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Original Article

Characterization of chromosomal segment substitution lines developed in the genetic background of rice variety K 343

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Abstract

In this study, BC_3F_2 convergent population [(K343*3/RML22 × K343*3/DHMAS) × K343] was constructed by marker-assisted backcross breeding using K343 as the recurrent parent. DHMAS and RML22 were used as donor parents for the rice blast resistance genes *Pi54* and *Pi9*, respectively. The population was first characterized using GGT 2.0 software, which showed 96.7% of the recurrent genome recovery covering 13953.6 cM, while DHMAS and RML22 showed 1.6% (235.5 cM) and 1.2% (177.1 cM) introgression respectively. The chromosomal segment substitution lines (CSSLs) were then identified using CSSL Finder software. A total of 36 CSSLs were identified, including 22 for DHMAS/K343 and 14 for RML22/K343. Introgression rates for donor substituted segments in DHMAS/K343 CSSLs ranged from 0.54% to 5.99%, with donor coverage of 44.5%, while in RML22/K343 CSSLs, introgression rates ranged from 0.54% to 4.75%, with donor coverage of 24.5%. The identified CSSLs would be a valuable genetic pool and could be used as genomic resources for the discovery and mapping of important genes and QTLs in rice genetic improvement.

Keywords: CSSL; Convergent population; DHMAS; RML22; K343

1. Introduction

Rice (*Oryza sativa* L.), a Gramineae or Poaceae family crop, is a staple food for half the world's population. More than 90 percent of rice is produced and consumed in Asia, with India and China leading the way. As the global population is rapidly increasing and is projected to reach nine billion by the middle of this century, the grain yield of rice must be increased by 70–10% to feed the growing global population [1]. In the year 2020-21, India produced 124.37 million tonnes of milled rice on a land area of approximately 43.7 million hectares [2]. To reach the targeted production levels, the rice grain yield must be increased for food security. Improvements in grain nutritional quality (GQ) traits, on the other hand, have made it very important for most breeding programs around the world to focus on improving rice grain quality. Quantitative trait locus (QTL) mapping in rice has been used for gene discovery and marker-assisted selection. Among various mapping populations, the chromosome segment substitution line (CSSL) population is preferred because it represents a useful genetic resource for QTL detection of complex agronomic traits in plants and can also identify naturally occurring favorable alleles in wild species. Chromosome segment substitution lines (CSSLs) are genetic stocks that represent the donor in the background of a recurrent parent as overlapping segments. Each CSSL should ideally

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have one or two chromosome segments from the donor with maximum recurrent parent genome coverage in the background [3]. In addition to the target genes, CSSL populations exhibit introgression of segments from donor lines; thus, each line in a CSSL library has a specific marker-defined large donor segment [4]. The successful application of novel QTLs in rice improvement via CSSLs is well documented [5, 6,7]. The CSSLs are powerful tools for investigating large-scale gene discovery in wild rice, which could be adapted for rice quality improvement. The successful application of novel QTL in rice improvement via CSSLs is well documented [5,6,7]. In addition, certain distinct morphological characteristics of wild rice that are derived from CSSLs were utilized as useful phenotypic markers in rice breeding programs [8]. Thus, this investigation aimed to develop two sets of CSSLs in the genetic background of the rice variety K343, followed by their characterization in terms of the genomic contribution of donor lines DHMAS and RML 22.

2. Materials and Methods

In the genetic background of the rice variety K 343 (recurrent parent), a BC_3F_2 convergent population (K343*3/RML22 × K343*3/DHMAS) consisting of 146 lines was developed at the School of Biotechnology, SKUAST-Jammu, Jammu and Kashmir (India) by repetitive crossing and backcrossing with the donor parents DHMAS and RML 22 using a marker-assisted backcross breeding strategy. The population had blast resistance genes *Pi54* and *Pi9* introgressed from DHMAS and RML22 varieties, respectively. BC_2F_2 convergent population was backcrossed with the K343 parent, followed by selfing, to form BC_3F_2 convergent population (Fig.1).

2.1. Extraction, quantification, and quality analysis of genomic DNA

DNA was extracted from young leaf samples using the CTAB method [9] and the quality of DNA fragments was evaluated using a 0.8% agarose gel. The samples with bright and intact bands were utilized for future investigations. The absorbance of DNA samples was measured at 260 nm and 280 nm using a Bio spectrophotometer (Eppendorf, Germany). The DNA samples with an OD ratio between 1.6 and 1.8 were used for genotypic analysis.

