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Marker-assisted pseudo-backcross breeding for improvement of amylose content and aroma in Myanmar rice cultivar Sinthukha

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ABSTRACT: Breeding for consumers preferring grain qualities has become a chief goal for rice breeding programs in the world. Amylose content (AC) and aroma are important qualities for consumers' preference and market price. To introgress the alleles of waxy (Wx^b) and fragrance (*badh2*) genes into Sinthukha, a widely adaptable high-yield rice variety in Myanmar which has intermediate AC and non-aroma, RNP20-145-1-9 line was used as a donor parent, and pseudo-backcross breeding was designed to shorten the backcross program. In this approach, only one backcross (BC_1F_1) and one self-pollinated (BC_1F_2) population was generated to select for the plants with donor alleles of waxy and fragrance genes in foreground selection, and the selected plants were screened for the highest percentage of recurrent genome content (%RGC) in background selection by amplified fragment length polymorphism (AFLP) analysis. The progenies with the highest %RGC, 84% and 92% were selected in BC_1F_1 and BC_1F_2 populations, respectively, and these selected plants possessed heterozygous alleles in fragrance and waxy genes. The agronomic and yield performance, grain size and shape of selected BC_1F_2 plants were most similar to those of Sinthukha. Nevertheless, amylose content of the selected plants was as low as that of RNP20-145-1-9 rice line. In this study, marker-assisted pseudo-backcross approach was useful in the introgression of low amylose and aroma genes from RNP20-145-1-9 line into Sinthukha, and it could accelerate backcross breeding program through the combination of marker-assisted foreground and background selections. AFLP analysis could save not only time consumption but also the cost of analysis and workload in background selection.

KEYWORDS: marker-assisted pseudo-backcross, waxy gene, fragrance gene, amylose content

INTRODUCTION

Rice is a major food crop for more than fifty percent of the world's population and one of the major economic crops around the world. Rice grain quality usually consists of four categories, i.e. appearance quality, cooking and eating qualities, milling quality and nutritional quality [1]. Among them, consumers pay more consideration on appearance and eating and cooking qualities than other qualities in rice. The rice with long slender grain, soft texture and pleasant aroma gets higher value than normal rice in domestic and international markets, even though consumers' preference on rice quality varies from one group of consumers to the others within country and among countries. AC is the chief criterion for cooking and eating qualities of milled rice [2]. The amylose synthesis needs a granule-bound starch synthase (GBSS) which is coded by the waxy (*Wx*) gene located on chromosome 6 of rice [3]. There are two wild-type alleles, Wx^a and Wx^b, prevailed at the waxy locus of the cultivated rice. Only one amino acid is different between gene products of Wx^a and Wx^b, and it is probable that their specific actions are similar [4, 5]. The functional alleles Wx^a and Wx^b are related to high AC (22–29%) and low AC (12–19%), respectively [5, 6]. Generally, geneticists and breeders widely accepted that high AC is controlled by a single dominant major gene, together with certain minor genes and/or modifiers [7, 8]. From ancient time, aromatic or fragrant rice has been grown mostly in south and southeast Asian countries. The scent or natural fragrance in the rice

kernel is the high valued quality factor. The most popular aromatic rice are Jasmine rice and Basmati rice from Thailand and India, respectively [9]. The most forceful compound of fragrance in Basmati and Jasmine-type aromatic rice is 2-acetyl-1-pyrroline (2AP) that imparts popcorn-like odor [10, 11]. 2AP has been isolated from all aerial parts of the rice plant, but it has not been found in the roots [10]. Recently, an eight base pair deletion and three SNPs in exon 7 of the gene (*Badh2*) encoding betaine aldehyde dehydrogenase 2 (BAD2) on chromosome 8 of *Oryza sativa* was recognized as the probable cause of aroma in Jasmine and Basmati style rice. A deletion in the gene encoding BAD2 on chromosome 8 results in a frame shift that generates a premature stop codon and presumably disables the BAD2 enzyme which is the most likely cause of fragrance in rice [12]. The aroma in Basmati and Jasmine type rice is controlled by a recessive trait [13] which results mainly from the occurrence of the higher levels of 2AP compound in all aerial parts of the rice plant.

