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

Khin S. Cho\textsuperscript{a,b}, Pasajee Kongsil\textsuperscript{a}, Thanakorn Wangsawang\textsuperscript{a,c}, Tanee Sreewongchai\textsuperscript{a,*}

\textsuperscript{a} Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900 Thailand
\textsuperscript{b} New Plant Variety Protection Section, Department of Agricultural Research, Naypyitaw 15030 Myanmar
\textsuperscript{c} Faculty of Agricultural Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathum Thani 13180 Thailand
\textsuperscript{*}Corresponding author, e-mail: taneesree@yahoo.com, agrtns@ku.ac.th

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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 (\textit{Wx}) and fragrance (\textit{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\textsubscript{1}F\textsubscript{1}) and one self-pollinated (BC\textsubscript{1}F\textsubscript{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\textsubscript{1}F\textsubscript{1} and BC\textsubscript{1}F\textsubscript{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\textsubscript{1}F\textsubscript{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 (\textit{Wx}) gene located on chromosome 6 of rice [3]. There are two wild-type alleles, \textit{Wxa} and \textit{Wxb}, prevailed at the waxy locus of the cultivated rice. Only one amino acid is different between gene products of \textit{Wxa} and \textit{Wxb}, and it is probable that their specific actions are similar [4, 5]. The functional alleles \textit{Wxa} and \textit{Wxb} 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 agro-climatic 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 agro-climate 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 F₁. The F₁ plants were checked to identify F₁ hybrids by using Naro 1 marker and then the F₁ plants that possessed heterozygous alleles of certain marker were backcrossed to Sinthukha to obtain BC₁F₁ population. Consequently, foreground selection was performed in BC₁F₁ and BC₁F₂ generations to select the plants that carried heterozygous alleles of two markers (Naro 1 and OSR19) for donor genes. In BC₁F₁ and BC₁F₂ 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 (Wxa⁺/Wxa⁻) [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 manufacturer’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 (AgNO₃) 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 EcoRI and 10 units of MseI 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 EcoRI and 75 pmol adapter for MseI, 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× dH₂O. 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 MgCl₂, 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
For selective amplification, a template was made from 2 µl of pre-amplification products and a mixture of 0.25 µM of primer MseI, 0.25 µM of primer EcoRI, 1 × Taq buffer, 1.5 mM MgCl2, 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 F1 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 F1 plants, the eighty-five 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 BC1F1 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 BC1F2. In BC1F2 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 (Wxα/Wxβ) 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 (Wxα/Wxβ) markers for AC and aroma were selected.

Evaluation of agronomic and yield component traits

The agronomic and yield component traits of BC1F2 plants were recorded from individual plants by comparing with two parents. The important agronomic traits and yield components of the top five BC1F2 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 BC1F2 population

For grain quality traits, AC, grain size and grain shape of the BC1F2 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 BC1F2 plants had the 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 BC1F2 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

| Marker | Type | Detection | Trait | Sequence forward (5′–3′) | Sequence reverse (5′–3′) | Ref. |
|--------|------|-----------|-------|--------------------------|--------------------------|-----|
| OSR19  | Specific marker (SSR) | (CT)n | amylose content | CTTGTTCATCTCAAGACAC | TGGCAAGAAGTTCCTGATG | [38] |
| Naro 1 | Functional marker (Indel) | 8-bp aroma | | AGGTGGCAATTTAATGGGAG | TGTTGCAATTTAATGGGAG | [23] |
Table 2 Important traits of top five BC$_1$F$_2$ 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$_1$F$_2$ 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$_1$F$_2$ 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$_1$F$_2$ progenies compared with two parents.

| Variety/line | Paddy grain | Brown rice grain |
|--------------|-------------|------------------|
|              | 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$_1$F$_1$ and BC$_1$F$_2$ generations. Stepwise screening was used in BC$_1$F$_1$ population because of its large population of more than one hundred individuals. The total of one hundred eighteen BC$_1$F$_1$ progenies revealed that the sixty-two plants had homozygous alleles (Badh2/Badh2) which were similar to Sinthukha 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$_1$F$_2$ 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)
and/or OSR19 marker \((Wx^c/Wx^d)\) 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\(_1\) and BC\(_2\) populations. Each primer combination produced the clear fragments between 14 and 40 in BC\(_1\) generation and between 17 and 38 fragments in BC\(_2\) generation, from which 2–8 and 4–11 fragments of each primer combination showed polymorphism between two parents in BC\(_1\) and BC\(_2\) populations, respectively. These represented overall 20\% and 24\% of total polymorphic fragments, and approximately 51 and 60 fragments were scored in BC\(_1\) and BC\(_2\) progenies, respectively. The total number of fragments similar to Sinthukha in each progeny ranged from 24–43 fragments in BC\(_1\) population and 23–55 fragments in BC\(_2\) population which were the representatives of the range of %RGC from 47–84\% and 38–92\% in BC\(_1\) and BC\(_2\) generations, respectively (Table 6 and Fig. 2). The plant no. 29 with highest %RGC (84\%) was selected in BC\(_1\) population, and it was self-pollinated to generate BC\(_1\)\(_2\). 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\(_1\) population, but the plant no. 29-20 was not healthy.

### 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\(_1\) and BC\(_2\) 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\(_1\)\(_2\) generation which was
similar to theoretical %RGC (93.7%) of BC$_2$F$_1$ 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 standpoint, the average percentage of recurrent genome in BC$_1$F$_1$ generation is 75% for the whole population whereas actually in BC$_1$F$_1$ 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$_1$F$_1$ 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$_2$F$_2$ 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$_1$F$_2$ 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$_1$F$_2$ 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$_2$F$_2$ 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$_2$F$_2$ plants with heterozygous alleles of OSR19 markers (Wx$^c$/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$_2$F$_2$ 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$_2$F$_2$ 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$_1$F$_1$ comprised 47–84% by background selection using AFLP analysis although the average percentage of RGC in BC$_1$F$_1$ population was less than the theoretical %RGC (75%) in conventional backcross. After selecting the highest %RGC (about 84%) in BC$_1$F$_1$ population, the recovery percentage was increased in BC$_2$F$_2$ progenies to 92%. By comparing the frequency distribution of %RGC in BC$_1$F$_1$ and BC$_2$F$_2$ populations, the larger number of plants had been between 70% and 85%RGC in BC$_1$F$_1$, but after selecting the plant with the highest %RGC (84%) for BC$_2$F$_2$ 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$_2$F$_2$ 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$_2$F$_3$BILs of the pyramiding multiple traits in PinK3 rice variety [18] and 84–93% in BC$_1$F$_2$ of the improvement of cooking quality traits in Manawthukka 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 amylase in CH1 rice variety [36] could select the plants which possessed 97.7% RGC in BC₁F₂ 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 BC₁F₁ and BC₁F₂ 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 BC₁F₂ 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 BC₁F₂ 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 BC₁F₁ and BC₁F₂ 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 BC₁F₂ 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 F1 population by using Naro 1 marker. “1” indicates Naro 1 marker of homozygous dominant (Badh2/Badh2) alleles; “2” indicates Naro 1 marker of heterozygous (Badh2/badh2) alleles; and “3” indicates Naro 1 marker of homozygous recessive (badh2/badh2) alleles.