selfing and selection, involving a time of two to three years. This approach demonstrates the effective use of marker assisted backcross breeding for introgression of qDTY1.1 in a molecular breeding programme.

Key words: *Rice, Drought, qDTY1.1, Marker Assisted Breeding (MAB), Foreground Selection, Recombinant Selection, Background Selection*

Introduction

Rice is the major stable crop worldwide which feeds approximately 2.7 billion people. Along with feeding more than half of the world population, cultivation of rice also provides employment to more than 3.5 billion people. Ballooning human population has argued the need to double the rice production by 2050. Shrinking farmland, decreasing irrigation facilities, various environmental stresses and changing climatic conditions has slow down the pace of rice production and productivity. In India, majority of areas are rainfed in nature hence vulnerable to vagaries of monsoon (Adhya *et al*., 2008). Among several constrains, drought stress is the main limiting factor of rice production which cause yield losses

averaging 18 million tonnes globally (Toole, 2004). A large area of 23 and 13.6 mha in Asia and Eastern India, respectively, are being affected by drought stress (Dixit *et al.,* 2014, Vikram *et al.,* 2011). The mega rice varieties grown in drought affected areas are bred for irrigated condition so incidence of drought cause sudden decline in rice production (Kumar *et al*., 2008). Development of drought tolerant varieties is urgently required to minimize the yield losses due to incidence of drought stress. Progress is being made in developing drought tolerant rice germplasm through QTL introgression which provides excellent approach by understanding the genetic entities governing the complex traits (Shamsudin *et al*., 2016).

In the present research work improvement in yield of Sita during drought has been carried out through markers. Here emphasis has been given on introgression of QTL qDTY1.1 as other researchers have also reported its importance in drought introgression programme (Kumar *et al*., 2014; Vikram *et al*., 2011). Location of qDTY1.1 on chromosome 1 for yield under drought has been studied (Vikram et al. 2015). This QTL is tightly linked with *sd-1* gene due to presence of linkage. Here, RM431 is present very close to qDTY1.1 so it was used for foreground selection (Ghimire *et al.,* 2012, Vikram *et al.,* 2015). Nagina-22 was used as donor parent. It is very popular drought donor in rice. It was released as a cultivar in 1978 and shows only 20% reduction in yield when drought occurs (Singh *et al.,* 2018). Taking into consideration of the above points the present research was being carried out with the objective of introgression of drought QTL (qDTY1.1) in the background of rice variety Sita through backcross programme from Nagina-22.

Materials and Methods

The present experiment was performed at Rice section of Bihar Agricultural University, Sabour, Bhagalpur, Bihar. Geographically, University Farm is situated between 25º′ 15′40′′ºN latitude to 87º2′42′′ºE longitude at 46 m above mean sea level. The soil type is alluvial which was enriched with vermicompost to support plant growth. All types of facilities necessary for rising of successful crop including field preparation, inputs, irrigation facilities and laborers were provided by Rice Section.

Plant Materials

Sita is a popular high yielding cultivar among farmers was released from RAU Pusa. It is tall plant (110 cm) having long duration (130-135 days) performs better in normal condition producing 40-45 quintals per hectare but is most severely affected in drought. So, to make it drought tolerant Nagina-22 was used which is donor of QTL qDTY1.1. Nagina-22 is a short duration from landrace Rajbhog from Nepal having maturity 95-100 days with yield of 27 quintals per hectare.

Hybridization

The present research work started from June 2015 with staggered sowing of Sita with Nagina-22. Crossing was done by transferring pollen from Nagina-22 to the emasculated anthers of Sita. F_1 obtained were sown in June 2016. It was validated with foreground selected marker RM431. The true F_1 containing heterozygous bands were crossed with Sita (recurrent parent). BC_1F_1 obtained were sown in June 2017 whose validation of heterozygosity was confirmed with RM431. Positive plants were backcrossed with Sita to obtain BC_2F_1.

Genotyping

DNA extraction and amplification of PCR products Rice genomic DNA was isolated from young leaf using the standard cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987).

