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AGRONOMIC CHARACTERIZATION AND BACKGROUND SELECTION OF BC3F1 INPARI-30 × CABACU RICE LINES USING SSR MARKERS FOR DROUGHT AND SUBMERGENCE TOLERANCE

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SUMMARY

Rice with extensive adaptation is needed in maintaining rice production. Breeding through backcrossing between the recurrent parent (RP) cultivar 'Inpari-30' and the donor parent (DP) 'Cabacu' was carried out to obtain new varieties tolerant to drought and submergence stresses. Cabacu was confirmed to carry QTL *qRPF 2.1, qGPP 2.1,* and *qSPP 4.1*, and Inpari-30 had *Sub1*, which were related to drought and submergence. Backcross breeding is time-consuming but can be shortened by using marker-assisted backcrossing (MABC) selection method. In the present study, MABC was carried out in background selection aimed at obtaining individuals with a high percentage of genomic recovery from the RP supported by agronomic trait observation. The population used was BC3F1 lines that were selected for foreground selection. Agronomic characterization was carried out after the generative phase. The 61 selected polymorphic microsatellites were used to genotype 10 lines and were scattered on all 12 linkage groups. Agronomic characterization showed that line D21.18 had similar agronomic characters as those of the parent cultivar Inpari-30 and root characters as those of the DP genotype 'Cabacu'. The percentage of the obtained recurrent parent genome (RPG) recovery ranged from 84.10% to 95.30%. The highest percentage of RPG recovery was observed in line D21.18 (95.30%). Therefore, rice line D21.18 can be used as the next selection material for further investigation in rice breeding programs.

Keywords: Background selection, SSR marker, genome recovery

Key findings: The selected lines with four pyramided QTL provided a high percentage of recurrent parent genome recovery supported with its agronomic character.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple foods in Asia. The biggest problem of rice cultivation is reduced productivity by abiotic stress that can be caused by climate change. Rice is a seasonal plant that is optimally grown on land with enough water availability (Wu *et al*., 2017). Drought conditions will adversely affect growth and reduce rice yield (Zhang *et al*., 2018). Similarly, floods or submergence caused by heavy rains can occur at any stage of crop growth and affect the decreasing productivity of rice in Asia, particularly in Southeast Asia (Ismail *et al*., 2013). Drought and submergence are considered as opposing abiotic stresses based on water status (Rahman and Zhang, 2016). Genetic improvement can be done to obtain new rice cultivars. Combining drought and submergence water stress tolerance traits with yield and desirable economic traits is the most promising solution (Sabar *et al*., 2019).

The backcross breeding scheme begins by crossing between drought-tolerant varieties and commercial varieties that have the submergence tolerance trait. INPARI-30 is lowland rice that is used as a recurrent parent (RP) because it has high productivity, good taste, and high yield and is favored by farmers (Sudarto *et al*., 2018). Lowland rice usually has a submergence trait that is largely controlled by a major gene, which is designated *Sub1* (Xu *et al*., 2004). Cabacu is upland rice that is chosen as a donor parent (DP) because it is tolerant of drought. This variety has QTL related to the characteristics of drought stress tolerance, such as *qLRS1.1* (leaf rolling rate), *qRPF2.1* (root pulling force), *qGPP2.1* (grains per panicle), *qSPP4.1* (spikelet per panicle), *qDTH8.1* and *qDTH10.1* (days to heading), and *qPH1.1* (plant height) (Trijatmiko *et al*., 2014).

Conventional backcross breeding relying on the observations of morphological characters is time-consuming, and its accuracy is relatively low. Molecular markers can be used (instead of morphology) in assisting backcross breeding to improve efficiency and accuracy and shorten selection time (Hasan *et al*., 2015). A marker-assisted background selection approach can recover up to 99% of the RP genome (RPG) in just three backcross cycles, whereas conventional breeding takes up to six backcrosses to recover 99% of the RPG. The marker-assisted backcrossing (MABC) method consists of three stages, namely, foreground selection, recombinant selection, and background selection. The use of molecular markers in background selection increases selection efficiency and selection precision and reduces the number of linkage drags compared with conventional selection (Fatimah *et al*., 2014). Background selection on rice has been previously carried out, such as selection for the identification of salt-tolerant rice genotypes (Alam *et al*., 2012), the introgression of three dominant blast resistance genes into an aromatic rice cultivar Mushk Budji (Khan *et al*., 2018), the background selection of early-maturing rice lines and high Ciherang

productivity (Prasetiyono *et al*., 2008), and marker-assisted background analysis for pyramiding bacterial blight resistance genes into Basmati rice (Baliyan *et al*., 2018).