Rice is a staple food crop and also one of the main economic crops in Myanmar. It is extensively cultivated throughout the country in all agroclimatic environments. Sinthukha or IR Yn1068-7-1 (Rc2010-195) is one of the widely grown rice varieties in Myanmar because of its high yield, non-photosensitivity, widely adaptability and moderate resistance to bacterial leaf blight (BLB) disease compared to other varieties (Department of Agricultural Research, Myanmar). It was obtained by the crossing of Manawthukha and IR BB 21 to improve BLB disease resistance [14]. Many rice varieties with good grain quality and aroma in Myanmar are low yield, low tiller number, high sterility, photoperiod sensitive, poorly responsive to fertilizer and susceptible to lodging due to their tall stature. Nowadays, consumers in Myanmar and most Asian countries increase the preference on long slender grain with good eating qualities and pleasant aroma. Since the demand of good quality and aromatic rice is increasing among consumers, recently, the development of new rice cultivars combining high yield potential and superior grain quality becomes the foremost priority for rice breeding programs in Myanmar.

Up to now, there are very limited breeding successes to develop high-yield and good grain quality varieties by conventional breeding methods, owing to the complex nature of grain quality traits and environmental factors. Marker-assisted backcrossing (MABC) is an efficacious technique for plant breeding programs, and presently this technique is efficiently and extensively utilized in actual molecular plant breeding. Contrary to the conventional backcross method, MABC is the process of using markers associated with or linked to interested gene(s)/QTL(s) to choose the target loci, diminish the donor segment length comprising a target locus and/or hasten the recovery of the genome content of recurrent parent throughout the backcrossing procedure [15, 16]. There are two types of selection in MABC: foreground selection (to assess the presence of target gene in BC progenies) and background selection (to hasten the recovery of genetic background of recurrent parent excluding the target gene(s)) [17]. Pseudo-backcross breeding, a modified form of marker-assisted backcross breeding, was used to shorten the backcross breeding program by combination of foreground and background selections with the aid of markers [18]. The original pseudo-backcrossing design comes from tree breeding methods in order to elude inbreeding depression [19], and it is frequently used in perennial crops such as oil palm [20] and grapes [21]. Ruengphayak et al [18] firstly described the utilization of pseudo-backcross approach in rice to shorten the time needed for gene/QTL pyramiding significantly. Hence, this research reported the useful application of pseudo-backcross approach to accelerate backcross breeding program through the combination of foreground and background selections with the assistance of molecular markers for the improvement of low amylose content and aroma in Sinthukha rice variety by using RNP20-145-1-9 rice line which is one of the popular aromatic rice lines in Thailand as a donor parent.

MATERIALS AND METHODS

Plant materials

Sinthuka is a high-yield and photoperiod insensitive rice variety, and it is widely adaptable for every agroclimate condition including rain-fed lowland and irrigated areas in Myanmar. It also has intermediate AC (23–25%), non-fragrance and moderate resistance to BLB disease. RNP20-145-1-9 line is one of the aromatic rice lines that possesses photoperiod insensitivity, long slender grain, low AC (15–19%) and good eating and cooking qualities [22]. To improve low AC and aroma in Sinthukha, RNP20-145-1-9 rice line was used as a donor to transfer the alleles of waxy (Wx^b) and fragrance (*badh2*) genes into Sinthukha genetic background.

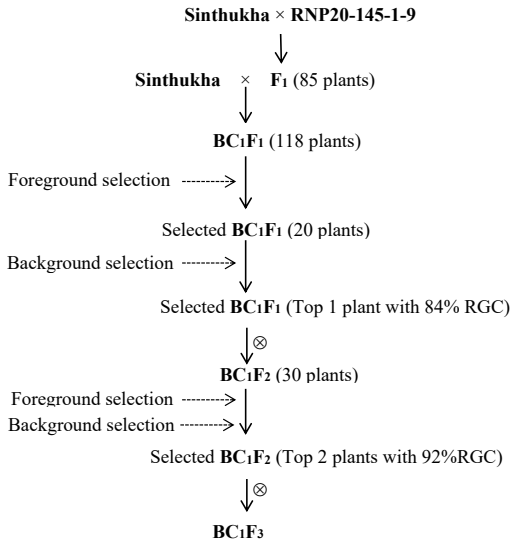

Fig. 1 Marker-assisted pseudo-backcross breeding scheme for gene introgression of low AC and aroma into Sinthukha.