PCR amplification through SSR primers

Genomic DNA samples were subjected to PCR amplification using SSR primers. List of SSR markers were mentioned in Table 1. SSR marker amplification was executed in 10 µl volume containing 5 µl of Premix Taq® Version 2.0 (Xcelris Lab Ltd. Ahmedabad, Gujarat), 0.2 µM each of forward and reverse primer and 50 ng of template DNA in a thermal cycler (Agilent Technologies). The amplification reaction involved an initial 94 ^{0}C for 5 min for denaturation followed by 35 cycles of 1 min at 94 ^{0}C, 30 sec at 55 ^{0}C, 45 sec at 72 ^{0}C and final extension at 72 ^{0}C for 5 min. Amplified products were electrophoresed on 3.5 % agarose gel and image were visualized using gel documentation system (UVITEC, Cambridge).

Morphological screening of BC_2F_1 plants

Screening for drought tolerance was carried out in

BC_2F_1 generation. Drought screening was performed at rainout shelter, BAU Sabour as described previously (Kumar *et al*., 2014). Drought stress was imposed by withholding irrigation 15 days before flowering to 10 days after flowering. Morphological data like days to anthesis (days), plant height (cm), panicle length (cm), number of panicles, grains per panicle and grain yield per plant were observed by following standard procedure.

Biochemical screening of BC_2F_1 plants

Proline content (µMol g^{-1})

The free proline content of dried leaves was determined using a modified procedure (Bates *et al*., 1973).

Relative Water Content (%)

Weight of fresh leaf was taken on weighing balance. The leaf samples were then immersed in water overnight blotted dry and then weighed to get the turgid weight. The leaves were then dried over night in a hot air oven at 70 °C and reweighed to obtain the dry weight.

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

Total Chlorophyll Content (mg g^{-1})

Total Chlorophyll Content was determined as described previously (Arnon 1949).

Results

Parental polymorphism survey

Parental polymorphism were studied among Sita (drought susceptible) and Nagina-22 (drought tolerant). Total 198 SSR markers were used in this study. Out of which 52 SSR markers showed polymorphism (Table 1), which were used to validate the qDTY1.1 in the backcross population.

Development of F_1 generation

The crossing of Sita and Nagina-22 yielded 25 F_1 seeds, which were harvested and sown in June 2016. These F_1 plants were screened using foreground marker RM431. Presence of heterozygous band in F_1 confirms the introgression of desired QTL. Twenty five F1 plants showing heterozygous banding pattern were selected for further analysis (Figure 1). Amplification of 250 bp band using RM431 confirms

Table 1. List of polymorphic SSR markers used in the study

S. No.	Chromosome	Markers	Amplicon Size	
			Sita	Nagina-22
1.	1	RM1	90bp	100bp
2.		RM5	110bp	120bp
3.		RM212	120bp	140bp
4.		RM259	170bp	160bp
5.		RM431	240bp	250bp
6.		RM495	160bp	150bp
7.		RM3746	90bp	100bp
8.		RM3825	130bp	150bp
9.		RM12091	150bp	130bp
10.		RM12146	110bp	120bp
11.	2	RM208	170bp	180bp
12.		RM263	190bp	170bp
13.		RM341	170bp	150bp
14.		RM3874	130bp	120bp
15.	3	RM22	200bp	190bp
16.		RM36	200bp	210bp
17.		RM55	210bp	220bp
18.		RM514	240bp	270bp
19.		RM517	250bp	270bp
20.		RM545	220bp	210bp
21.		RM3202	100bp	110bp
22.		RM3513	100bp	105bp
23.		RM5801	80bp	90bp
24.	4	RM127	200bp	210bp
25.		RM551	180bp	210bp
26.		RM17303	160bp	130bp
27.	5	RM164	270bp	250bp
28.		RM334	200bp	170bp
29.		RM413	70bp	80bp
30.	6	RM190	100bp	130bp
31.		RM225	150bp	130bp
32.		RM276	150bp	100bp
33.		RM314	110bp	120bp
34.	7	RM11	150bp	130bp
35.		RM3799	190bp	170bp
36.		RM5499	210bp	220bp
37.	8	RM25	150bp	140bp
38.		RM44	100bp	110bp
39.		RM80	140bp	120bp
40.		RM152	170bp	150bp
41.		RM256	100bp	140bp
42.		RM447	100bp	110bp
43.	9	RM205	140bp	170bp
44.		RM219	210bp	215bp
45.		RM242	210bp	200bp
46.	10	RM271	210bp	190bp
47.		RM333	200bp	180bp
48.	11	RM287	100bp	115bp
49.		RM552	170bp	210bp
50.		RM5961	140bp	160bp
51.	12	RM235	100bp	120bp
52.		RM27406	180bp	200bp

