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IMPROVEMENT OF HIGH AMYLOSE CONTENT IN CH1 RICE VARIETY BY MARKER ASSISTED PSEUDO-BACKCROSS BREEDING

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ABSTRACT

The objective of this research was to introgression of high amylose content into CH1 rice variety by using pseudo-backcrossing breeding .Crossing between CH1 and RD49 was performed to produce F_1 progenies .After that, the progenies will backcross to CH1 to develop BC_1F_1 population .Then, the selected plants from the BC_1F_1 population were continuously selfed to develop BC_1F_2 and BC_1F_3 populations, respectively .For marker assisted selection, the OSR19 DNA marker that is specific to *Wx* gene was used for assisting the selection of plants with high amylose content in foreground selection to choose favorite genotype. Total 67 SSR markers used genetic marker assisted selection was utilized for BC_1F_1 and BC_1F_2 populations .The results showed that selection could be achieved for BC_1F_1 and BC_1F_2 plants having high amylose content and first highest ranking of genetic background similar to recurrent parent exhibited 91 and 97.8 percent, respectively .The marker assisted selection could accelerate in backcross breeding program .The BC_1F_3 seeds of 6 selected lines were planted in rice field for preliminary yield test .It was found that some agronomic characters (Days of 50% flowering, flag leaf length, panicle length, number of tillers per plant and harvest index) and yield of the selected lines were not statistically different from those of CH1 variety and high amylose content as donor parent. Thus, the advantage of the pseudo-backcross method is that it can save not only time and workload, but also the cost of analysis and leaping generations in the normal backcross method. Moreover, genetic similarity results in relatively similar morphological characteristics.

Keywords: CH1, high amylose content, pseudo-backcross breeding, RD49, *Wx* gene

INTRODUCTION

Rice is the main food crop that is consumed within Thailand . In addition, rice is also a very important economic crop for Thailand in 2018 (Saosaovaphak et al., 2022). Yanjie et al. (2018) examined rice with AC ranging from 13 to 20% and found that the correlation of AC with eating quality was positive; this different finding compared to those just cited may be due to the small range of AC in the samples used. Moreover, preference depends on culture (Chen, 2019). For example, it has been reported that consumers in some parts of Vietnam and China prefer low-amylose rice, while people from Philippines, Malaysia, Pakistan and Iran favor intermediate amylose, and high-amylose rice is more popular in Sri Lanka and Myanmar (Calingacion et al., 2014). In Thailand, rice with moderate to high amylose content (AC) is more preferable to eat than rice with low AC (Cruz and Khush, 2000). Qiqnizhan (CH1) rice variety originated from China with high yield potential, new plant type, tall plant and good ability to cross (Rattanapol et al., 2011). AC is one of the most critical criteria determining cooking and eating quality (Juliano, 1971). In fact, AC is primarily a hereditary feature that is governed by the waxy locus (*Wx*) on chromosome 6 (Bao et al., 2008). The *Wx* gene has a polymorphic microsatellite DNA marker with a dinucleotide cytosine-thymine repeat (CT_n) (Bligh et al., 1995).

The novel platform based on pseudo-backcross breeding shortens both multiple foreground selection and background genome recovery. This study proposes a modified form of pseudo-backcrossing to shorten the backcross breeding cycle. Pseudo-backcrossing evolved from tree breeding methods, in which F_1 plants from a single cross are backcrossed to alternate recurrent parents to avoid inbreeding depression (Bouquet, 1986). Within four years, the entire project was completed in seven cycles consisting of a single backcross, two cycles of pseudo-backcrossing, and three cycles of line fixation (Ruengphayak et al., 2015). To ensure a high recovery of the recurrent parent phenotype after transferring multiple resistance genes using conventional marker assisted selection (MAS), at least three to four backcrosses are required (Suh et al., 2013).

