

## Recurrent Genome Recovery in Drought Tolerance Backcross Inbred Lines of IR 64

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### Abstract

Rice is the primary food for more than half of the world's population, especially in developing countries of Asia, where water scarcity/drought is becoming an imminent threat for sustaining rice productivity. Rice uses two to five times more water than other cereals especially wheat or maize and consumes about 30 per cent of the freshwater used for agricultural crops worldwide. Drought is the most serious environmental stress which is estimated to affect 50 percent of the world's rice production to a greater or lesser extent. Introgression of drought tolerance to the cultivated varieties is a way to overcome the scarcity of water. But the time and breeding cycle needs to be reduced to use this effectively. To overcome the time scarcity, minimize the limitation of conventional breeding and recover the parental genome within 2-3 generations, the usage of marker assisted backcrossing technique is most effective way. Here in this study Marker assisted backcross breeding (MABB) technique is used to identify the lines with highest recurrent parent genome (RPG) recovery. IR64 is an adopted variety with drought susceptibility which was crossed with Apo an upland rice. Sixty seven BC<sub>2</sub>F<sub>3</sub> populations were screened for foreground selection for three drought QTLs namely qDTY<sub>2.2</sub>, qDTY<sub>3.1</sub> and qDTY<sub>8.1</sub>. Four lines which were homozygous for the different QTL combination were identified. A total of 596 SSR markers were tested to identify the polymorphism between the parents and 98 of them found to be polymorphic. Background analysis revealed in the BC<sub>2</sub>F<sub>2</sub> generation the average RPG recovery was above 80 % and that for the selected plant was 85%. This study revealed the usefulness of marker assisted backcrossing for the quick recovery of a parental genome in a backcrossing population.

**Keywords:** Marker assisted backcrossing; Recurrent parent genome; Rice; Drought.

### Introduction

Rice being one of the widely cultivated cereals across diverse agroecological systems is a great consumer of water, requiring around 5,000 litres of water to produce one kg of rice and is prone to high yield

losses due to recurring droughts (Shen et al., 2001). Rice the novel system of aerobic rice, in which it is cultivated in non-puddled and non-flooded soil just like wheat and maize, drastically reduces water requirements and allows cropping of rice where a

lack of water has made this impossible till now. Abiotic stress can impose limitations on crop productivity and also limit land availabilities for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress.

From past few years, use of genotypic variation for genomic research on drought tolerance has been enhanced by development of introgression lines from drought tolerant donor cultivars into elite cultivars and the selection of drought tolerant backcross populations (Li et al., 2005; Lafitte et al., 2006). Substantial progress has been made in detecting QTLs conferring drought tolerance in lowland and irrigated rice (Serraj et al., 2011). To date, several QTLs with large and consistent effects on grain yield under drought stress have been identified from drought tolerant rice genotypes viz., Apo, Nagina 22 Way rarem etc., varieties

(Bernier et al., 2007; Venuprasad et al., 2009; Venuprasad et al., 2011). QTLs showing large and consistent effects can be utilized through marker-assisted breeding (MAB), a rapid and precise approach to develop high-yielding drought-tolerant rice varieties.

Breeding a completely new line is mostly not being now-a-days followed as it consumes several years to bring all the desirable traits into one genotype. Hence the identified trait or the trait of interest is being transferred to already adopted modern cultivars to produce its own isogenic lines. The advantages of such lines are: they already harbor all the desired traits, they are farmers adopted varieties, and transfer of single gene/qtl is being easier these days due to the advance in the molecular breeding. The background genome recovery could be studied with the help of polymorphic background markers between the two parents making the isogenic lines available within two to three backcrosses. In traditional backcross breeding programme usually about 7-8 backcross with the recipient parent is done in order to produce morphologically identified isogenic lines. Backcross breeding is generally performed with the specific objective to keep the target gene and reduce the amount of donor segment as much as possible. With every successive backcrossing the amount of donor genome segment decreases and the amount of recurrent parent segment increases (Lewis and Kernodle 2009). Molecular assisted Backcross breeding (MABB) can be used to precisely remove the adjacent donor segment attached to the gene of interest, and also to measure the amount of parental genome recovery

in each generation. The objective of this study was to determine the amount of recurrent parent genome recovery from new lines that were developed by crossing Apo and IR 64.

