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Research article

Efficiency of short-grain rice breeding program between pedigree and single seed descent methods for yield, biotic resistance and cooking quality derived from indica × japonica

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Abstract

Importance of the work: Japanese food is popular in tropical countries; however, high-yielding and good-quality temperate japonica rice supplies are limited in tropical climates.

Objectives: To compare two breeding methods: pedigree and single seed descent applied for a short-grain rice (japonica-like) breeding program.

Materials & Methods: A temperate japonica rice (Koshihikari) and a tropical indica fragrant rice (Pathumthani1) were used as parents. The pedigree and single seed descent methods were used to select breeding lines from F_2 to F_5. Preliminary yield trials and yield traits of selected breeding lines were investigated in F_5 to F_7.

Results: From the preliminary trial, based on short-grain, plant type and grain yield criteria, two breeding lines (PS171-31-10 from the pedigree method and SSD125-7 from the single seed descent method) were selected in which genetic markers for bacterial leaf blight resistance (*Xa21*) and blast resistance (*Pi-ta*) were detected. In the yield trial experiments, the mean grain yields of PS171-31-10 (5.07±3.46 t/ha) and SSD125-7 (4.43±3.00 t/ha) were higher than those of the japonica parent (4.30±1.99 t/ha) but lower than those of the indica parent (5.80±1.17 t/ha). In addition, the highest overall scores of sensory tests were for Ps-171-31-10 (37.58 overall score), the second highest was for SSD-125-7 (35.37 overall score), while Koshihikari had the third highest sensory score. The physical and chemical properties of the milled grain from the two lines were consistent with standard japonica rice. In addition, the *badh2* gene was detected in SSD125-7, which is identified as a japonica fragrant rice variety.

Main finding: The pedigree and single seed descent methods created new short-grain varieties with comparable performance: adaptability to tropical environments, production of satisfactory grain yields and good cooking quality, similar to that of japonica rice.

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Introduction

Rice (*Oryza sativa* L.) is one of the most common food crops in the world and represents a staple food for more than half of the world's population (Calingacion et al., 2014). Rice cooking quality largely determines its market price and consumer acceptance because consumers pay particular attention to high eating quality (Hori and Yano, 2013). Rice cooking quality preference varies from country to country and region to region; currently, Asian countries have been increasing their consumption of japonica rice, particularly in the tropics (Miyamoto, 2017). This trend has occurred despite the higher price of japonica varieties compared with local indica rice varieties (Ha et al., 2011). For example, for japonica rice eating quality, a low-amylose short-grain rice is preferred because it is soft and sticky after cooking (Hossain et al., 2009).

In Thailand, Japanese foods have become popular in urban areas, with the number of Japanese restaurants, especially in Bangkok, rapidly increasing in the last decade, with Japanese cuisine seemingly positioned to remain the second largest food industry following Thai cuisine (Miyamoto, 2017). Thai people in urban areas choose Japanese food because it is considered "healthy", appropriately "luxurious", but still affordable (Miyamoto, 2017). Thus, the demand for japonica rice, which is an important material for Japanese food, is increasing substantially. Many companies in Thailand have encouraged farmers, especially those in northern regions, to cultivate Japanese rice (Seemanon et al., 2015).

At present, shortages of grains with good quality and quantity are still an obstacle to Japanese rice production in Thailand (Seemanon et al., 2015). In addition, in Thailand, there are only two varieties of japonica rice, namely, DOA1 and DOA2. Currently, the genetics of these varieties have changed greatly from those of the original varieties of Sasanishiki and Akitakomachi (Nakwilai et al., 2020). Furthermore, the production of temperate japonica rice in tropical regions is very limited; for example, in Thailand, the tropical climate and topography are suitable for indica rice cultivation but not for temperate japonica rice (Nakwilai et al., 2020). The tropical japonica rice varieties found in Thailand are distributed in mountainous regions and known as upland rice; however, they have low productivity and low cooking quality (Chimthai et al., 2021). Therefore, the japonica rice used for cultivation under tropical conditions should be improved (Saichompoo et al., 2021).