Using conventional breeding methods, the donor segment can remain very large even with many backcross generations. Without marker-assisted background selection, the percentage of recurrent genome content (%RGC) in BC$_2$ progeny was only 87.5% (Hasan et al., 2015). However, when combined with MAS and phenotypic selection, % RGC in F$_2$ increased to 93.1% (Wangsawang et al., 2019). Indeed, using genome-wide molecular markers for background screening during backcrossing has been proposed as the most effective method for improving low %RGC (Cho et al., 2020). Although pseudo-backcrossing is the quickest method for gene/QTL pyramiding, it may not be the best breeding platform for creating elite recurrent varieties. However, early-stage marker-assisted genome-wide scanning can be implemented to aid in the reconstruction of favorable genomic backgrounds at the end of the pseudo-backcrossing scheme. Therefore, breeders should consider if they want to shorten the time but incur additional costs or it will take a longer time but cost less than the above method. Finally, the breeders must consider the trade-offs. However, using molecular markers to help in selection may bring additional costs. In an ideal world, new high-throughput, low-cost genome-wide scanning technologies would be used in tandem with skilled breeder selection (Ruengphayak et al., 2015).

The current study's goal was to select new rice lines with high AC using a pseudo-backcrossing breeding method by crossing CH1 with the RD49 rice variety.

MATERIALS AND METHODS

Introgression of Wx^a conferring high AC from RD49 into CH1 by Pseudo-backcross

The pseudo-backcross platform is divided into the RD49, Thai rice with good cooking qualities, a high AC, and higher yields strain was crossed with the rice variety CH1, a new Chinese plant type variety with the potential to provide higher yields (Rattanapol et al., 2011), to develop the F$_1$ generation as shown in Figure 1. Individual F$_1$ plants with heterozygous alleles of specific markers were chosen and backcrossed to CH1 using mixed pollen to produce the BC$_1$F$_1$ generation. Through marker-assisted selection, the BC$_1$F$_1$ progenies were Wx^a/Wx^b allele. The BC$_1$F$_2$ generation was created by self-pollinating of the BC$_1$F$_1$ progenies with top ten highest ranking of genetic background as similar as CH1. The BC$_1$F$_2$ progenies which were homozygous Wx^a/Wx^a allele as RD49 and Wx^a/Wx^b allele were achieved through marker-assisted selection. The BC$_1$F$_3$ generation was created by self-pollinating of BC$_1$F$_2$ progenies with the highest ranking of genetic background as similar as CH1. Six BC$_1$F$_3$ pseudo BILs with Wx^a allele as RD49 were successfully developed.

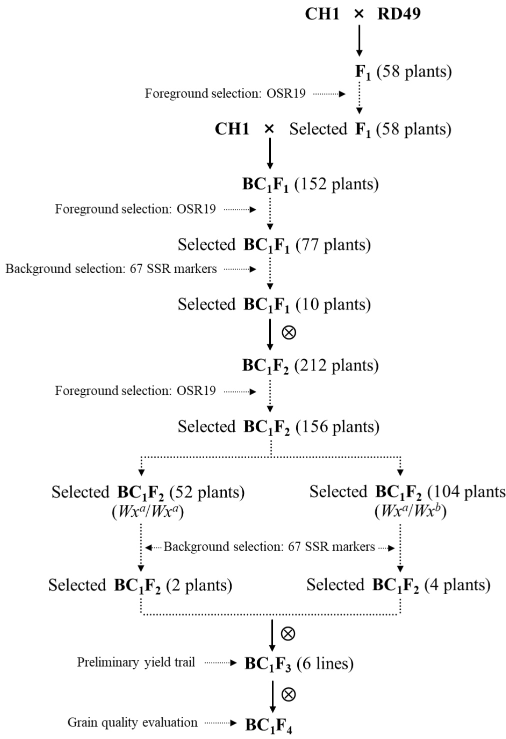


Figure 1. Scheme for the development of high AC using pseudo-backcrossing breeding method through crossing CH1 with the RD49 rice variety.

Genomic scanning of foreground and background

Individual plants carrying the donor allele were identified using marker assisted selection in one backcross generation, BC$_1$F$_2$ generation, and BC$_1$F$_3$ generation. The SSR marker, OSR19 which contain CT$_n$ alleles of the *Wx* gene was used to identify the plants carrying the favorite *Wx* gene (Akagi et al., 1996). The primer pair OSR19F (5'-CTCTCTCACCATTCCTTCAG-3') and OSR19R (5'-GATCTGAATAAGAGGGGAAAC-3') was developed by Sreewongchai et al. (2014). The plants carrying the band which similar with RD49 were selected. The PCR protocol was following the applied method of Rattanapol et al. (2011).