Development of pseudo-backcross lines

Initially, Sinthukha rice variety was crossed with RNP20-145-1-9 line to develop F_1. The F_1 plants were checked to identify F_1 hybrids by using Naro 1 marker and then the F_1 plants that possessed heterozygous alleles of certain marker were backcrossed to Sinthukha to obtain BC_1F_1 population. Consequently, foreground selection was performed in BC_1F_1 and BC_1F_2 generations to select the plants that carried heterozygous alleles of two markers (Naro 1 and OSR19) for donor genes. In BC_1F_1 and BC_1F_2 generations, the plants which had the highest genomic recovery of the Sinthukha variety were selected by AFLP analysis as shown in Fig. 1.

Markers used

In foreground selection, Naro 1 and OSR19 (RM190) markers were utilized in this research (Table 1). The functional Naro 1 marker developed by Rattanapol et al [23] based on the position of eight base pair deletion of exon 7 in the *Badh2* gene located on chromosome 8, which is associated with the aromatic trait in rice, was used to separate aromatic and non-aromatic alleles. The gene specific OSR19 marker, CTn microsatellite marker, positioned in the 5′ untranslated region (UTR) of exon 1 in waxy gene on chromosome 6 was applied to distinguish waxy alleles (Wx^a/Wx^b) [24].

PCR analysis

Foreground selection was based on polymerase chain reaction (PCR) analysis using Phire® Plant Direct PCR Kit (Thermo Fisher Scientific, Inc.) without DNA isolation as described in manufacture's protocol. In brief, 0.35 mm punched leaf disc from Harris Uni-Core was put into 10 µl PCR reaction with 1 µl of target forward and reverse primers. Before starting the PCR, one drop of mineral oil was put to the samples to inhibit evaporation during PCR process. Reaction was amplified 40 cycles with condition of 98 °C for 5 s, 58 °C for 5 s, 72 °C for 20 s and final incubation at 72 °C for 1 min to complete the extension of primer. After completing the PCR process, 5.0 µl of loading dye buffer was added. The polymorphism of PCR amplified products was detected by silver nitrate ($AgNO_3$) staining after the electrophoresis on 6% polyacrylamide gel had been finished. Finally, the polymorphic bands were scored, and Chi-square test was used to examine the segregation ratio of the progenies in the DNA analysis with respective markers.

AFLP analysis

AFLP analysis using ten primer combinations was performed in background selection to evaluate the recurrent genome recovery of individuals in each population. The leaf samples were sent to DNA Technology Laboratory, Kasetsart University (Kamphaeng Saen), Nakorn Pathom, Thailand for analysis. Ten primer combinations with three selective nucleotides in each primer were used in selective amplification. AFLP analysis was operated as described by Vos [25] with some modifications. Initially, genomic DNA (100 ng) was digested for 3 h at 37 °C to a final volume of 25 µl with 10 units of *Eco*RI and 10 units of *Mse*I in 1 × R/L restriction/ligation buffer (33 mM Tris-HCl, pH 7.5, 10 mM potassium chloride, 0.5 mM DTT). To this mixture, 10 µl of ligation mix containing 7.5 pmol adapter for *Eco*RI and 75 pmol adapter for *Mse*I, 1.2 units T4-DNA ligase, 1.2 mM ATP and 1 × ligation buffer was added. The ligation reaction was performed at 37 °C for 3 h after which a DNA template was prepared by diluting DNA with $10 \times dH_2O$. From the resulting digestion-ligation mixture (DNA template), 3 µl was used for PCR pre-amplification by adding 0.25 mM of primer, 1.5 mM $MgCl_2$, 1 × *Taq* buffer, 200 mM dNTPs, and 0.3 units of *Taq* DNA polymerase, in a final volume of 10 µl. The thermal conditions for PCR were: 24 cycles of 30 s at 94 °C, 1 min at 56 °C and 1 min at 72 °C. A GeneAmp® PCR System 9700

Table 1 Molecular markers utilized in foreground selection.