There are several plant breeding methods, such as plant selection based on natural variation and plant selection originating from directed crosses with recombinant desirable genes (Lalić et al., 2008). Pedigree selection (PS), the bulk method and single-seed descent (SSD) have been proposed for improving the grain yield of self-pollinated crops. Most rice breeders still use the pedigree method (79%), according to Collard et al. (2017) and Fahim et al. (1998) reported that the PS and bulk methods presented much higher yields compared to SSD. Similarly, Kanbar et al. (2011) confirmed that PS was superior to the bulk and SSD methods for improving rice grain yield. In contrast, PS and SSD were equally effective in improving the yield of yardlong bean (Sarutayophat and Nualsri, 2010). However, the cost of applying SSD is lower than that of the PS method (Fahim et al., 1998).

In general, breeding to improve cooking quality is done using the marker-assisted backcrossing method (Kim et al., 2021). However, Jantaboon et al. (2011) successfully combined submergence tolerance and cooking quality from IR57514 × KDML105 based on the SSD method by using *Sub1, Wx badh2* and *SSIIa* markers in marker-assisted selection (MAS). In addition, Sreewongchai et al. (2014) used the PS method with MAS to improve the yield and cooking quality using a cross (KDML105 × Qiqnizhan). Recently, Saichompoo et al. (2021) successfully improved the yield and cooking quality of novel short-grain rice for tropical climates using a cross between temperate japonica and indica based on backcross and pedigree methods with MAS.

Therefore, knowledge about the relative efficiency of the different methods may help rice breeders choose a better method to improve elite rice varieties. Thus, the current research aimed to compare performance of the PS and SSD methods to improve yield and cooking quality of japonica-like rice (short grain) derived from intersubspecific crossing between japonica Koshihikari (KH) and indica Pathumthani1 (PTT1), with the aim of producing a cross suitable for growing in tropical regions.

Materials and Methods

Growth conditions

The breeding program was conducted from 2016 to 2020 at Tana Grain Polish, Ltd., Phan district, Chiang Rai province, Thailand. The rice plants were seeded in a field nursery. After 30 d, the rice seedlings were transplanted into breeding plots. The soil in the Phan district consisted of 2.16% organic matter, 0.07% total N, 19.98 mg/kg available P, 91.58 mg/kg exchangeable K, 1134.58 mg/kg exchangeable Ca and 160.61 mg/kg exchangeable Mg and had a pH of 5.75. In addition, 18-22-0 (N-P-K) fertilizer was applied at a rate of 187.5 kg/ha at 15 d after planting, and 46-0-0 fertilizer was applied at 125 kg/ha at the booting stage (65 d). Water management was applied to a water depth of 10 cm above the soil and a constant surface water depth was maintained at this level from transplanting until 2 weeks before harvest. Other management practices were performed in accordance with conventional high-yield cultivation approaches (Rice Department, 2021). Weather data (air temperature, relative humidity and amount of rain in the field) were measured every 3 h in each year using a data logger (WatchDog 2000 Series Micro Stations; Spectrum Technologies, Inc.; USA).

Breeding schemes

Koshihikari (KH), a premium short-grain rice variety, containing genes for starch (*wx*^{b}), gelatinization temperature (*SSIIa*), short grain (*GS3*) and blast resistance (*Pi-ta*), was used as the female parent (Kobayashi et al., 2018). The Thai indica variety Pathum Thani1 (PTT1), a high-yielding variety, containing genes for fragrance (*badh2*), brown planthopper resistance (*Bph3*), blast resistance (*Pi-ta*) and bacterial leaf blight resistance (*Xa21*), was used as the male parent (Chakhonkaen et al., 2012).