Some populations of BC3F1 lines selected from foreground selection need to be further selected by background selection. Several polymorphic SSR markers are used in background selection, and it was hoped that the obtained selected lines will be confirmed to have most of the genes of the RP. Several observations of morphological characters were also carried out to support the selection of lines. The selected lines will be used in the next breeding stage to obtain new varieties as expected.

MATERIALS AND METHODS

This research was done from November 2018 to February 2019 in the Molecular Biology Laboratory and glasshouses of The Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development, Bogor, Indonesia.

Plant material

The plant material consisted of the *indica* rice (lowland) variety Inpari-30 as a RP and (upland) Cabacu as a DP with drought tolerance genes. Crosses were made between Inpari-30 and Cabacu, and the obtained F1 plants were backcrossed with Inpari-30 (Fatimah *et al*., 2018) until the third generation (BC3F1). Ten progeny lines of BC3F1 selected based on agronomic performance and foreground selection and confirmed to carry QTL *qRPF 2.1, qGPP 2.1, qSPP 4.1*, and *Sub1* were planted in the glasshouse under normal conditions.

Morphological observation

Some important agronomic characters, such as plant height (cm), panicle length (cm), panicle number, number of filled and unfilled grains, 100-grain weight (g), total grain weight, number of roots, root length (cm), and dry root weight (g) were observed. Plant height was measured at ±90 days after seedling (DAS) or after the vegetative stage. Plant height was measured from the soil surface to the tip of the tallest panicle (awns excluded). The number of filled grains, number of unfilled grains, weight of 100 grains, and weight of total grain were measured after grains were separated from panicles when the rice had been harvested (±130 DAS). The number of filled grains and the number of unfilled grains were counted manually with three repetitions. The number of roots, root length, and dry root weight were destructive observation. Root length was measured from the root base to the longest root tip of the primary root. The dry root was dried at 50 °C in an oven.

DNA extraction and PCR amplification of SSR markers

Two hundred mg of leaves from 3–4-week-old rice plants were taken for DNA isolation by following the methods of Doyle and Doyle (1990). A total of 3 µL of DNA samples mixed with 1 µL, 7.5 µL of My Taq Bioline, and 1.5 µL of primers were amplified with SSR markers using Thermo Cycler BioRad 100 TM. The template DNA was initially denatured at 94 °C for 5 min, followed by 34 cycles of PCR amplification with the following conditions: denaturation at 94 °C for

45 s, annealing at 55 °C for 45 s, 1 min of primer extension at 72 °C followed by final extension at 72 °C for 10 min.

The amplified PCR product was resolved through electrophoresis on 8% polyacrylamide gel, stained with silver nitrate and 0.5 mg/mL ethidium bromide in 1.0× TBE buffer, and visualized under UV light. A total of 61 simple sequence repeat (SSR) polymorphic primers, which were distributed on 12 rice chromosomes, were used in this study. The positions of the SSR markers used in this study were based on Temnykh *et al*. (2001).