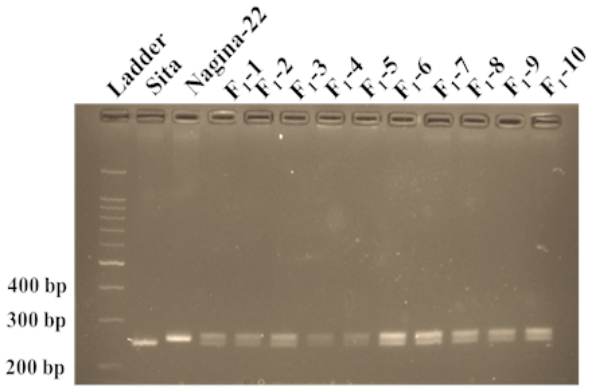

Fig. 1. Foreground selection of F_1 generation. The presence of double band corresponding to parents Sita and Nagina 22 were selected as true hybrid.

the presence of qDTY1.1 in Nagina-22.

Development of BC_1F_1 and BC_2F_1 generation

Twenty five plants were selected in F_1 generation by validating through RM431 which confirmed the presence of heterozygosity. These plants were further backcrossed with Sita to produce 67 BC1F1 seeds. On screening of obtained BC_1F_1 plants a total of 37 plants were found to be heterozygous using RM431. These 37 heterozygous plants obtained were again backcrossed with Sita to produce 179 BC_2F_1 plants which were checked with RM431 through which 98 plants were found heterozygous which confirms the presence of heterozygosity. The selected plants were subjected to recombinant selection using flanking markers RM3825 and RM12091. Six plants were confirmed by these markers which were used for background selection. These plants were screened for background recovery using 49 SSR (Table 1). Maximum background recovery percentage ranged from 69.38% to 79.59%. Plant number 119 had recovery of 79.59% followed by plant number 94 which was 77.55%, plant number 97 had 75.51%, plant number 65 had 74.48%, plant number 23 had 73.46% followed by plant number 63 which had only 69.38% recovery.

Morphological and Biochemical screening of BC_2F_1 plants

Morphological observations were recorded in all the BC_2F_1 plants and compared to parents. The DFF of selected recombinant BC_2F_1 plants were varied from 93-97 days which were similar to Sita (Table 2). The plants height of selected BC_2F_1 plants was numerically higher than Nagina 22 but lower than Sita (Table 2). The length of panicle of all selected recombinant BC_2F_1 plants was similar to Sita. Grains per panicle selected recombinant BC_2F_1 plants were varied from 165-182. The yield per plant of selected recombinant BC_2F_1 varied from 27.46 – 32.8 g. The proline content of selected recombinant BC_2F_1 varied 22.46-27.84 µMol g^{-1} which was higher than Sita (20.21 µMol g^{-1}) and closer to Nagina-22 (28.25 µMol g^{-1}). The relative water content of selected recombinant BC_2F_1 varied from 68.56 – 74.56% which was higher than Sita (65%) and at par with Nagina-22 (76.80%). The chlorophyll content varied from 17.12 to 20 mg g^{-1} in selected recombinant BC_2F_1 plants. The chlorophyll content of selected recombinant BC_2F_1 plants was higher than Sita (15.76 mg g^{-1}) and much nearer to Nagina-22 (20.24 mg g^{-1}).