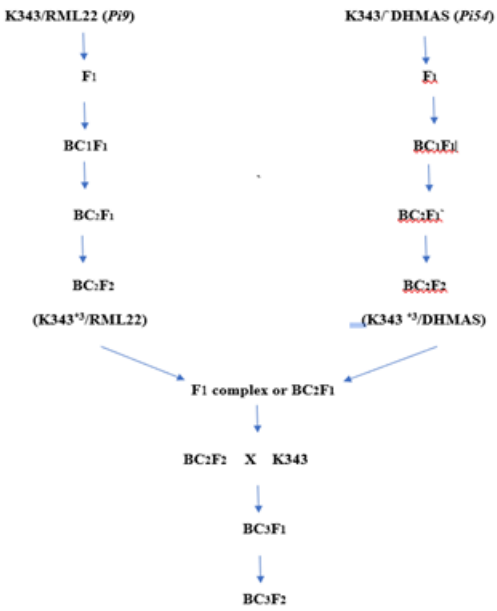

Fig. 1. Scheme for development of two BC_2F_2 genetic stocks and BC_3F_2 convergent population.

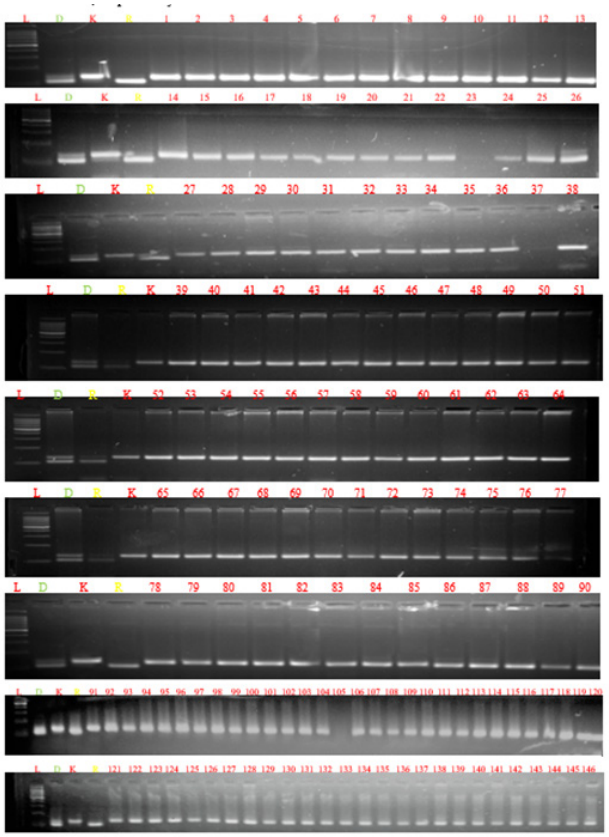

Fig. 2. Band amplification pattern of SSR marker RM 334 (K= K343, D=DHMAS, and R= RML22; 1-146 = Population [BC_3F_2(K343*3/DHMAS/ K343*3/RML 22) × K343]; Red color indicates plants similar to K343, green color and blue color similar to RML22 and DHMAS, respectively.

2.2. Characterization of B_C2F_2 (convergent population)

A total of 110 genome-wide SSR primers (oligos synthesized by Sigma-Aldrich, USA) were used in the study, with 75 primers showing parental polymorphism and being used for CSSL population genotyping. The information regarding the sequences of all of the markers was obtained from the database of SSR markers found on the website www.gramene.org [10]. The SSR allelic bands for each plant in the BC_3F_2 convergent population were counted and manually scored as "A" for similarity to DHMAS, "B" for similarity to K 343, "C" for similarity to RML 22, "H" for heterozygous bands, and "-" if no band was present. The sizes of the bands were estimated by comparing them to a 100bp or 50bp standard marker ladder, as well as their parents (Fig. 2). The graphical representation of molecular marker data was made using the computer program GGT 2.0. [11].