Marker	Type	Detection	Trait	Sequence forward (5′–3′)	Sequence reverse (5′–3′)	Ref.
OSR19	Specific marker (SSR)	(CT)n	amylose content	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	[38]
Naro 1	Functional marker (Indel)	8-bp	aroma	AGGTTGCATTTACTGGGAG	TGGCTACTAGAATGATGCT	[23]

(Applied Biosystem) was used.

For selective amplification, a template was made from 2 µl of pre-amplification products and a mixture of 0.25 µM of primer *Mse*I, 0.25 µM of primer *Eco*RI, 1 × Taq buffer, 1.5 mM $MgCl_2$, 200 mM dNTP, and 0.3 units *Taq* DNA polymerase (Euroclone) to a final volume of 10 µl. The following PCR conditions were observed and the annealing temperature was reduced every cycle by 1 °C: 29 cycles of 30 s starting at 94 °C down to 65 °C and a further 1 min at 72 °C. In the next stage, a further 30 cycles for 30 s at 94 °C, 30 s at 56 °C, 1 min at 72 °C was operated and hold at 4 °C until the reaction was complete. It was stopped by adding the 5 µl of loading buffer (10 mM EDTA pH 8.0, 98% formamide, Bromophenol Blue & Xylenecyanol). Selective PCR was performed in A GeneAmp® PCR System 9700 (Applied Biosystem). Amplified fragments were separated by 4.5% (w/v) polyacrylamide gel electrophoresis and silver staining. The DNA bands were visualized by autoradiography and manually scored for their presence or absence. The clear fragments (bands) were scored with 1 indicating the corresponding fragment of Sinthukha (RP) and 2 representing the fragment of RNP20-145-1-9 line (DP), and the number of bands that had similar fragments of Sinthukha from each progeny was counted and calculated to estimate the %RGC of individuals.

RESULTS

Development of pseudo-backcross lines

The F_1 plants generating from the cross of Sinthukha and RNP20-145-1-9 line were identified as hybrids using functional Naro 1 marker, and among the total eighty-seven F_1 plants, the eighty-five heterozygous plants (Fig. S1) were backcrossed to Sinthukha to create BC_1F_1. In a total one hundred eighteen BC_1F_1 plants, the fifty-six plants which had the heterozygous alleles of the Naro 1 marker for aroma were stepwise screened by OSR19 marker for waxy gene, and twenty plants with heterozygous alleles for both markers were selected. These twenty selected BC_1F_1 plants were profiled by AFLP markers for background selection to pick out the plants that had highest %RGC. From this AFLP analysis, the topmost plant which possessed 84% RGC was chosen and self-pollinated to obtain BC_1F_2. In BC_1F_2 population, the total of thirty plants were simultaneously screened by foreground and background selections by Naro 1 and OSR19 markers and through AFLP analysis, respectively. In the foreground selection, ten plants with homozygous alleles of Naro 1 (*Badh2*/*Badh2*) and/or OSR19 (Wx^a/Wx^a) markers as Sinthukha were discarded. From the AFLP profiling, two plants with the highest percent of RGC (92%) which also occupied the heterozygous alleles of Naro 1 (*Badh2*/*badh2*) and OSR19 (Wx^a/Wx^b) markers for AC and aroma were selected.

Evaluation of agronomic and yield component traits

The agronomic and yield component traits of BC_1F_2 plants were recorded from individual plants by comparing with two parents. The important agronomic traits and yield components of the top five BC_1F_2 plants with highest %RGC and low AC and fragrance genes were shown by comparing with two parents in Table 2 and Table 3, respectively.

Evaluation of grain quality in BC_1F_2 population

For grain quality traits, AC, grain size and grain shape of the BC_1F_2 plants were recorded by phenotypic evaluation compared with two parents. AC was determined by following the procedure of the method of Juliano [26] with some modifications. The fragrance was not examined based on phenotypic evaluation since almost all of the selected BC_1F_2 plants had the heterozygous alleles of Naro 1 marker. Hence, the fragrance may not be determined in heterozygous condition because the fragrance might be phenotypically detected only in homozygous recessive alleles. The AC percentage and grain size and shape of top five BC_1F_2 plants and two parents were shown in Table 4 and Table 5, respectively.

Foreground selection

The functional Naro 1 marker and the gene specific OSR19 marker were implemented in foreground selection for fragrance and waxy genes, respectively. These two markers showed polymorphism between

Table 2 Important traits of top five BC_1F_2 plants and two parents.