Discussion

Looking into the significant advantages of MAB over conventional breeding, an attempt was made to introgress qDTY1.1 from Nagina-22 to farmer-preferred variety Sita rice variety through MAB. A plethora of reports advocate the use of MAB in introgression of QTLs in crop plant including rice (Singh et al. 2018). The advantages of introgression of qDTY1.1 in mega varieties of rice for improving

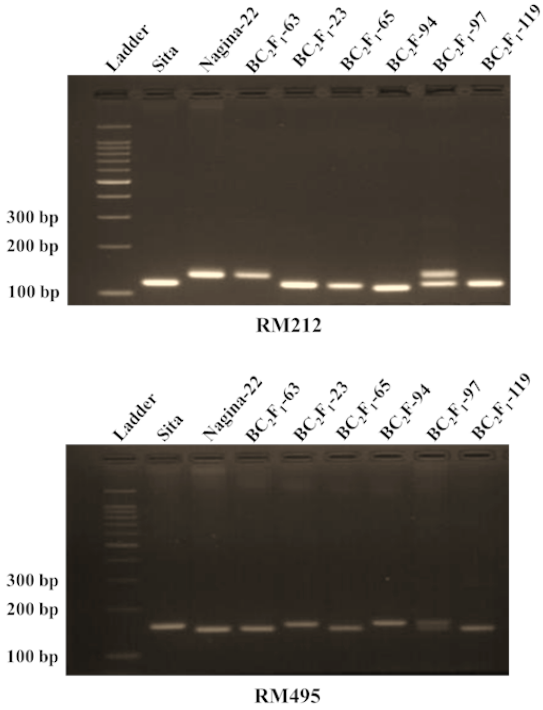

Fig. 2. Background selection of BC_2F_1 generations

grain yield under drought are well documented (Ghimire et al. 2012, Vikram et al. 2011). As previously reported, RM431, tightly linked SSR marker with qDTY1.1 was used for foreground selection (Ghimire et al. 2012, Vikram et al. 2015) in this study also. RM431 also helped in identifying the trait of interest since appearance of heterozygous bands confirms the presence of QTL qDTY1.1 in the recombinant lines. In the present research work RM431 was used for validation of heterozygous lines in F1, BC_1F1 and BC_2F_1 generations. Several reports confirmed the use of RM431 for validation of qDTY1.1 in MAB (Vikram *et al*., 2011, Vikram *et al.,* 2015, Hemamalini *et al.,* 2000, Shen *et al.,* 2001, Kumar *et al*., 2007, Awasthi and Lal 2014). Identification of molecular markers that co- segregate with the desired trait is a critical step for success of MAB. The most favourable case for MAB is when the molecular marker is located directly within the gene of interest. Recombinant selection was performed using flanking markers RM3825 and RM12091 as qDTY1.1 is located in between these two flanking SSR markers. Using RM3825 and RM12091, six and three plants, respectively, were selected which are similar to recurrent parent Sita. For cases like drought tolerance, where gene-linked markers are not available, the identification of peak markers and flanking markers in the recipient background within the QTL region is necessary for initiation of any MAB programme (Shamsudin *et al*., 2016). Recently, RM3825 and RM12091 have been used for recombinant selection for QTL qDTY1.1 (Verma *et al.,* 2014, Sandhu and Kumar 2017). The use of molecular markers for background selection accelerated the identification of backcross progenies possessing maximum recovery of the recurrent parent genome (Servin and Hospital, 2002, Visscher *et al.*, 1996). A few well-placed markers (two to four markers on a chromosome of 100 cM) provide adequate coverage of the genome in backcross programs. The 12 chromosomes in rice have different sizes hence different number of markers was screened for different chromosomes depending on their availability. In the present investigation, background selection was performed using 49 polymorphic markers. Selected BC_2F_1 recombinants were checked using these markers which showed a recipient allele recovery from 69.38 to 79.59%. In general background recovery of BC_2 generation should range from 85 to 90%. In our experiment the background recovery percentage was found to be low as compared to other reports

Table 2. Morphological and biochemical observations of recombinant positive plants obtained in BC_2F_1 generation