In a genome-wide survey, the 233 SSR markers from known chromosomal positions distributed evenly across the 12 chromosomes were used to identify the polymorphism between the two parents. The 67 polymorphic SSR markers between the two parents were used for the background profiling in BC$_1$F$_1$ generation and BC$_1$F$_2$ generation compared with CH1 (Supplementary table 1). The nucleotides of 67 primers reference in McCouch et al. (2002).

Supplementary Table 1. The 67 SSR markers used for genetic background selection in BC_1F_1 and BC_1F_2 populations.

Primer name	Chromosome	Primer name	Chromosome
RM1	1	RM1054	5
RM129	1	RM334	5
RM283	1	RM5371	6
RM472	1	RM400	6
RM405	2	RM125	7
RM71	2	RM501	7
RM475	2	RM25	8
RM106	2	RM433	8
RM526	2	RM444	9
RM573	2	RM105	9
RM208	2	RM3912	9
RM48	2	RM201	9
RM6	2	RM6791	9
RM545	3	RM215	9
RM7	3	RM258	10
RM251	3	RM222	10
RM563	3	RM3717	11
RM16	3	RM332	11
RM416	3	RM536	11
RM85	3	RM287	11
RM3524	4	RM286	11
RM471	4	RM457	11
RM6341	4	RM6440	11
RM518	4	RM206	11
RM551	4	RM224	11
RM241	4	RM19	12
RM317	4	RM247	12
RM280	4	RM83	12
RM559	4	RM277	12
RM7444	5	RM1261	12
RM598	5	RM519	12
RM87	5	RM463	12
RM437	5	RM235	12

Genomic DNA was extracted from fresh frozen leaves of rice plants using the CTAB method with little modification of Murray and Thompson (1980). The PCR amplifications were carried out using a Phire® Plant Direct PCR Kit (Finnzymes; Keilarata, Espoo, Finland), which was designed to amplify DNA directly from rice leaf samples with little modification (Murray and Thompson, 1980). After electrophoresis on 6% polyacrylamide gels, polymorphism in each PCR product was detected using silver nitrate ($AgNO_3$) staining following (Benbouza et al., 2006).

Field experiments and data collection

At Bang Sai, Phra Nakhon Si Ayutthaya, Thailand, agronomic characteristics and yield performance were studied. The experiment was carried out from June to December, 2017. The experiment was designed with three replications using a Randomized Complete Block Design (RCBD). In the experiment, two rice varieties, RD49 and CH1, were used as controls. The seeds of 6 lines and checks were sown in a seed nursery .One-month-old seedlings were then manually transplanted into the rice field with one seedling per hill .Each plot had five rows, each with seven plants, and a planting density of 20 cm between plants (within a row) and 20 cm between rows. On each experimental plot, fertilizing and field management were explained by Wangsawang et al. (2019). Days of 50% flowering (DOF), plant height (PH), flag leaf length (FLL), panicle length (PL), number of tillers per plant (T/P), harvest index (HI), number of panicles per plant (P/P), number of grains per panicle (S/P), number of filled grain per panicle (G/P), 100-grain weight (GW), and total grain weight per plant (W/P) were measured.

Using the STAR 2.0.1 software, an analysis of variance was performed, and Duncan's multiple range test (DMRT) was used for multiple mean comparisons.

Grain quality evaluation

Rice grains from six lines and their parents were harvested when they reached physiological maturity and naturally dried in a greenhouse. Prior to evaluating the grain quality traits, the dried grains were stored at room temperature for one month. Grain samples weighing 100 g were collected from each replicate and combined. The grain quality test was then conducted using 50 g of mixed samples. A mini polisher was used to mechanically deshell grain samples.

The procedures described by (Lanceras et al., 2000) were used to evaluate AC, gel consistency (GC), and gelatinization temperature (GT). The GC was determined by measuring the length of the grain starch slurry in a culture tube of cold gel; the length of the gel, measured from the bottom of the tube to the front of the gel migration, was measured in millimeters one hour later. The longer gel is thought to be softer than the shorter gel. GT is a cooking time indicator. The alkali spreading value (ASV) was used to estimate the GT indirectly; a larger ASV indicates increased alkali spreading and thus a lower GT, whereas a smaller ASV indicates a higher GT.