Variety/line	%RGC	50% flowering (day)	Plant height (cm)	Flag leaf length (cm)	Panicle length (cm)	No. tillers
29-27	92	115	110	29	27	12
29-28	92	115	115	32	28	13
29-18	90	115	108	32	26	12
29-17	87	114	107	33	26	14
29-22	87	109	99	29	23	13
Sinthukha	100	110	110	48	24	13
RNP20-145-1-9	0	103	114	50	30	10

Table 3 Major yield component traits of top five BC_1F_2 plants and two parents.

Variety/line	No. panicles (per plant)	Total grains (per panicle)	No. filled grains (per panicle)	Filled grain (%)	Grains weight (g/100 grains)	Total grain weight (g/plant)
29-27	11	183	86	47.0	1.5	14.4
29-28	13	195	30	15.4	1.4	5.5
29-18	12	211	7	3.3	1.5	1.3
29-17	14	178	57	32.0	1.4	11.5
29-22	13	154	31	20.1	1.5	5.9
Sinthukha	12	181	100	55.2	1.4	16.4
RNP20-145-1-9	10	122	13	10.7	2.2	2.8

Table 4 Amylose content percentage (AC %) of top five BC_1F_2 progenies by comparing with two parents.

Variety/line	OSR19 allele	AC (%)	AC Class
29-27	Wx^a/Wx^b	16.0	Low
29-28	Wx^a/Wx^b	17.7	Low
29-18	Wx^a/Wx^b	18.8	Low
29-17	Wx^a/Wx^b	25.0	Medium
29-22	Wx^a/Wx^b	17.6	Low
Sinthukha	Wx^a/Wx^a	22.2	Medium
RNP20-145-1-9	Wx^b/Wx^b	18.4	Low

Wx^a, allele of OSR19 marker as Sinthukha.
Wx^b, allele of OSR19 marker as RNP20-145-1-9 line.

Table 5 Grain size and shape of top five BC_1F_2 progenies compared with two parents.

Variety/line	Paddy grain		Brown rice grain				
	L	W	L	W	L/W	Size	Shape
29-27	8.6	2.1	6.5	1.8	3.7	M	S
29-28	8.5	1.9	6.5	1.7	3.8	M	S
29-18	8.8	2.1	6.5	1.7	3.8	M	S
29-17	9.0	2.1	6.3	1.7	3.6	M	S
29-22	8.7	2.1	6.1	1.8	3.5	M	S
Sinthukha	8.5	2.1	6.4	1.8	3.6	M	S
RNP20-145-1-9	11.0	2.0	7.7	1.7	4.5	EL	S

L, length (mm) ; W, width (mm);
EL, extra-long; M, medium; S, slender.

two parents and were used to select the plants which possessed donor alleles of RNP20-145-1-9 line in fragrance and waxy genes in BC_1F_1 and BC_1F_2 generations. Stepwise screening was used in BC_1F_1 population because of its large population of more than one hundred individuals. The total of one hundred eighteen BC_1F_1 progenies revealed that the sixty-two plants had homozygous alleles (*Badh2/Badh2*) which were similar to Sinthuka and the fifty-six plants had heterozygous alleles (*Badh2/badh2*) of Naro 1 marker. It was segregated as the expected Mendelian segregation ratio (1:1) by Chi-square analysis. The fifty-six heterozygous plants were picked out for screening with OSR19 marker. Among these plants, the seven plants died because of brown plant hopper infestation and only forty-nine heterozygous plants were used to identify by OSR19 marker. Among those forty-nine plants, eight plants did not show any band, and twenty-one plants showed homozygous alleles (Wx^a/Wx^a) as Sinthukha, and twenty plants with heterozygous alleles (Wx^a/Wx^b) of OSR19 marker were selected for background analysis. It was also segregated with Mendelian pattern (1:1 ratio). In BC_1F_2 population, the total of thirty plants were analyzed by Naro 1 and OSR19 markers simultaneously. In this analysis, the alleles of two markers were segregated independently, and observed frequencies were conformed to the expected frequencies of unlinked dihybrid cross (9:3:3:1 ratio). Ten plants which had homozygous alleles of Naro 1 marker (*Badh2/Badh2*)