## Materials and Methods

The biological material for this study consisted of a set of Backcross Inbred Lines of IR64 ( $BC_2F_3$ ) which were introgressed with drought yield QTLs viz.,  $qDTY_{2.2}$ ,  $qDTY_{3.1}$  and  $qDTY_{8.1}$  from Apo an indica cultivar with combinations of two and three in IR64 background.

**DNA extraction:** Fresh leaves from 2 weeks old seedlings were collected and DNA was extracted using modified CTAB method (Doyle and Doyle 1990). Quantification of DNA was performed using 1 % agarose gel electrophoresis at 80 V for 30 min, followed by visualization on Molecular Imager\_ Gel Doc System (Bio-Rad, USA). DNA samples were diluted with TE buffer to a concentration of 50 ng and kept in a refrigerator at  $-20^{\circ}C$  for further use.

**Molecular analysis:** Foreground selection was carried out using SSR markers tightly linked with the  $qDTY$ s and showing polymorphic characters. The foreground markers used in the study were RM71 for  $qDTY_{2.2}$ , RM520 for  $qDTY_{3.1}$  and RM256 for  $DTY_{8.1}$ . All the four individuals were screened across the genome with SSR markers for background analysis and to study the genome constitution of the recombinant population. Polymorphism survey identified 98 polymorphic SSR primers out of 596 SSR primers between IR64 and Apo. These primers (98 SSRs) were used to screen IR64 BILs for background analysis. Genotypic data of these individuals were subjected to graphical genotyping software GGT ver. 2.0 (Graphical Genotypes) which gave out a graphical representation of the molecular marker data. The graphical representations and comparisons were made across the entire population as linkage group wise and also the entire genome level as individual wise.

**Allele scoring and data analysis:** Different alleles of the genotypes were scored as A, B, and H, i.e., A for the recipient allele (IR 64) in a homozygous state, B for the donor allele (Apo) in a homozygous state, and H for heterozygous alleles. The marker data were analyzed using GGT 2.0 to estimate the percentage of recurrent parent chromosomal segments in the selected segregates of the backcross population.

## Results and Discussion

Sixty seven  $BC_2F_4$  plants were selected from the cross between IR64x Apo based on the fore ground selection by crossing. The QTL combinations were

confirmed using three SSR foreground markers i.e., RM71, RM520 and RM256, which were polymorphic between the parents. Among the plants surveyed the QTLs in each individual were present in different combinations viz., CB 229-BIL of IR64 with 3 QTL (qDTY<sub>2,2'</sub>, qDTY<sub>3,1</sub> and qDTY<sub>8,1</sub>), CB 193-1- BIL of IR64 with 2 QTL (qDTY<sub>2,1</sub> and qDTY<sub>3,1</sub>), CB 193-2 - BIL of IR64 with 2 QTL (qDTY<sub>2,2'</sub> and qDTY<sub>8,1</sub>) and CB 193-3- BIL of IR64 with 2 QTL (qDTY<sub>3,1'</sub> and qDTY<sub>8,1</sub>). Out of 67 plants the best 4 plants were selected based on their foreground and recombinant selection which were homozygous for QTLs. The other plants were showing heterozygosity for any one of the QTL. The details of number of plants showing positive for the target QTLs are given in the Table.1.

**Table 1:** Details on number of BC<sub>2</sub>F<sub>4</sub> progenies harboring the target QTLs in various combinations under homozygous conditions.