2.3. Selection of a subset of CSSLs

The CSSLs were then identified using the CSSL Finder software (http://mapdisto.free.fr/CSSLFinder/) in the BC_3F_2 convergent population. The software is utilized to select a subset of introgressed lines that cover the entire

donor genome. CSSL Finder (http://mapdisto.free.fr/CSSLFinder/) determines the size of the introgressed segments, the percentage of the donor genome, and the number of introgressed segments per chromosome, followed by the graphical genotyping analysis of the selected lines using GGT 2.0. software [11].

2.4. Statistical analysis

The genotyping data obtained was analysed using CSSL Finder software for identification of DHMAS-K 343 and RML 22-K 343 CSSLs. The molecular data with respect to BC_3F_2 convergent population and CSSLs was analysed for graphical representation using GGT 2.0 [11].

3. Results

The BC_3F_2 convergent population was characterized using GGT 2.0. software showing a graphical representation of all the individual plants chromosome-wise as depicted in Fig. 3, where blue chromosomal segments represented homozygous regions of the recipient parent genome, i.e. K 343, and red and light blue chromosomal segments represented homozygous genomic regions of the donor parents, DHMAS and RML 22, respectively. The statistical analysis revealed a 96.70 percent genomic background of the K 343 parent with a coverage of 13953.6 cM.; and a 1.6 percent genomic introgression from one donor parent, i.e DHMAS, with a coverage of 235.5 cM. Likewise, approximately 1.2% of the total genome, or 177.1 cM, was contributed by the other donor parent, RML 22. About 0.4 percent of the genome, or 64.9 cM, belonged to the heterozygous regions.

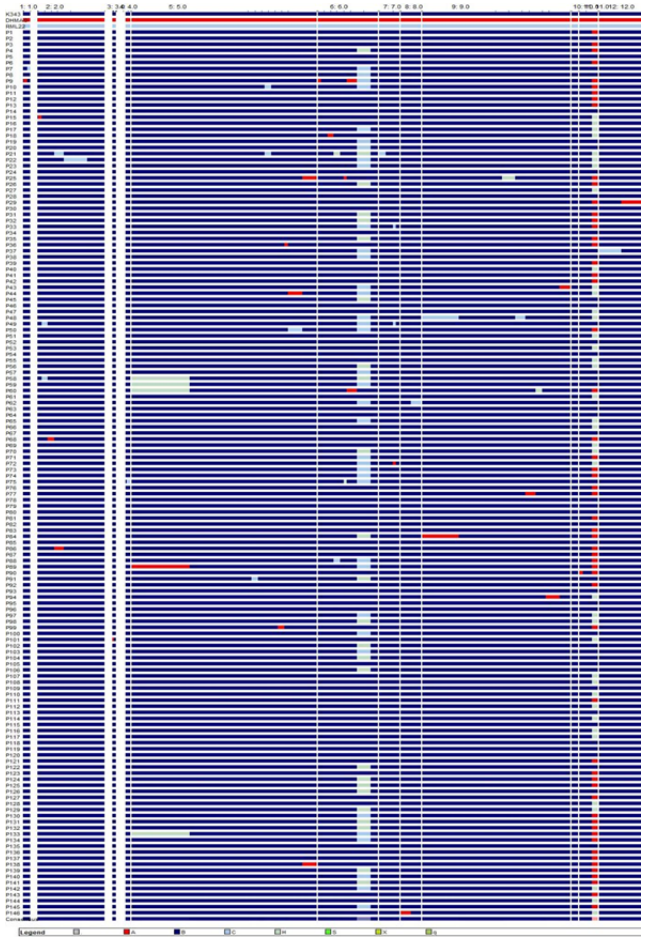

Fig. 3. Graphical genomic representation of BC_3F_2convergent population.

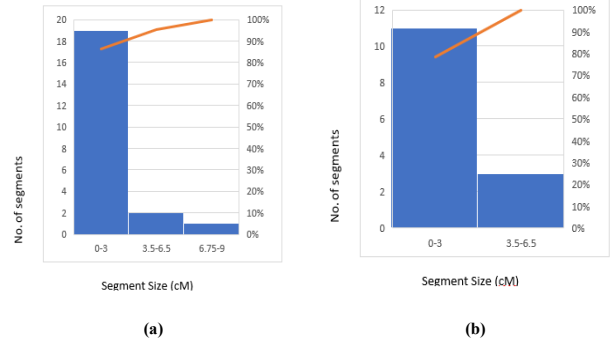

Fig. 4. Distribution of chromosomal substituted segments (a) DHMAS-K343 CSSL population (b) RML22-K343 CSSL population.