		Positive recombinant plants obtained in BC2F1 generation						Parents	
		63	23	65	94	97	119	Sita	Nagina-22
A. Morphological Observations	Days to Anthesis (days)	93	94	97	95	94	95	97	85
	Plant Height (cm)	108	109	106	108	107	108	110	100
	Panicle Length (cm)	26.8	27.1	27.3	26.4	27.0	27.6	28.0	24.0
	Number of Panicles	7	6	7	7	6	7	6	7
	Grains per Panicle	170	165	173	180	177	182	150	185
	Grain Yield per Plant	29.34	27.46	30.12	31.76	30.45	32.8	22.3	33.7
B. Biochemical Observations	Proline Content (µMol g^{-1})	22.46	23.84	24.64	26.80	25.92	27.84	20.21	28.25
	Relative Water Content (%)	68.56	70.26	73.46	72.87	71.57	74.56	65	76.80
	Total Chlorophyll Content (mg g^{-1})	17.12	17.76	18.12	19.84	19.46	20.00	15.76	20.24

(Hospital, 2001; Siangliwa *et al.,* 2007; Awasthi and Lal, 2014; Shamsudin *et al*., 2016; Linh *et al.,* 2013). The low background recovery in our experiment might be due to low population size.

Several metabolic changes occur within the plant during drought with respect to fresh and dry weights of the leaves, proline content and total chlorophyll content (Shehab *et al.,* 2010; Usman *et al.,* 2013). The RWC was identified as a key parameter to select tolerant plants under drought stress (Bunnag and Pongthai, 2013; Choudhary *et al.,* 2009, Teulat *et al.,* 2003). In the present research work relative water content has been taken during drought when leaf rolling was observed in rice plants. In