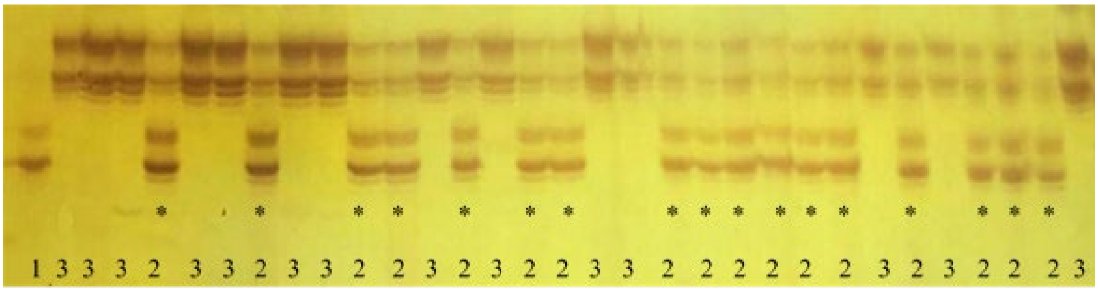
The appearances of the endosperm, as well as the size and shape of the kernel, are morphological characteristics of rice grains. Ten milled rice kernel seeds were measured for length and breadth with vernier caliper, and length/breadth ratios (L/B) were calculated. The appearance of the rice endosperms was determined visually using the procedure described by (Tan et al., 2000).

RESULTS

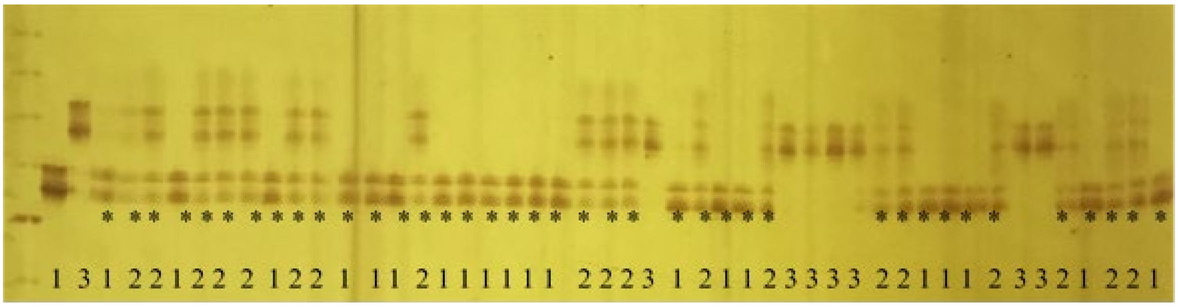
Development of high AC pseudo-backcross inbred lines and marker Assisted Selection

A total of 58 F_1 progenies were produced from the crosses of CH1 and RD49. Each F_1 were backcrossed into CH1 by targeting MAB to generate BC_1F_1 progenies. The BC_1F_1 plants were genotyped using the *Wx* gene specific marker (OSR19). The Wx^a locus for high AC was discovered in RD49. Out of 152 plants, 77 were heterozygous (Wx^a/Wx^b) and 75 were homozygous (Wx^b/Wx^b) as CH1 (Table 1). At a 1:1 ratio, the chi square test was accepted. Hence, only 77 plants were chosen for high AC (Supplementary figure 1). Among 77 plants of BC_1F_1 progenies, 10 plants which had Wx^a/Wx^b allele and highest genetic background similar with CH1 were selected. The 10-BC_1F_1 plants were generated to 212 pseudo-backcrossed BC_1F_2 progenies . Plants carrying the heterozygous Wx^a/Wx^b gene were screened using the OSR19 marker at the seedling stage in the pseudo-BC_1F_2 BILs. The 52 pseudo-BILs were tagged for the homozygous Wx^a/Wx^a gene as RD49, the 104 plants were heterozygous Wx^a/Wx^b, and the 56 plants were homozygous Wx^b/Wx^b gene as CH1 (Table 1 and supplementary figure 2). At a 1:2:1 ratio, the chi square test was accepted. As a result, the 156 pseudo-BILs were examined for genetic background profiling. Using target MAS, 2 fully homozygous (Wx^a/ Wx^a) and 4 heterozygous (Wx^a/ Wx^b) pseudo-backcrossed BC_1F_2 plants were selected and selfed to generate BC_1F_3 progeny. Finally, six pseudo backcrossed inbred lines (pseudo-BILs) carrying the donor gene's positive Wx^a allele were achieved.