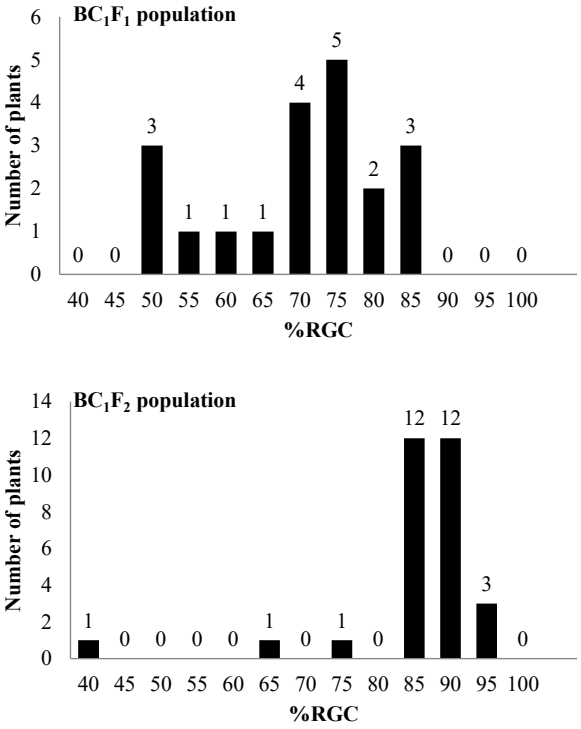

Fig. 2 Frequency distribution of percentage of recurrent genome content (%RGC) in BC_1F_1 and BC_1F_2 populations.

and/or OSR19 marker (Wx^a/Wx^a) which were corresponding with non-aroma and intermediate AC as Sinthukha variety were excluded.

Background selection

Ten primer combinations were applied in the AFLP analysis for the background selection in BC_1F_1 and BC_1F_2 populations. Each primer combination produced the clear fragments between 14 and 40 in BC_1F_1 generation and between 17 and 38 fragments in BC_1F_2 generation, from which 2–8 and 4–11 fragments of each primer combination showed polymorphism between two parents in BC_1F_1 and BC_1F_2 populations, respectively. These represented overall 20% and 24% of total polymorphic fragments, and approximately 51 and 60 fragments were scored in BC_1F_1 and BC_1F_2 progenies, respectively. The total number of fragments similar to Sinthukha in each progeny ranged from 24–43 fragments in BC_1F_1 population and 23–55 fragments in BC_1F_2 population which were the representatives of the range of %RGC from 47–84% and 38–92% in BC_1F_1 and BC_1F_2 generations, respectively (Table 6 and Fig. 2). The plant no. 29 with highest %RGC (84%) was selected in BC_1F_1 population, and it was self-pollinated to generate BC_1F_2. The plants no. 29-20, 29-27 and 29-28 were the highest percentage of recurrent genome content (92%) and had similar plant type as Sinthukha in BC_1F_2 population, but the plant no. 29-20 was not healthy.

Table 6 Percentage of recurrent genome content (%RGC) in BC_1F_1 and BC_1F_2 populations.

BC_1F_1 generation			BC_1F_2 generation		
Plant no.	NFS	%RGC	Plant no.	NFS	%RGC
2	35	69	29-1	49	82
4	34	67	29-2	45	75
11	35	69	29-3	52	87
14	37	73	29-4	23	38
16	24	47	29-5	38	63
17	24	47	29-6	54	90
18	24	47	29-7	50	83
23	36	71	29-8	51	85
29	43	84	29-9	51	85
30	37	73	29-10	51	85
42	39	76	29-11	52	87
65	36	71	29-12	51	85
67	41	80	29-13	51	85
68	42	82	29-14	53	88
80	30	59	29-15	52	87
81	40	78	29-16	49	82
85	28	55	29-17	52	87
92	34	67	29-18	54	90
105	31	61	29-19	54	90
115	37	73	29-20	55	92
			29-21	52	87
			29-22	52	87
			29-23	51	85
			29-24	50	83
			29-25	53	88
			29-26	53	88
			29-27	55	92
			29-28	55	92
			29-29	50	83
			29-30	49	82

NFS, number of fragments similar to Sinthukha (RP).