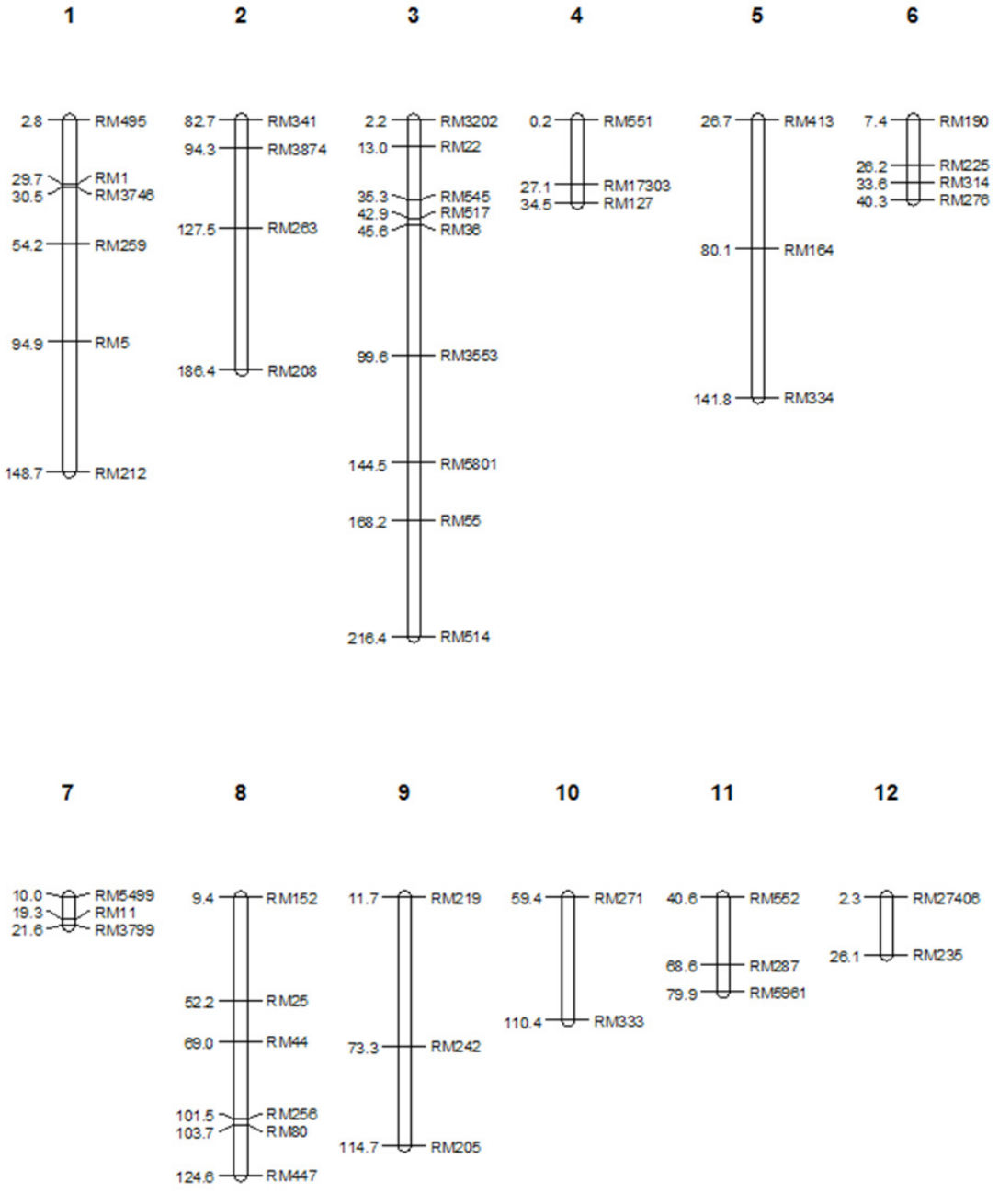

Fig. 3. Pictorial representation of 49 polymorphic markers which were checked for background selection

drought tolerant parent Nagina-22 it was 76.80% while it was 65% in susceptible parent Sita. Its value in recombinants ranged from 68.56 to 74.56%. All recombinants were showing RWC higher than susceptible parent (Sita) which was due to introgression of QTL $qDTY_{1.1}$ reflecting that these plants maintain higher metabolic activity in tissue. Kumar *et al.,* 2014 reported RWC 31.57% in rice genotypes. RWC in three genotypes of rice was found 76.5%, 75.2% and 74.1% (Kumar *et al.,* 2014). Tolerant plants maintaining higher RWC during drought helps them to recover easily in stress (Swamy *et al*., 1983; Nadarajan and Kumarevelu 1993; Saxena *et al*., 1996; Cabuslay *et al*., 1999; Kumar and Kujur, 2003, *Chutipaijit et al*., 2010; Mahender *et al.,* 2014; Larkunthod *et al*., 2018).

Proline accumulation in tolerant genotypes of rice plays an important role in osmotic adjustment during drought. Its accumulation in stress condition functions as osmoprotectant compound and reserve energy which reduces lipid peroxidation by acting as a free radical scavenger. During drought, proteins degrade and consequently proline content increases faster than other amino acids in plants. More proline accumulation in tolerant plants expressed more yield (Deivanai *et al*., 2010; Mahender *et al.,* 2014; Larkunthod *et al*., 2018). Hence, its accumulation is used as criteria for drought stress tolerance in plants. In present investigation, proline content was estimated to be 20.21 and 28.25 $\mu Molg^{-1}$ in Sita (drought susceptible) and Nagina-22 (drought tolerant), respectively. The proline content in recombinants BC_2F_1 plants was higher than Sita and similar to Nagina-22 was due to introgression of qDTY1.1.

In the present investigation, chlorophyll content in recombinant BC_2F_1 plants was closer to Nagina-22 which was correlated with similar yield in recombinant BC_2F_1 plants and Nagina-22. A plethora of report positively correlates the drought stress with chlorophyll content (Kumar *et al*., 2014; Yooyongwech *et al*., 2012).

In the present study RM431 was found to be more suitable for foreground selection as it is tightly linked with qDTY1.1. Recombinant selection was performed with RM3825 and RM12091 which revealed that 6 recombinants were similar to recurrent parent Sita. The background recovery percentage was varied from 69.38 to 79.59% in selected BC_2F_1 plants. Field screening showed reliability of MAS for introgression.

Authors' contribution

Conceptualization of research (RK, PK, BDP, S); Designing of the experiments (PK, AK, BDP, S); Contribution of experimental materials (PK, AK); Execution of field/lab experiments and data collection (RK); Analysis of data and interpretation (RK, PK, BDP, MK); Preparation of the manuscript (RK, PK, BDP).

Declaration

The authors should declare that they do not have any conflict of interest.

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