Six pseudo-BC_1F_3 BILs were chosen based on their highest genetic background similarity to CH1 for field testing. Entirely, donor and recipient were intensively crossed and selected for five seasons : two seasons to generate BC_1F_1, another two seasons to generate pseudo backcrossed BC_1F_2 and BC_1F_3 populations, respectively, and one season of selfing to fix the final best- selected pseudo-BC_1F_4 BIL for evaluating grain quality.



Supplementary Figure 1. Example of a DNA band obtained by selecting for the high AC in the BC_1F_1 crossing between the RD49 and CH1 using the OSR19 marker.
Note: 1 is Wx^b/Wx^b as CH1, 2 is Wx^a/Wx^b, 3 is Wx^a/Wx^a as RD49, and * is the BC_1F_1 selected plants.



Supplementary Figure 2. Example of a DNA band obtained by selecting for the high AC in the BC_1F_2 crossing between the RD49 and CH1 using the OSR19 marker.
Note: 1 is Wx^b/Wx^b as CH1, 2 is Wx^a/Wx^b, 3 is Wx^a/Wx^a as RD49, and * is the BC_1F_2 selected plants.

Table 1. The use of the OSR19 marker to segregate alleles associated with AC in the BC_1F_1 and BC_1F_2 population.

Genotype	Number of BC_1F_1 plants	Genotype	Number of BC_1F_2 plants
Wx^a/Wx^a (RD49)	0	Wx^a/Wx^a (RD49)	52
Wx^a/Wx^b	77	Wx^a/Wx^b	104
Wx^b/Wx^b (CH1)	75	Wx^b/Wx^b (CH1)	56
Total	152	Total	212
$\chi^2_{(1:1,0.05)}$ test	0.0313^{ns}	$\chi^2_{(1:2:1,0.05)}$ test	0.0188^{ns}

Remarks: ^{ns} = non-significant.

Genetic background of the pseudo-BILs

Background analysis confirmed the presence of substituted chromosome segments in the 77 BC_1F_1 plants. The study was made using genome-wide molecular markers. The 233 SSR markers were used in the background analysis. Marker polymorphism between CH1 and RD49 was 28.76% (67 markers). Each plant contains an SSR marker that defines the recipient's genetic background, CH1. Ten pseudo-BILs were chosen from BC_1F_1 progenies with the highest genetic similarity to CH1. The average genetic background percentage of the plants ranged from 82.09% (Q-6) to 91.04% (Q-8) (Table 2). The expected background genome recovery for these ten plants was 85.72%. In the study, the Q-8 plant inherited the most genetic background from CH1.

The frequency of CH1 alleles at non-target loci for the individual plant with homozygous introduced gene (Wx^a/Wx^a) derived from RD49 ranged from 94.78) %Q-8-2, Q-8-66, Q-8-72 and Q-8-74 (to 97.01) %Q-8-17 (and the genetic background profiling with Wx^a/ Wx^b ranged from 95.52% (Q-8-6) to 97.76% (Q-8-143) after one self-pollination generations BC_1F_2 : pseudo-backcross (Table 2).

Table 2. Genetic background profiling of BC_1F_1 BILs, pseudo-BC_1F_2 BILs with Wx^a/Wx^a and pseudo-BC_1F_2 BILs with Wx^a/Wx^b performed using polymorphic simple sequence repeat markers between CH1 and RD49.

BC_1F_1 BILs	GB (%)	Pseudo-BC_1F_2 BILs (Wx^a/Wx^a)	GB (%)	Pseudo-BC_1F_2 BILs (Wx^a/Wx^b)	GB (%)
Q-8	91.04	Q- 8-17	97.01	Q-8-143	97.76
Q-16	89.87	Q- 8-64	96.27	Q-8-182	97.76
Q-55	87.50	Q- 8-4	95.52	Q-8-145	97.01
Q-18	85.07	Q- 8-82	95.52	Q-8-178	97.01
Q-63	85.07	Q- 8-112	95.52	Q-8-183	97.01
Q-67	85.07	Q- 8-216	95.52	Q-8-128	96.27
Q-68	85.07	Q- 8-2	94.78	Q- 8-15	96.27
Q-38	83.58	Q- 8-66	94.78	Q- 8-28	96.27
Q-20	82.84	Q- 8-72	94.78	Q- 8-73	96.27
Q-6	82.09	Q- 8-74	94.78	Q- 8-6	95.52
Average	**85.72**	**Average**	**95.48**	**Average**	**96.86**