DISCUSSION

In marker-assisted pseudo-backcross design, two parents, recurrent and donor parents were crossed in the first generation, and after that the plants with donor alleles from RNP20-145-1-9 line and the highest recovery of recurrent genome of Sinthukha were selected in BC_1F_1 and BC_1F_2 generations to hasten the breeding program. This marker-assisted pseudo-backcross breeding approach could select the plants with donor alleles of fragrance (*badh2*) and waxy (Wx^b) genes and high percentage of RP genome (92%) in BC_1F_2 generation which was

similar to theoretical %RGC (93.7%) of BC_3F_1 generation in conventional backcross. So this approach may accelerate backcross program when the marker-assisted foreground and background selections were combined. In conventional backcross, generally six to eight backcross generations are required for full recovery of the genome of RP, and high theoretical %RGC (about 96.9%) may be obtained in BC_4. But in practice, it may sometimes need to perform larger numbers of backcrossing (ten or more). Conversely, the route of the introgression of quantitative trait loci (QTLs)/genes and recurrent genome recovery may be hastened by using the molecular markers in the selection process and/or phenotypic selection simultaneously. MABC with background selection may recover recurrent genome about 98% in BC_3 [17]. From the theoretical stand point, the average percentage of recurrent genome in BC_1F_1 generation is 75% for the whole population whereas actually in BC_1F_1 population, some progenies may occupy more or less of RP genome than average theoretical %RGC (75%) [27]. If the progenies which contained the highest %RGC (more than 75%) were selected in BC_1F_1 population by background selection using markers, it would accelerate the backcross breeding program.

The 50% flowering days, plant height, panicle length and number of tillers of the top five BC_1F_2 plants were similar to those of Sinthukha (RP). But the flag leaf length of the top five plants was shorter than that of the two parents (Table 2). For yield component traits, the top five plants had similar values as Sinthukha in numbers of panicles/plant, total grains/panicle and 100-grain weight. Although the number of filled grains/panicle, filled grain percent and total grain weight/plant of all BC_1F_2 plants and two parents were very low because of the high temperature at the flowering period, these of the topmost plant and Sinthukha were higher than those of RNP20-145-1-9 rice line and other plants (Table 3). Therefore, the top five BC_1F_2 plants were most similar to Sinthukha in many agronomic and yield component characters.

In the AC analysis, Sinthukha variety had medium AC (22.2% AC), and RNP20-145-1-9 line had low AC (18.4% AC). The AC of top five BC_1F_2 plants ranged from 16.0–25.0% with the representative classes from low AC to medium AC. By comparing with genotypic and phenotypic evaluation, the BC_1F_2 plants with heterozygous alleles of OSR19 markers (Wx^a/Wx^b) were segregated into two groups comprising four progenies with low AC as RNP20-145-1-9 and one progeny (plant no.29-17) with medium AC as Sinthukha (Table 4). In the previous studies, it has been stated that high AC is incompletely dominant to low AC and is controlled by one major gene and several modifiers [28], and the transgressive segregation was observed in F_2 populations derived from low AC and intermediate AC parents [7].

In the measurement and classification of grain size and shape, the brown rice of RNP20-145-1-9 line was extra-long slender grain with the values of 7.7 mm length and 4.5 length/width ratio. Sinthukha was medium slender grain type with 6.4 mm length and 3.6 length/width ratio. The length of brown rice in top five BC_1F_2 progenies ranged from 6.1–6.5 mm and the length/width ratio ranged from 3.5–3.8. Hence, the grain size and shape of top five BC_1F_2 progenies were medium slender grain as in Sinthukha (Table 5).