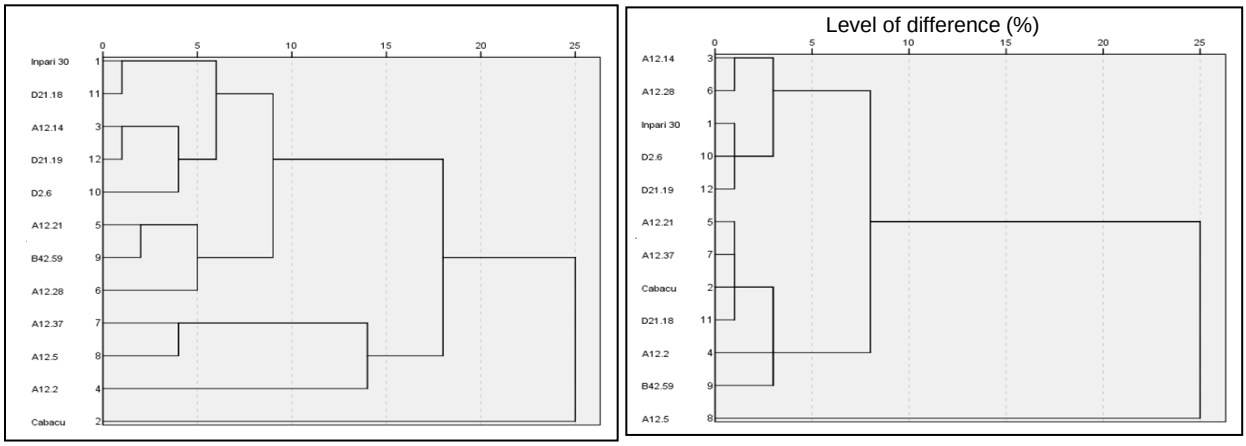
Data analysis

The data for morphological character observation were analyzed using SPSS version 25 and Microsoft Excel 2010 software for cluster analysis. For background analysis, 61 polymorphic SSR markers were used for the assessment of the relative contribution of the two parental genomes to the pyramided genotypes. Graphical genotypes version 2.0 (Berloo, 2008) software was used for the assessment of the genomic contribution of the parent to the selected genotypes based on SSR marker data. The scoring of the DNA band was carried out by comparing the progeny band with the two parent bands. There were three patterns of bands, namely, homozygous with Inpari-30 band (A), homozygous with Cabacu band (B), and heterozygous with both parent bands (H). The analysis of background recovery was done on a set of 10 selected BC1F3 lines in comparison with the RP Inpari-30.

RESULTS AND DISCUSSION

Cultivar Inpari has lower plant height and panicle length than Cabacu. On the other hand, Cabacu higher number of filled grains than Inpari, but has higher results for the grain weight and root characters (Figure 1). Therefore, both parents were used for comparison with the BC3F1 line. Inpari-30 is lowland rice that was used as the RP because it has high productivity, good taste, and high yield and is favored by farmers (Sudarto *et al*., 2018).

Plant height is related to biomass production, which affects yield performance. The height of Inpari-30 plants was more than 110 cm, which was moderate statute. Plant height varied from 75 cm to 103 cm among the studied genotypes. The ideal plant height is beneficial for rice lodging, but excessively short plants lead to insufficient growth and affect the yield potential of rice. Rice with a tall stature is harder to harvest than a plant with a short stature (Darsani and Koesrini, 2018). Ideal plant height ranges from 80–100 cm for high yield potential in lowland rice (Peng *et al*., 2008). Genotypes have been confirmed to have the *Sub1* gene from Inpari-30 (from foreground selection). The *Sub1* gene, which is known as a gene that suppresses cell elongation and carbohydrate metabolism, in rice genotypes can increase plant tolerance to complete submergence for 10 days or more (Nugraha *et al*., 2013). Therefore, a rice variety with increased panicle size and weight is considered as a potential high-yielding variety. Inpari-30 had more panicles than Cabacu, but its length was



(a) (b)

Figure 1. Dendogram grouping of BC3F1 lines based on (a) length, number, and root dry weight; (b) all variables except root.

Figure 2. Comparison of the panicle length of Inpari-30 and Cabacu parents with that of BC3F1 lines.

shorter. Figure 2 shows that Inpari-30 had more secondary rachis branches. According to Mo *et al*. (2012), highly positive correlations (>0.95) between secondary rachis branches and spikelet number per panicle have been reported. The results of panicle length varied from 22.20 cm (A12.12) to 28.50 cm (A12.37) among the studied genotypes. In this study, several lines had higher panicle length as represented by the number of grains (Figure 3). For example, A12.37, which had higher panicle length, had a filled grain number of 145 (Table 1).

Grain characteristics are a component that can vary depending on the rice variety. The number of panicles the higher potential yield of rice plants can be obtained. Lines with the highest total filled grain numbers were A12.37 (145 grains), A12.2 (141 grains), D21.18 (128 grains), and A12.5 (127 grains) (Table 1) and were preferred to be selected. New superior varieties ideally have 100–130 grains per panicle (Farmanta and Handoko, 2016). This was supported by the opinion of Ma *et al*. (2006), who stated that the amount of grains for ideal rice plants ranges from 180 grains to 240 grains with more than 85% of filled grains. Photosynthate, which is transported to the panicle, is sufficient for filling the lines, so the percentage of contents will be increased.