Remarks: GB (%) = Genetic background as recurrent parent (%)

Agronomic and yield performance

The six selected pseudo-BILs were grown under field condition in the BC_1F_3 generation. Tables 3 and 4 showed the results of an analysis of variance (ANOVA) for agronomic and yield performance. Significant differences were found between the two parents for DOF, PL, S/P, G/P, and GW traits. When comparing the pseudo-BILs to the recurrent parent CH1, significant genotype variances for PH, S/P, G/P, and GW were discovered. The pseudo-BILs had mean values ranging from 68.2 to 82 cm (PH), 5.7 to 6.7 (P/P), 189.2 to 290.3 (S/P), 159 to 243 (G/P), and 1.9 to 2.3 g (GW). However, no significant differences in DOF, FLL, PL, T/P, HI, P/P, or W/P were found between the pseudo-BILs and CH1. Six improved lines had mean values ranging from 71.3 to 78.3 days (DFT), 21 to 28.7 cm (FLL), 27.9 to 29.8 cm (PL), 6 to 6.9 (T/P), 0.38 to 0.48 (HI), 5.7 to 6.7 (P/P), and 16.7 to 19.8 g (W/P). Highly stable characters, such as plant height and 100-grain weight, emerged differently from the repeated parent. Plant height has decreased and thousands of plants have increased. Although their genetic similarity is seen in molecular studies, their morphological features appear to be significantly different.

Table 3. Major important agronomic traits of the pseudo-BC_1F_3 BILs compared with parental varieties.

Pseudo-BILs/ Varieties	DOF	PH (cm.)	FLL (cm.)	PL (cm.)	T/P	HI
Q-8-17	77.0 b	68.2 d	23.0	29.1 a	6.7	0.47 a
Q-8-64	71.7 b	71.9 cd	22.9	27.9 ab	6.9	0.48 a
Q-8-143	78.3 b	75.3 bcd	21.0	28.1 ab	6.6	0.43 abc
Q-8-145	72.0 b	79.3 abc	22.1	29.7 a	6.2	0.41bc
Q-8-182	73.7 b	80.9 ab	28.7	29.0 a	6.0	0.38 c
Q-8-183	71.3 b	82.0 ab	25.4	29.8 a	6.4	0.44 ab
Average (line)	74.0	76.3	23.9	28.9	6.5	0.44
CH1	76.0 b	84.3 a	26.7	29.9 a	6.2	0.43 abc
RD49	90.0 a	81.8 ab	18.3	25.4 b	8.3	0.42 bc
Average (parent)	83.0	83.1	22.5	25.4	7.3	0.42
F-test	**	**	ns	*	ns	*
C.V. (%)	5.72	5.79	15.86	5.20	12.42	6.05

Remarks :ns= non-significant; *, = ** significance at 0.05 and 0.01 probability levels, respectively .Means within each column of each agronomic traits followed by the same letter are not significantly different according to DMRT. Days of 50% flowering (DOF), plant height (PH), flag leaf length (FLL), panicle length (PL), number of tillers per plant (T/P) and harvest index (HI).

Table 4. Major important yield component of the pseudo-BC_1F_3 BILs compared with parental varieties.

Pseudo-BILs/ Varieties	P/P^z	S/P	G/P	GW (g)	W/P (g)
Q-8-17	6.6	189.2 bc	164.3 c	2.1 bc	16.8
Q-8-64	6.7	204.3 bc	165.7 c	2.3 ab	17.4
Q-8-143	6.4	225.7 b	194.6 bc	2.1 cd	16.7
Q-8-145	6.2	213.4 b	159.0 cd	2.0 cd	16.8
Q-8-182	5.7	289.8 a	243.0 a	2.0 cd	17.8
Q-8-183	6.2	290.3 a	236.3 a	1.9 d	19.8
Average (line)	6.3	221.9	193.8	2.1	17.5
CH1	6.2	274.8 a	217.9 ab	2.0 cd	20.3
RD49	8.3	162.3 c	122.9 d	2.4 a	19.1
Average (parent)	7.2	218.6	170.4	2.2	19.7
F-test	ns	**	**	**	ns
C.V. (%)	12.85	10.16	11.14	3.08	18.45