MABC without background selection necessitates at least three to four backcrosses to secure a higher recovery of the phenotype of recurrent parent [29–31]. Background selection can significantly hasten the backcross program when compared with traditional backcross method [27]. In this study, the %RGC in the progenies of BC_1F_1 comprised 47–84% by background selection using AFLP analysis although the average percentage of RGC in BC_1F_1 population was less than the theoretical %RGC (75%) in conventional backcross. After selecting the highest %RGC (about 84%) in BC_1F_1 population, the recovery percentage was increased in BC_1F_2 progenies to 92%. By comparing the frequency distribution of %RGC in BC_1F_1 and BC_1F_2 populations, the larger number of plants had been between 70% and 85%RGC in BC_1F_1, but after selecting the plant with the highest %RGC (84%) for BC_1F_2 generation, the largest number of progenies had been shifted to between 85% and 95% (Fig. 2). Finally, the progenies which consist of the highest %RGC (92%) were chosen only in BC_1F_2 population, and it was the best efficiency of background selection with the assistance of molecular markers. A low background recovery rate has been described in previous studies. The %RGC was only 82% in BC4F7 progeny in the introgression of stripe rust resistance in wheat [32], 74.50–81.30% in the pseudo-BC_3F_3BILs of the pyramiding multiple traits in PinK3 rice variety [18] and 84–93% in BC_4F_2 of the improvement of cooking quality traits in Manawthukha rice variety [29] without marker-assisted background selection. Nonetheless, the %RGC was enhanced to 85–92% in BC_3 in wheat through

the combination with phenotypic selection [33–35]. Conversely, background selection using SSR markers in marker-assisted pseudo-backcross breeding to improve high amylose in CH1 rice variety [36] could select the plants which possessed 97.7% RGC in BC_1F_2 by using large enough population size (more than 200 plants), and it was observed on a single gene (waxy gene). But this study focused on two genes (fragrance and waxy genes), and the population size was not quite large, only 20 and 30 progenies in BC_1F_1 and BC_1F_2 populations, respectively. The efficacy of marker-assisted backcrossing relies on many aspects, including the distance of the target locus and the markers used, the size of the population of every backcross generation and the number of utilized background markers [27]. The minimal population sizes increase exponentially through the number of target genes and turn out to be one of the most limiting factors [17]. Through the use of AFLP analysis in the background selection, many loci were analyzed simultaneously, so it could save time consuming and reduce the cost of analysis and workload. Since it needed no DNA sequence data in the AFLP analysis and genome abundance of these markers was high, AFLP analysis was easy to use in background profiling [37].

CONCLUSION

The plants with high recovery of recurrent genome (92% RGC), donor alleles of fragrance and waxy genes from RNP20-145-1-9 rice line were selected in BC_1F_2 generation in this study using the foreground selection together with background selection through the assistance of molecular markers. The plant type, agronomic and yield performance of the selected BC_1F_2 plants were most similar to those of Sinthukha. Even though the grain size and shape of the selected plants were also medium slender grain as in Sinthukha, AC of the selected plants were as low as that of RNP20-145-1-9 rice line. This marker-assisted pseudo-backcrossing was designed to shorten the breeding program, and it needed only one backcross and successive self-pollinated generations, and the highest %RGC was selected in BC_1F_1 and BC_1F_2 populations. Therefore, this design was useful in the introgression of low AC and soft aroma from RNP20-145-1-9 rice line into Sinthukha background and could accelerate the backcross breeding program. AFLP analysis could save not only time consuming and workload but also the cost of analysis in background selection. If the population of each generation was large enough, it would select higher recovery of recurrent genome than 92% in BC_1F_2 population.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2020.070.

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Appendix A. Supplementary data

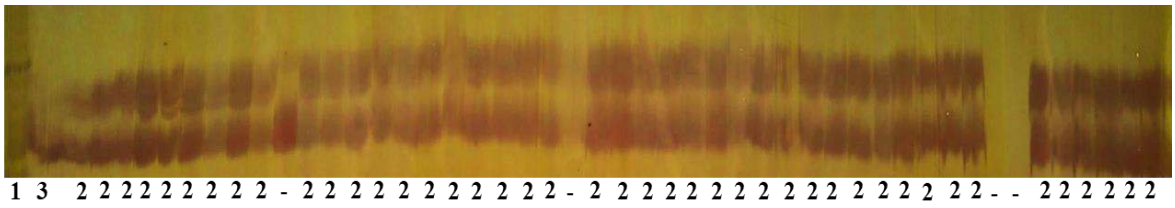

Fig. S1 Identification of F_1 population by using Naro 1 marker. 1, Naro 1 marker of homozygous dominant (*Badh2/Badh2*) alleles; 2, Naro 1 marker of heterozygous (*Badh2/badh2*) alleles; and 3, Naro 1 marker of homozygous recessive (badh2/badh2) alleles.