BILs (BC <sub>2</sub> F <sub>4</sub> )	Number of BILs harboring target QTL under homozygous condition
CB 229	16
CB 193-1	24
CB 193-2	21
CB 193-3	17

To assess the recovery of recurrent parent genome in the 67 progenies, parental polymorphism survey was carried out between IR64 and Apo using 596 random SSR markers covering all the 12 chromosomes rice genome. Out of 596 markers surveyed, 98 markers were found to be polymorphic between IR64 and Apo and used for background genotyping of 67 BILs along with the parents. A total of 477 markers were used to identify polymorphism between two parents, BT7 and FL478 (Linh et al. 2012). In another study found that the frequency of polymorphic SSR markers was 12.6 % (Huyen et al. 2012a, b). Basavaraj et al. (2010) reported 54 polymorphic markers out of a total of 309 STMS markers between PRR78 and Pusa 1460, which accounts for a frequency of 17.47 %. A study of polymorphism in two parents, Bac Thom 7 and IR64,

revealed that 15.1 % of the SSR markers were polymorphic (Khanh et al. 2013). This study also indicates that the polymorphic percentage of markers between the parents was around 16.44 percent.

The percentage of recurrent parent genome among the BILs lines ranged from 85.55 % (CB 229) to 81.40 % (CB 193-3). Four BILs viz., CB 229 (Fig 1), CB 193-1 (Fig 2), CB193-2 (Fig 3) and CB 193-3 (Fig 4) were found to retain >80% of recurrent parent IR64 genome (Table 2). BIL CB 229 was found to retain maximum recurrent parent (IR64) genome (85.55 %) with 13% donor genome and 2.9 % heterozygosity (Table 3). In MABC, polymorphic markers are typically used for background study to facilitate the recovery of the parental genome as quickly as possible. Normally, in conventional breeding, it takes 6-7 generations to recover the parental genome. In contrast, with MABC, selection takes only 2-3 generations to recover the parental genome, which saves time and money. Here, we observed 85 % RPG recovery with just two backcrosses. A non-carrier chromosome recovery depends on several factors, such as saturation of the molecular marker map, availability of technical resources at a given time and the required levels of line conversion (Ribaut et al. 2002). DNA markers that are distributed evenly throughout the genome were used to reduce the donor genome content in offspring in several studies (Hospital et al. 1992; Visscher et al. 1996; Young and Tanksley 1989; Frisch et al. 1999).

**Table 2.** Recurrent parent genome recovery analysis in BC<sub>2</sub>F<sub>4</sub> progenies.

BILs (BC <sub>2</sub> F <sub>4</sub> )	Number of plants possessing recurrent parent genome.			
	50-60 %	61-70 %	71-80 %	Above 80 %
CB 229	4	5	5	1
CB 193-1	2	5	7	1
CB 193-2	1	4	9	1
CB 193-3	4	7	3	1

**Table 3.** Recovery of recurrent parent genome among the selected four BILs.

Genotype	Introgressed drought yield QTL	A (%)	B (%)	H (%)	Total recovery (%)
CB 229	3(qDTY <sub>2,2'</sub> , qDTY <sub>3,1</sub> and qDTY <sub>8,1</sub> )	84.10	13.00	2.90	85.55
CB 193-1	(qDTY <sub>2,2</sub> and qDTY <sub>3,1</sub> )	79.70	14.90	5.40	82.40
CB 193-2	(qDTY <sub>2,2</sub> and qDTY <sub>8,1</sub> )	83.30	13.20	3.50	85.05
CB 193-3	(qDTY <sub>3,1</sub> and qDTY <sub>8,1</sub> )	78.60	15.90	5.60	81.40

**A-Recurrent parent genome, B-donor parent genome, H-heterozygote.**

The markers were selected by keeping the basic

requirement of atleast one marker for an interval of 5cm. The crossing over interference is usually considered within at least 15 cm, and the number of