Remarks: ns= non-significant; =** significance at 0.01 probability levels. Means within each column of each yield component followed by the same letter are not significantly different according to DMRT. Number of panicles per plant (P/P), number of grains per panicle (S/P), number of filled grain per panicle (G/P), 100-grain weight (GW), and total grain weight per plant (W/P)

Grain quality

Six pseudo-BILs, the original CH1, and the donor parent, RD49, were tested for three quality traits. Table 5 showed the results of grain quality testing. High AC of the pseudo-BILs ranged from 23.8% (Q-8-183) to 28.8% (Q-8-17). CH1 and RD49 had average AC of 17.4% and 29.1%, respectively. There was no difference in AC between the BILs and RD49. On the other hand, CH1 had intermediate average AC .High AC was perfectly associated with the *Wx* in allele of RD49. CH1 and RD49 had dissimilar ASV of 2.0 and 6.2, and gel consistency (GC) of low and high, respectively. BILs with similar alkali spreading values had mean ASV ranging from 4.7 to 7.0. The GC of pseudo-BILs (Q-8-17, Q-8-64, Q-8-143, Q-8-145, Q-8-182 and Q-8-183) differed as low, low, intermediate, low, intermediate, and intermediate, respectively.

The kernel length and width of CH1 were 7.7 and 1.9 mm, respectively, with an L/W ratio of 4.1. RD49, on the other hand, had kernel length and width of 9.0 and 1.9 mm, respectively, with an L/W ratio of 4.7. There was no significant difference in grain length and width or kernel length and width between the pseudo-BILs and the recipient parent CH1.

Table 5. Comparison of grain quality performance of the pseudo-BC_1F_4 BILs and two parents calculated by combined analysis of variance data in all traits of each experiment.

Pseudo-BILs/ Varieties	**AC (%)**	**ASV (GT)**	**GC**	**Kernel size**		
				Length (mm)	**Width (mm)**	**L/W**
Q-8-17	28.8 a	7.0 a	Low	8.2 bc	1.9 ab	4.4 bc
Q-8-64	27.8 a	6.9 a	Low	8.5 b	1.9 abc	4.5 ab
Q-8-143	24.6 ab	4.7 b	Intermediate	8.1 bcd	1.8 bc	4.5 ab
Q-8-145	24.8 ab	6.10ab	Low	8.0 cde	1.9 abc	4.3 bc
Q-8-182	24.6 ab	5.5 ab	Intermediate	7.8 de	1.9 abc	4.2 c
Q-8-183	23.8 b	5.5 ab	Intermediate	8.1 bc	1.8 c	4.5 ab
CH1	17.4 c	2.0 c	Low	7.7 e	1.9 a	4.1 c
RD49	29.1 a	6.2 a	High	9.0 a	1.9 a	4.7 a
F-test	**	**	-	**	*	**
C.V. (%)	14.85	11.92	-	4.38	4.28	6.33

Remarks :ns= non-significant; *, = ** significance at 0.05 and 0.01 probability levels, respectively. Means within each column of each yield component followed by the same letter are not significantly different according to DMRT.

DISCUSSION

A cross between CH1 and RD49, followed by pseudo-backcross selection, could result in rice lines with high AC accompanying with the goal of improving a favorable variety having favorable genetic background. To best encourage the efficient integration of the *Wx* gene into a CH1, an improved breeding platform based on pseudo backcrossing was developed. Recurrent backcrossing is a traditional breeding method used to transfer alleles from a donor to an elite variety at one or more loci. By six backcrosses process, the expected recurrent parent genome recovery would be 99.2%, which is most similar to improved variety (Wangsawang et al., 2018). However, introducing trait until the end result of a backcrossing program is to obtain lines as similar to the recurrent parent as possible, this approach can be tedious and time-consuming (Hasan et al., 2015). In a nutshell, this modern platform based on pseudo-backcross breeding relates to dual foreground selection and background genome recovery. The entire scheme was completed in two and a half years, consisting of two seasons to generate BC_1F_1, another two seasons to generate pseudo backcrossed BC_1F_2 and BC_1F_3, respectively, and one season of selfing to fix the final best-selected pseudo-BC_1F_4 BILs. This multiple pseudo-backcrossing platform reduces the time required to develop new rice varieties with complex, long-lasting resistance to biotic and abiotic stresses in desirable backgrounds (Ruengphayak et al., 2015).