Some lines showed total grain weights that were more than those of both parents, namely A12.2, A12.5, and D21.18, which were selected. The increase in total weight in some progenies was due to the presence of a *transgressive* segregation effect caused by differences in genomes or the far genetic distance between *indica* and *japonica*. *Transgressive* segregation results in progenies that have a better appearance than both parents (Wang *et al*., 2015). 100-grain weight can be influenced by the size of the grain. The grain with a large size will have a heavier 100 grain weight than that with a small size. The BC3F1 lines were expected to have large grains similar to Cabacu. As shown in Figure 4, BC3F1 grains tended to resemble Inpari 30 grains.

The root variable becomes one of the variables related to environmental adaptation. Cabacu is a drought-tolerant rice variety with deep root lengths and positively correlated with the number of roots in penetrating the soil layer (Figure 5) (Suardi, 2002). A MABC study conducted by Fatimah *et al*. (2018) on a BC1F1 Inpari-30 × Cabacu population showed that 32% of these lines have the ability to penetrate the soil, which was similar to their parents. This indicates the existence of the target QTL (*qRPF2.1*). Root strength is influenced by many characters, such as root length, root number (root density), root diameter, and root weight (Comas *et al*., 2013). Many other researchers also reported that under drought stress, genotypes modified their root distribution and penetrated into deeper soil to extract water for their survival and growth (Allah *et al*., 2010 and Lobet *et al*., 2014).

The estimation of background genome recovery using SSR markers has been reported previously. Furthermore, the estimation of RPG recovery (%) may be suggested to be an option for the evaluation of background genome content in backcross-derived lines. The comparison between DP and RP based on RPG recovery (%) may help to

Table 1. Mean performance of rice parental cultivars and lines for various traits.

Genotypes	Plant height (cm)	Panicle length (cm)	Number of panicle	Number of grains		Weight (g)		Root		
				Filled	Empty	100 grains	Total	Length (cm)	Number (blade)	Dry weight (g)
Inpari-30	85.67	24.24±0.65	10	116±7.51	24±4.93	2.24	22.12	28.60	116	4.14
Cabacu	115.33	25.07±1.44	6	62±13.75	25±9.54	3.04	10.24	34.13	155	9.48
A12.14	88.00	25.20±1.47	6	97±10.12	25±9.17	2.35	10.50	30.10	73	2.23
A12.2	87.00	28.00±3.53	17	141±6.03	28±9.17	1.94	29.60	33.10	133	14.05
A12.21	87.00	22.20±0.26	12	83±6.56	22±3.06	2.14	15.83	33.50	152	7.07
A12.28	75.00	22.33±2.67	4	95±4.73	22±7.55	2.32	7.64	29.40	81	2.17
A12.37	101.00	28.50±0.26	6	145±23.54	29±14.18	2.46	15.84	33.20	167	8.10
A12.5	90.00	27.63±1.11	9	127±7.21	28±20.07	2.47	26.65	38.40	266	21.30
B42.59	85.00	22.77±1.35	8	62±2.00	23±27.47	2.26	7.97	37.30	111	9.91
D2.6	103.00	25.07±0.55	10	78±9.02	25±13.43	2.26	15.21	26.60	129	5.25
D21.18	91.00	24.40±2.82	12	128±22.37	24±16.7	2.16	28.44	33.50	192	7.23
D21.19	88.00	26.50±2.18	8	105±19.7	27±28.57	2.21	13.86	25.40	150	3.39