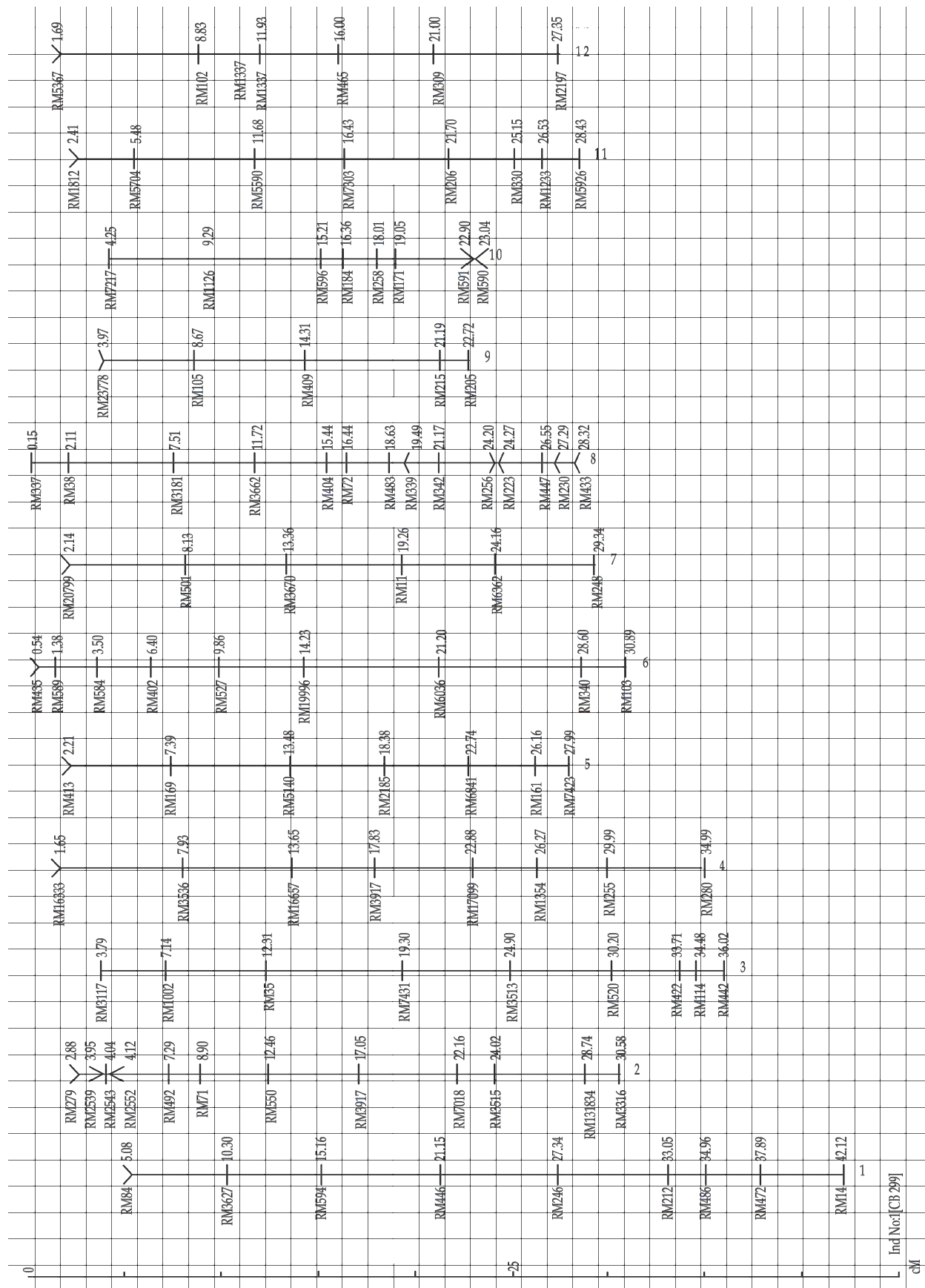


Fig. 1: Genomic constitution of CB 229 based on position of SSR markers.