AC is an important determinant of rice quality. In this study, the Wx^a allele was introduced from RD49 into CH1 via MAS. MAS is a very efficient and cost-effective breeding technology because it is used in most of the steps of the breeding program and greatly increases the success of selecting desirable lines because it directly targets the genotype without the influence of environment and thus speeds up the conventional selection procedures (Collard et al., 2005). Pseudo-BILs had a high AC and agronomic performance comparable to CH1. OSR19 was used in this experiment to identify individuals with the positive allele derived from RD49. All of the BILs with the positive allele of OSR19 had a high AC, indicating that (CT)n repeats were present (Akagi et al., 1996), in these genetic materials, it was able to distinguish between high and intermediate AC. MAS for foreground selection may be especially useful for traits with time-consuming or laborious phenotypic screening processes. It can also be used to select the reproductive-stage traits in seedlings, allowing the best plants for each backcrossing to be identified.

Two pseudo-BC_1F_3 BILs and four pseudo-BC_1F_3 BILs were largely homozygous and heterozygous, respectively in the MAS- based target loci with agronomic traits similar to CH1, having high AC . In BC_1F_1 progenies, background genotype recovery ranged from 82.09% to 91.04%. In the experiment, the Q-8 plant inherited the most genetic background from CH1. This was more or less the expected value, as the recipient genome should theoretically be recovered to 50% in BC_1. The average genetic background percentage in BC_1F_2 progenies ranged from 94.78% to 97.01% as homozygous gene (Wx^a/Wx^a) and 95.52% to 97.76% as Wx^a/Wx^b. The average background genotype recovery rate should be 75% with one time of backcross and self-pollination (BC_1F_2), and that background recovery rate is lower than that of the selected plants in this study because MAS was used to select population in each generation. In addition, the results from this research gave the same results as Cho et al. (2020). This result showed that the pseudo-backcross method with marker assistance can increase the percentage genetic background of plants more than the standard backcross method and skips generations in the normal backcross method.

It is critical that the genes used in MAS and gene introgression do not introduce undesirable traits due to linkage drag (Sun and Mumm, 2015). In addition to high AC, the major positive agronomic traits of RD49 were similar to pseudo BILs. The results showed that there is no negative effect in pseudo BILs after the *Wx* gene introgression. The *Wx* gene was found to be primarily responsible for quantitative GC inheritance (Zhou et al., 2003). The findings of this research showed that *Wx* gene associated with OSR19 had no effect on GC. The ASV of the pseudo-BILs differed significantly from CH1. Lower ambient temperature during the grain filling period may cause a higher ASV because lower environmental temperatures decreased the relative amount of long amylopectin chains and increased the relative amount of short chains, resulting in a high GT (Fan et al., 2005). Furthermore, high temperatures increased the GT significantly (Zhong et al., 2005). The grain size and shape of the pseudo-BILs matched that of the original CH1.

CONCLUSION

The pseudo-BC_1F_2 BILs used in this study had background genome recovery of 97.76% with the RD49 allele of the *Wx*^{a} gene using foreground and background simultaneous selection. This pseudo-backcross design could aid in the introgression of high AC from the RD49 rice variety into the CH1 background, thereby speeding up the backcross breeding program. This study found that the pseudo-backcross method with marker assistance could increase the genetic background percentage of plants than the traditional backcross method. Thus, the advantage of the pseudo-backcross method is that it can save not only time and workload, but also the cost of analysis and leaping generations in the normal backcross method. However, there are still disadvantages of selecting with pseudo-backcross bases SSR markers. The selection of plants in the rice field may have other environmental factors that cause the experimental results to be inaccurate. In the present study, marker assisted pseudo-backcrossing breeding accelerated the development of superior qualities in the genetic background of CH1. The improved CH1 should be immediately useful for Thai farmers and will help farmers to increase their incomes.

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