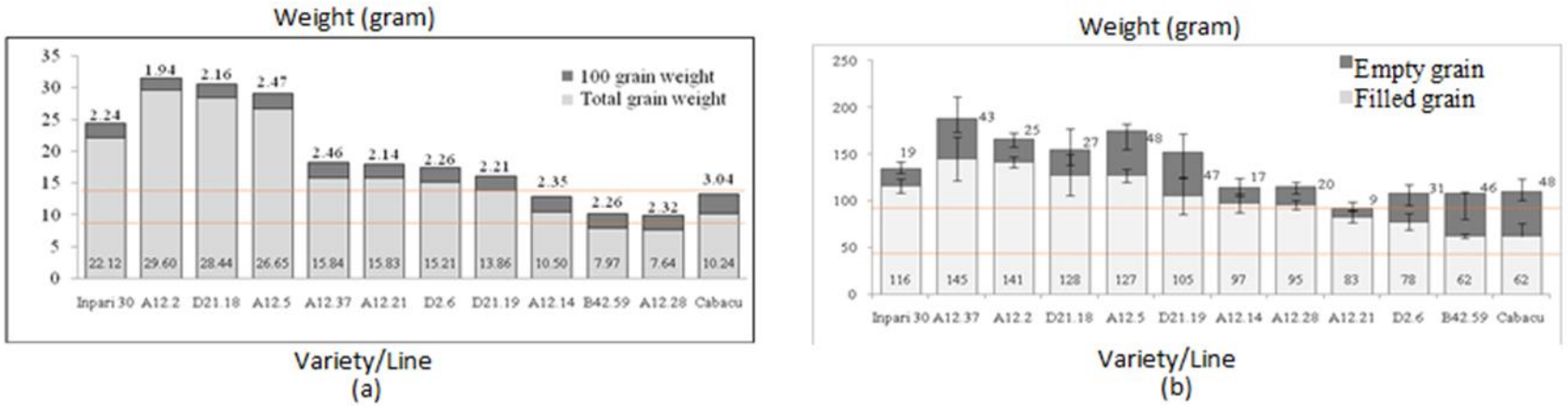


Figure 3a, b. Comparison graph (a) 100-grain weight and total grain weight per panicle. (b) Number of filled and unfilled grains between BC3F1 lines and both parents.

Figure 4. Comparison of grains between selected BC3F1 lines and both parents.

Figure 5. Comparison of roots between Inpari-30 and Cabacu with selected BC1F3lines: (a) A12.2; A12.5; A12.14; A12.21 (b) A12.28; A12.37; B42.59 (c) BC3F1 D2.6; D21.18; D21.19.

decide the necessity of the further advancement of backcross generations in a more accurate manner. Based on Fatimah *et al*. (2018) stated that the amounts of ideal polymorphic primers that can be used in background selection are 10–15 primers in each chromosome. The limited number of polymorphic markers causes many regions with unknown genome composition. Prasetiyono *et al*. (2008) and Holland (2004) suggested that the position of optimum markers used in background selection should range from 10 cM to 20 cM between primers to reduce linkage drag. Polymorphic primers that are increasingly used cause a greater chance of the faster and more precise recovery of the restoring genome (Prasetiyono *et al*., 2008). Background selection was carried out in the areas of the non-target locus on 12 rice chromosomes. There were distant areas, such as areas on 11 chromosomes, that were not reached by the markers that were used in this study as visualized by polyacrylamide gel (Figure 6).

According to Fatimah *et al*. (2014), the majority of the remaining donor genomes are on the chromosomes where the target genes are positioned. This can be caused by the introduction of additional chromosome segments from the donor segment or from the linkage drag on the target chromosome. At several loci, there were still heterozygous segments (Figure 7) that have the chance to obtain a homozygous Inpari-30 locus on the next cross. According to Hospital (2003), the recovery of the genome of the restoring parents in BC3F1 populations carried out with the help of molecular markers reached 92.2%–98.0%; conventionally, the maximum RPG recovery was 93.70% (Figure 8). The RPG result showed that all genotypes had RPG recoveries of 84.10% to 93.50% (Table 2). Therefore, the individual selected for the next selection was D21.18. This is also supported by data on agronomic characterization. D21.18 individuals have agronomic characters that were most similar to the Inpari-30 parent. QTL is generally influenced by a group of genes (polygenic), which affect different variations in the phenotype (Novembre and Barton, 2018).

Table 2. Percentage of RPG recovery in various rice genotypes.

No.	Line Number	Percentage of RPG recovery (%)
1	A12.2	89.20
2	A12.5	84.10
3	A12.14	89.90
4	A12.21	84.10
5	A12.28	85.70
6	A12.37	87.80
7	B42.59	88.80
8	D2.6	89.00
9	D21.18	95.30
10	D21.19	92.60