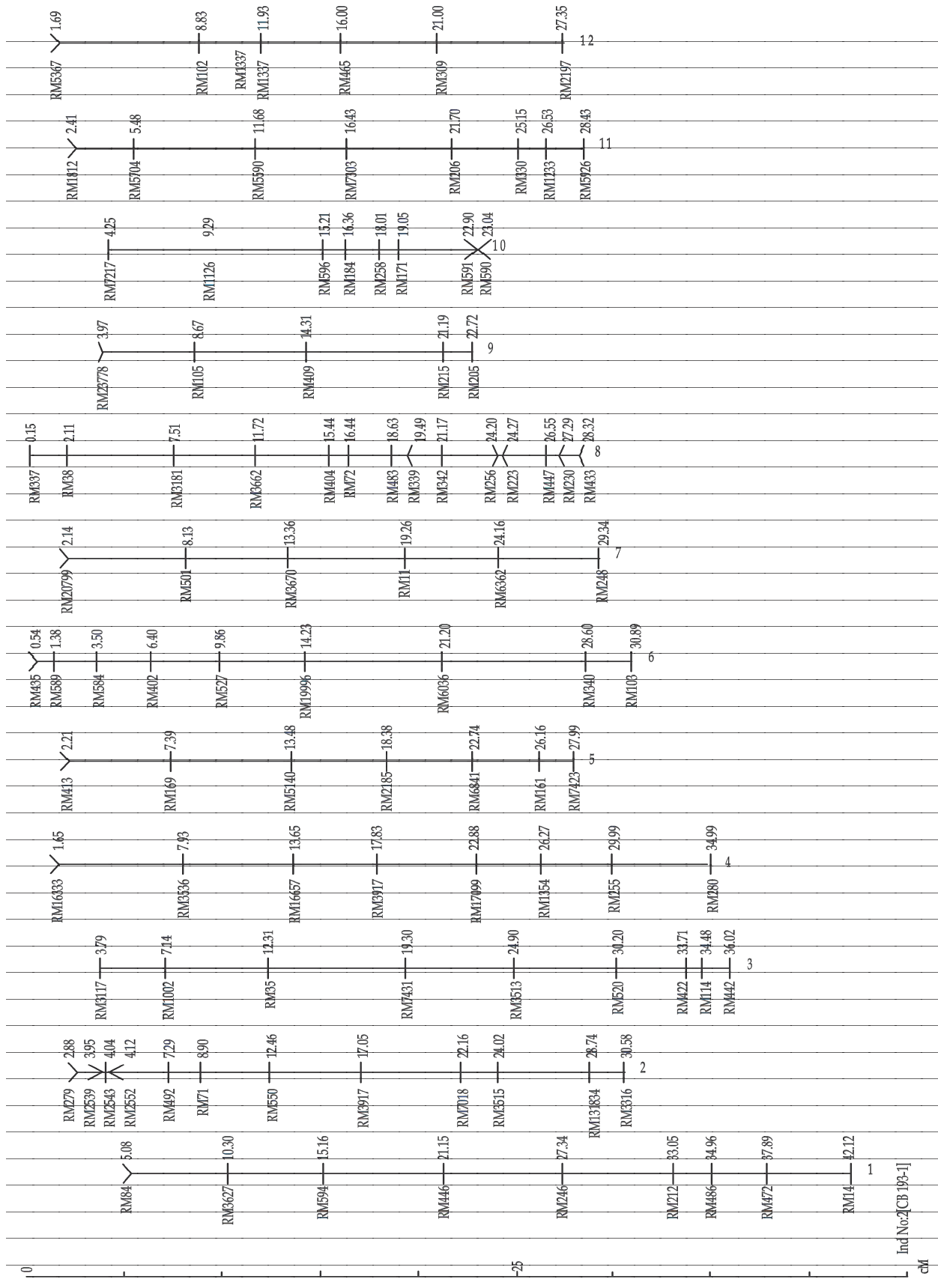


Fig. 2: Genomic constitution of CB 193-1 based on position of SSR markers.