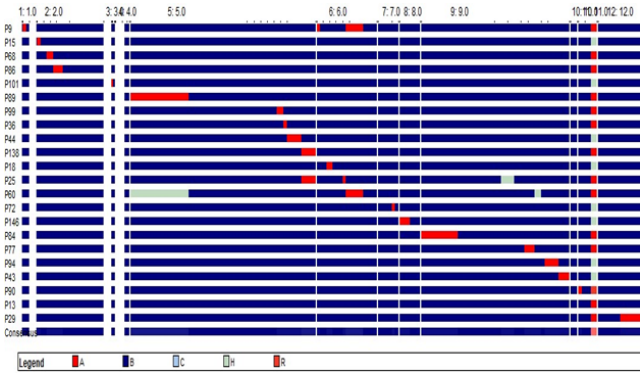

Fig. 5. Graphical genomic representation of 22 DHMAS-K343 CSSLs (blue- K343 genomic region, red - DHMAS introgressed segments, green-heterozygous genomic regions).

Fig. 6. Graphical genomic representation of 14 RML22-K343 CSSLs (blue- K343 genomic region, light blue – RML22 introgressed segments, green-heterozygous genomic regions).

Characterization of BC_3F_2 convergent population using CSSL Finder led to identification of two different subsets of CSSL populations. Subset A contains 22 lines (DHMAS-K343 CSSLs) and subset B contains 14 lines (RML22-K343 CSSLs). Subset A covered 2.57 percent of the donor genome and 96.80 percent of the recurrent parent genome (Fig.5). Subset B covered 97.66 percent of the recurrent parent genome and 2.26 percent of the donor genome, as shown in Tables 1 and 2, while only 0.6 percent of the genomic region corresponded to heterozygous loci (Fig. 6). The donor genome introgression in the CSSL population ranged from 0.54 percent to 5.99 percent in subset A and 0.54 percent to 4.75 percent in subset B. A shown in Table S1 and Table S2, the donor introgressed segments were, on average, 2.05 cM and 1.88 cM in length and covered 41.33 percent and 33.22 percent, respectively, of the A and B CSSL subsets (Fig. 4 a & b). The introgressed donor segments in the K343 genome were specified by SSR markers

Table 1. Distribution and characterization of chromosomal substituted segments of DHMAS-K343 CSSL population.

S. No.	Line	Chromosome no.	Markers specifying introgressed segments	Best segment size (cM)	Donor parent genome (%)	Recurrent parent genome (%)
1	8	1	RM 201	0.56	4.90	95.20
2	15	2	RM 263	0.56	0.54	99.46
3	68	2	RM 221	1.08	1.04	98.96
4	86	2	RM 110	1.63	2.56	97.44
5	102	3	RM 16	0.55	1.51	98.49
6	84	5	RM 18105	9.87	9.57	90.43
7	100	5	RM17990	1.13	1.09	99.91
8	37	5	RM 274	0.57	1.53	98.47
9	44	5	RM 304	2.45	3.35	96.65
10	138	5	RM 413	2.45	3.35	96.65
11	18	6	RM 541	1.09	2.20	98.96
12	19	6	RM 330	0.56	1.04	93.81
13	60	6	RM 276	2.91	3.10	85.95
14	72	7	RM 160	0.56	0.54	99.46
15	146	8	RM 3120	1.74	1.68	98.32
16	85	9	RM 242	6.18	5.99	94.01
17	79	9	RM 3808	1.71	1.66	98.34
18	94	9	RM 219	2.38	2.47	97.69
19	43	9	RM 3	1.82	2.31	97.54
20	96	11	RM 286	0.56	0.54	99.46
21	25	11	RM 206	1.01	0.98	99.02
22	28	12	RM 19	3.73	4.60	95.40
Average				2.05	2.57	96.80

Table 2. Distribution and characterization of chromosomal substituted segments of RML22-K343 CSSL population.