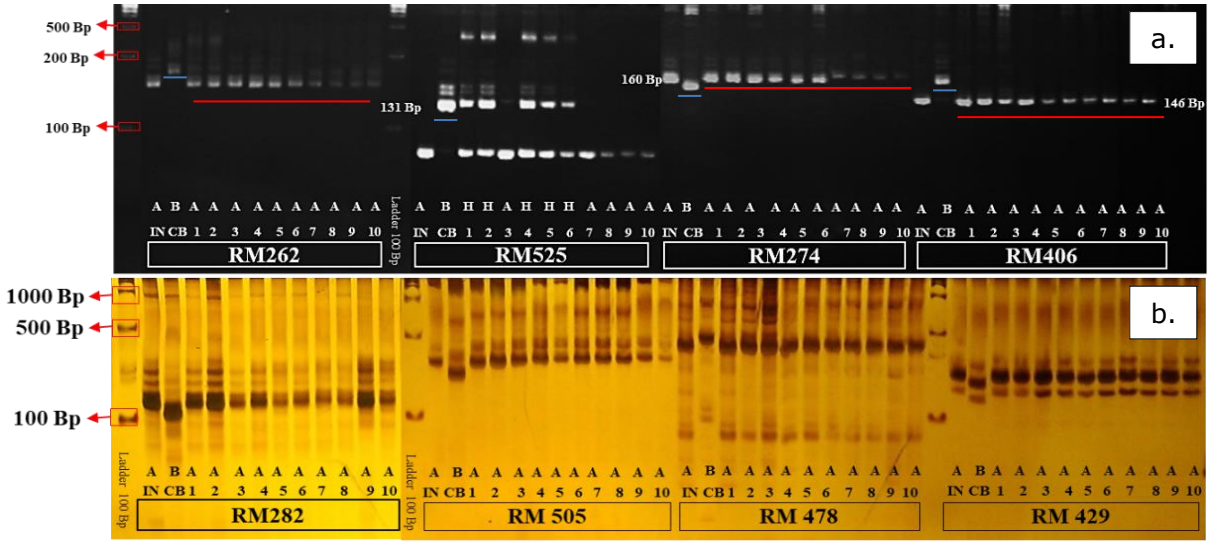


Figure 6. Results of the visualization of DNA bands using 8% polyacrylamide gel electrophoresis (a) EtBr staining (b) silver nitrate staining. IN: Inpari-30; CB: Cabacu; 1: A12.2; 2: A12.5; 3: A12.14; 4: A12.21; 5: A12.28; 6: A12.37; 7: B42.59; 8: D2.6; 9: D21.18; and 10: D21.19. Heterozygote (H), Homozygous RP (A) and Homozygous DP (B).

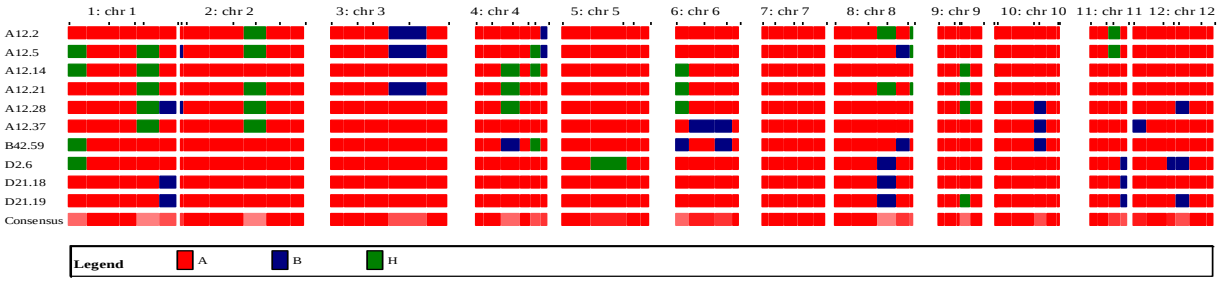


Figure 7. Genotype visualizations of 10 BC3F1 lines on 61 polymorphic marker loci distributed on 12 chromosomes. Red color: homozygous genotype with Inpari-30 RP, blue color: homozygous genotype with Cabacu DP, green color: heterozygous genotype with both parents. Chr: chromosome; cM: centiMorgan.

CONCLUSION

The rice line D21.18 had the most similar agronomic characters as the parent cultivar Inpari-30 (RP), and its root characters were similar to the those of its parent genotype Cabacu (DP) as inferred from the dendogram. Line D21.18 has a good performance based on agronomic characters that were compared with the ideal type. The highest percentage of genomic recovery (95.30%) was found in BC3F1 population of line D21.18. Therefore, the rice line D21.18 can be used as the next selection material in future rice breeding programs.