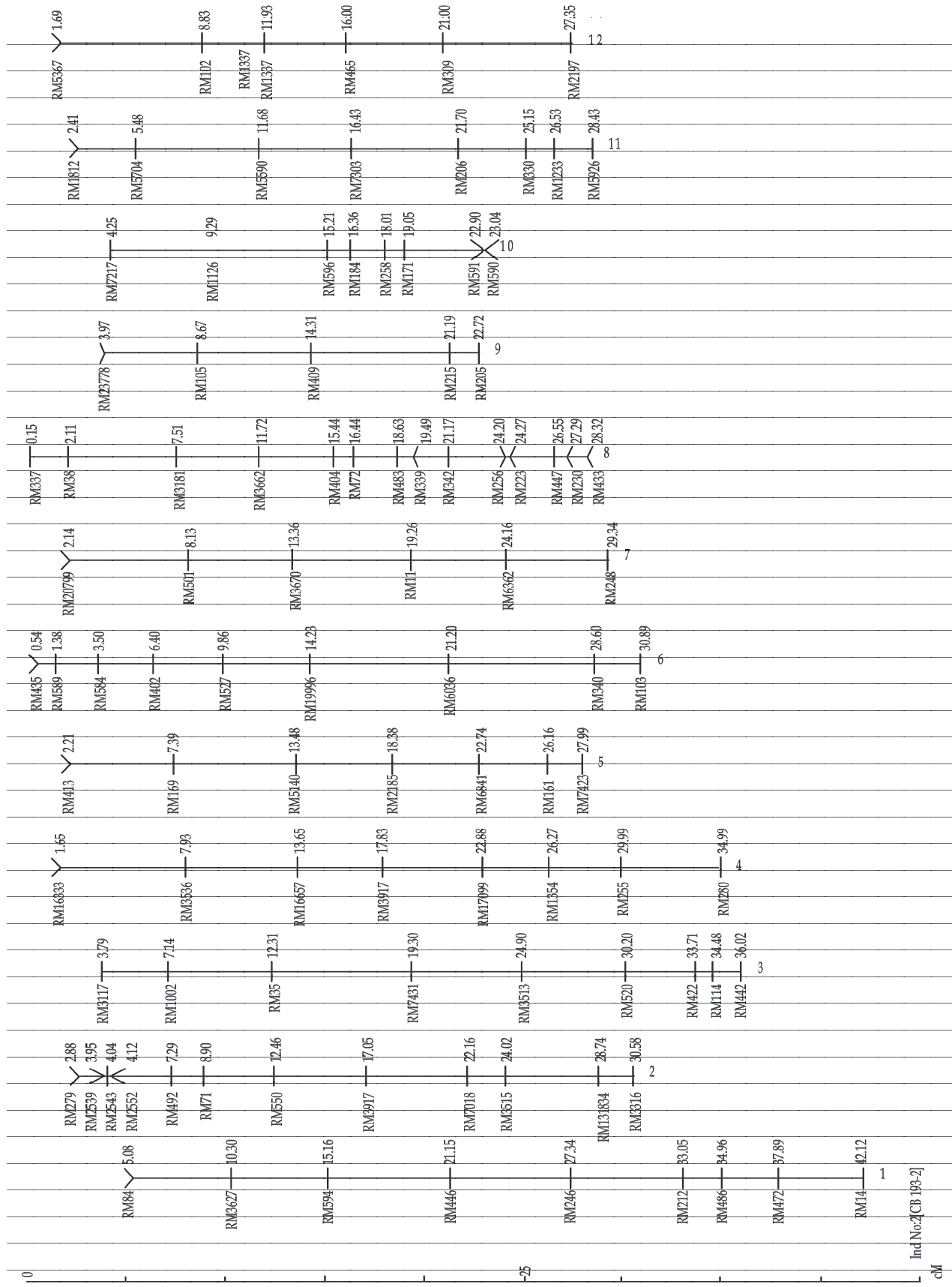


Fig. 3: Genomic constitution of CB 193-2 based on position of SSR markers.