S. No.	Line	Chromosome	Markers specifying introgressed segments	Best segment size (cM)	Donor parent genome (%)	Recurrent parent genome (%)
1	5	1	RM 310	0.56	0.54	99.46
2	49	2	RM 525	0.56	0.54	99.46
3	58	2	RM 221	1.10	1.85	96.55
4	21	2	RM 110	1.63	2.22	97.85
5	22	2	RM 240	3.99	3.45	97.78
6	76	4	RM 335	1.10	4.68	95.52
7	91	5	RM 18080	1.13	1.09	98.91
8	9	5	RM 3321	1.13	1.09	98.91
9	50	5	RM 304	2.45	2.37	97.63
10	86	6	RM 588	0.57	0.55	98.47
11	33	7	AP5930	0.53	0.51	99.49
12	60	8	RM 3496	1.96	3.98	96.02
13	48	9	RM 242	5.89	4.78	95.22
14	27	12	RM 202	3.76	4.00	96.00
Average				1.88	2.26	97.66

RM 201, RM263, RM221, RM110, RM16, RM18105, RM17990, RM274, RM304, RM413, RM541, RM330, RM276, RM160, RM3120, RM242, RM3808, RM219, RM3, RM286, RM206 and RM19 in the DHMAS-K343 CSSL population while the introgression of donor genome segments in the RML-K343 CSSL population was determined by SSR markers RM 310, RM 525, RM 221, RM 110, RM 240, RM 335, RM 18080, RM 3321, RM 304, RM 588, AP5930, RM 3496, RM 242 and RM 202.

4. Discussion

In the present study, the BC_3F_2 convergent population, which consisted of 146 lines and was characterized using the GGT 2.0 software, displayed parent genome recovery of 96.7%. In the genetic background of the variety K 343 of the BC_3F_2 convergent population, 22 lines exhibited the characteristics of a CSSL with segments substituted from the donor parent DHMAS (K343-DHMAS), whereas 14

lines exhibited the characteristics of a CSSL with segments substituted from the donor RML22 (K343-RML22). The introgressed segments were located on distinct chromosomes using flanking markers that were closely linked. In K343-DHMAS, CSSL-substituted segments were distributed throughout the genome except for chromosomes 4 and 10, whereas in K343-DHMAS, CSSL-substituted segments were not detected on chromosomes 3, 10, and 11. In both CSSL populations, however, significant genomic introgressions from donor parents could be observed.

Chromosome segment substitution lines (CSSLs) are important genetic resources that represent the donor genome as overlapping segments within the genetic background of a recurrent parent. Ideally, each CSSL has one chromosome segment from the donor with maximum recurrent parent genome coverage in the background [3]. A CSSL population is preferable to other populations because it allows for the precise identification of genes/QTLs controlling complex traits, as well as the identification of new breeding lines. CSSLs serve as a superior backcross population that can effectively reduce the interaction between QTLs, thereby enhancing the detection ability, facilitating fine-mapping, and cloning QTLs [12]

CSSLs have been used to clone novel genes and QTLs associated with rice grain quality traits [7]. Recently developed CSSLs with 192 (87%) lines revealed fewer substitution segments, allowing to investigation of reliable QTLs of complex characteristics [13]. According to previous studies [14,15,16], 95 percent, 92 percent, and 99.67 percent of recurrent parent genomes were recovered in backcrossed populations. Although CSSLs with greater chromosomal substitution or genomic introgression from donor parents have been produced [17,3], their findings are comparable to those of the current study. Similarly, a CSSL population with average donor introgression of 2% was developed [18] and it was observed that substituted segments of donor parent (Yangdao 6) constituted on an average of 5% of the whole rice genome [19]. Three sets of CSSLs with greater coverage of substituted segments were developed [8] using various donor-recipient combinations. In another study, 93.37% of the whole donor genome was covered [20]. In the present study, a low to moderate percentage of total donor coverage was observed, which could be increased by employing a greater number of markers and a larger population size [21,22,23]. The developed genetic resources could be precisely characterized and implemented in breeding programs.

5. Conclusions

The CSSLs demonstrated large-scale gene recovery and would be valuable genomic resources for identifying QTLs for agronomically important traits. The doner coverage and introgression of CSSLs could be modulated by adjusting markers that are employed. The developed CSSLs would be a potential source for exploring novel genes and QTLs for the quality improvement of rice. A comprehensive characterization of CSSLs may result in the discovery of novel breeding lines.

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Conflict of Interest

The authors declare no conflict of interest.

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