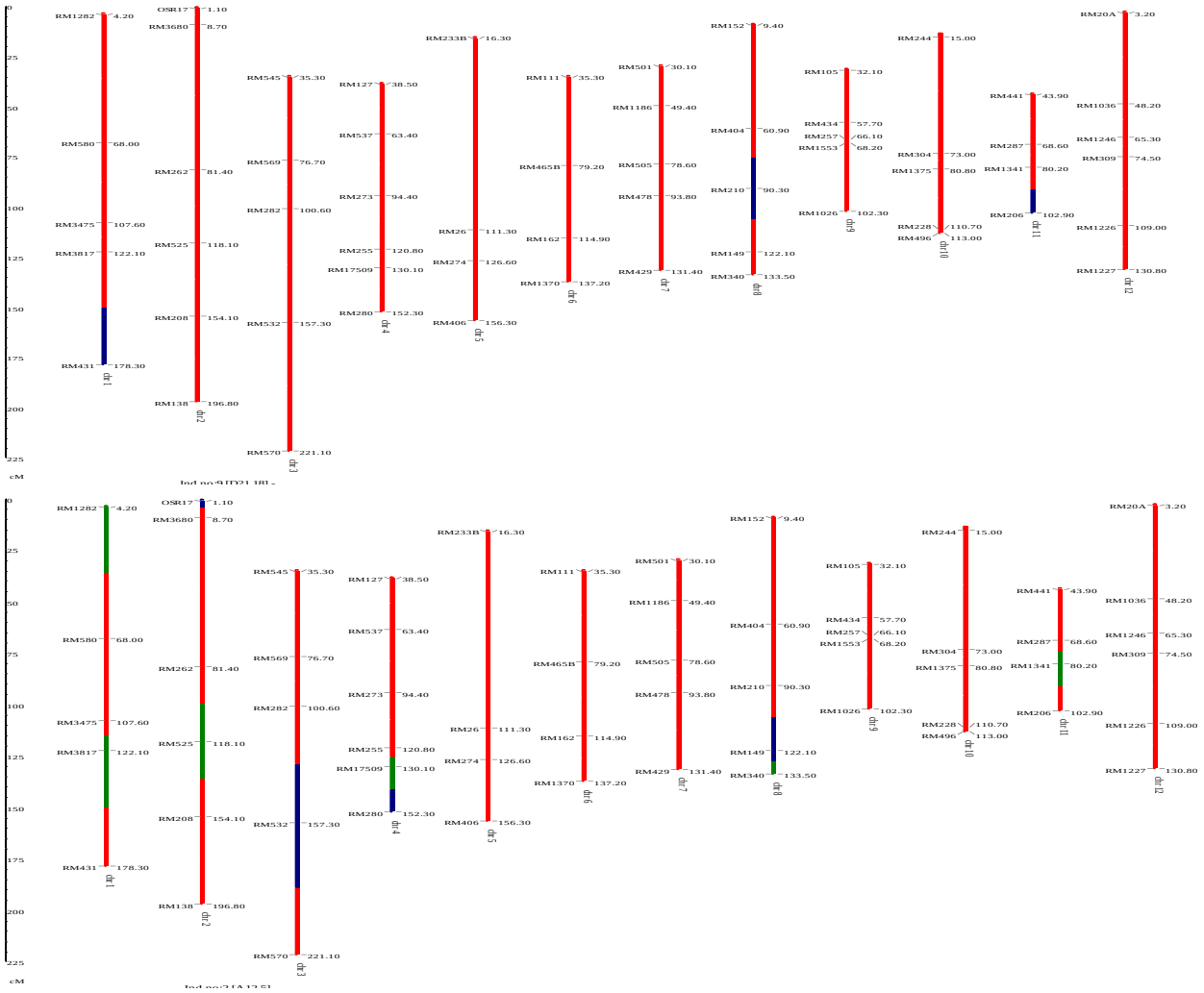


Figure 8. Visualization of the genotype of the BC3F1 (a) D21.18 (95.30%) (b) A12.5 (84.10%) with 61 polymorphic markers loci spread on 12 chromosomes (map of the Cornell SSR 2001 position [www.gramene.org]). Red color: homozygous genotype with Inpari-30 RP, blue color: homozygous genotype with Cabacu DP, green color: heterozygous genotype with both parents. Chr: chromosome; cM: centiMorgan.

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