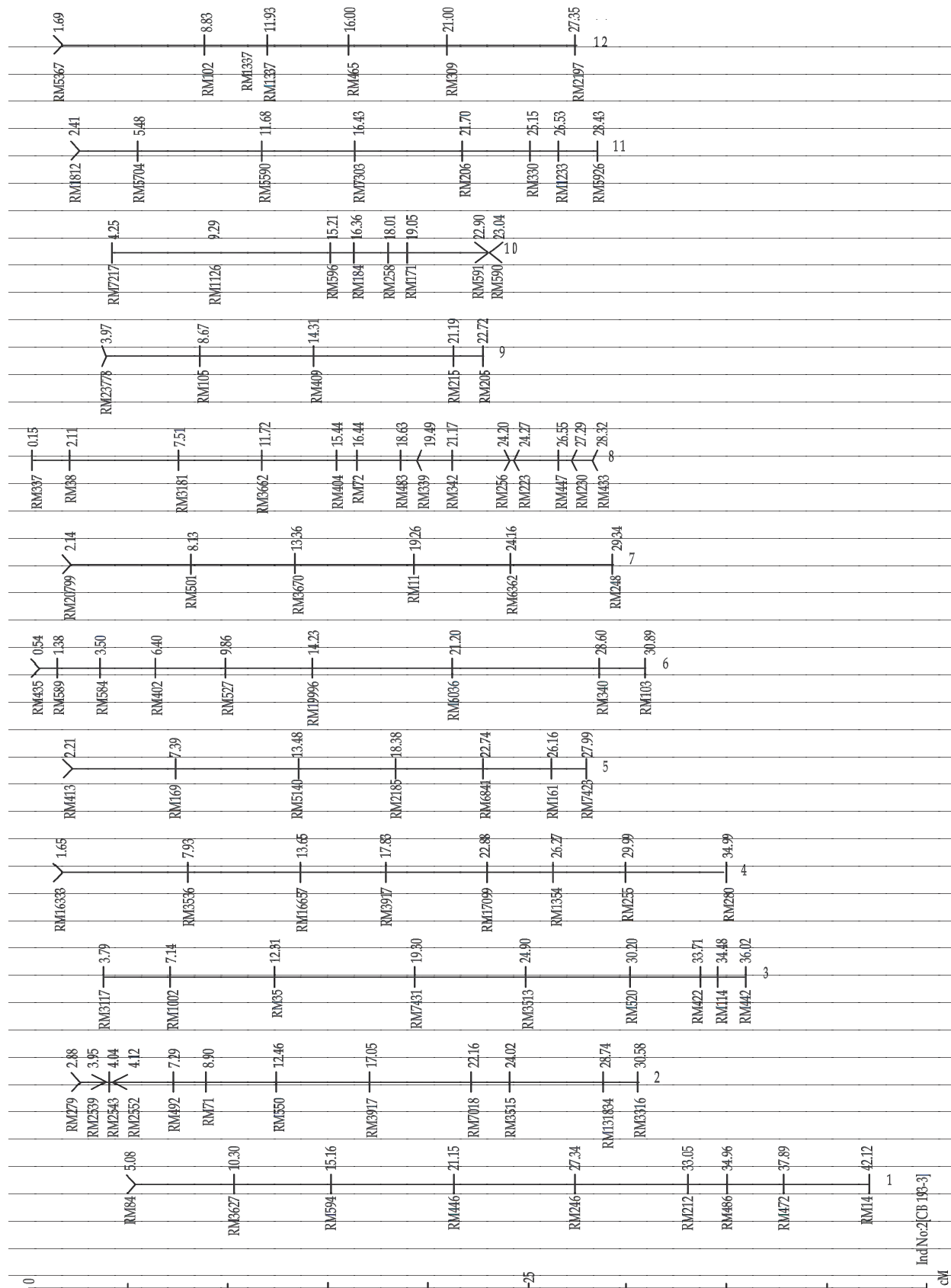


Fig. 4: Genomic constitution of CB 193-3 based on position of SSR markers.

crossovers per chromosome per meiosis rarely exceeds four (Kearsey and Pooni 1996). Thus this study helped in identification of four BILs with highest recipient genome recovery, thus saving the time and breeding cycle. In the study further agronomical characteristics of the selected plants were carefully monitored and variations in the tillering pattern, panicle length, grain width and grain length were noted (Fig 5). The drought screening was already done during the same season by inducing drought stress (Baghyalakshmi et al., 2016).

The MABC technique was successfully used in this study to introgress three QTL regions into the background of IR 64. A short time frame is sufficient to transform any popular rice variety into a drought tolerant variety and is beneficial for keeping the donor segment as small as possible. Methodological background genome recovery approach reduces the chance of insertion of an additional segment into the background of any adopted variety. Selecting modern high-yielding cultivars, instead of wild types as a donor could also accelerate the whole recovery process. As a result, the lines generated from this study can be used as a donor parent in the future, as its agronomic characteristics were better than other donors.



Fig. 5: Panicle characters of parents and BILs .

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