The breeding program involved pairwise methods of PS and SSD (Fig. 1). First, KH was crossed with PTT1 to obtain F_1 seeds and then allowed to self and produce F_2 seeds. Subsequently, 200 F_2 seeds were separately subjected to either the PS or SSD breeding methods. For the PS method,

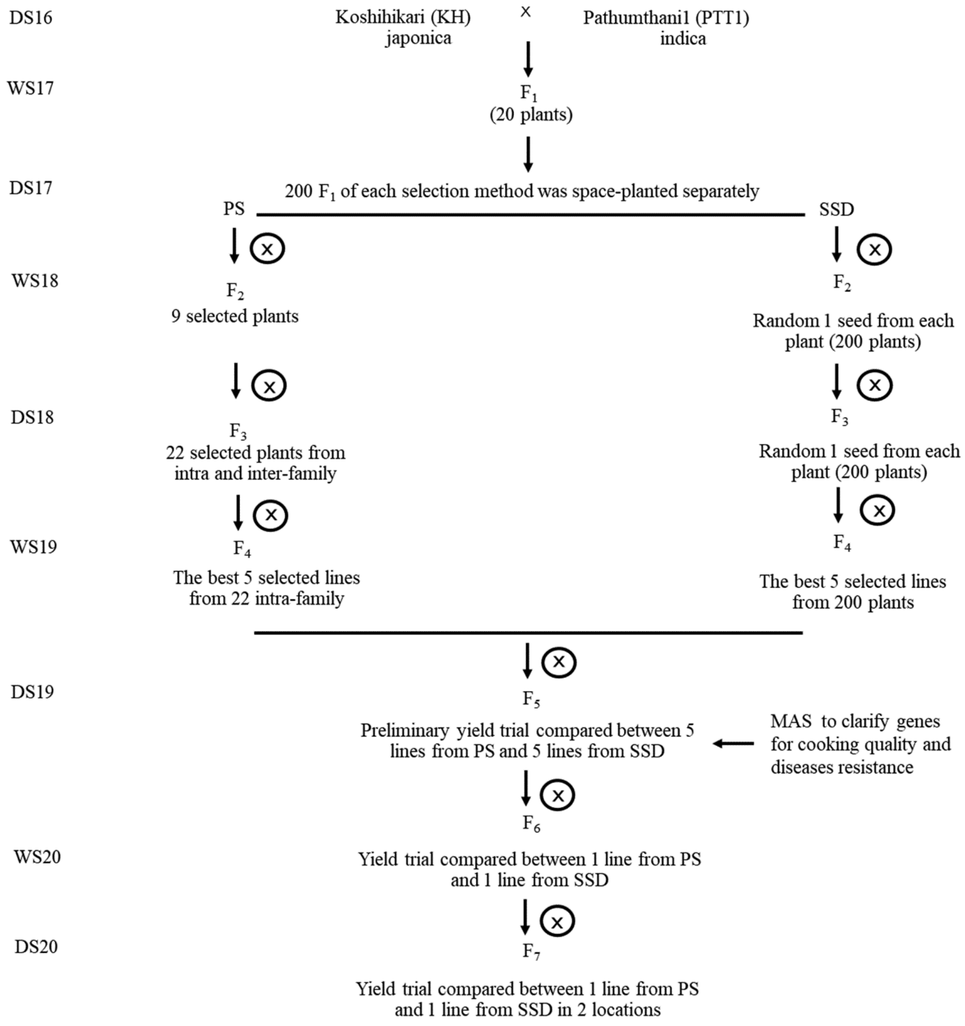

Fig. 1 Scheme of breeding programs for short-grain rice derived from Koshihikari x Pathumthani1 from dry season 2016 to dry season 2020 in Phan district, Chiang Rai province, Thailand

9 desirable individuals among 200 F_2 plants were selected based on the standard japonica grain shape (International Rice Research Institute, 2013) and the F_3 seeds were collected separately. Subsequently, nine F_3 progenies were planted in separate rows, and 42 desirable F_3 plants were selected among and within the family. In F_4, the top-five families were selected and each family was separately maintained. In the SSD method, one random seed from each of 200 F_2 plants was individually harvested and individually grown. The protocol was repeated in F_3 and F_4 generation. Then, 200 F_4 seeds were planted and the top-five desirable plants were selected based on the same criteria as for the PS method.

The five lines each from the PS and the SSD method that were selected based on the standard japonica grain shape, good plant type and grain yield were subjected to preliminary yield trials together on the F_5 generation during the dry season 2019 (DS19). In addition, KH, PTT1, DOA1 and DOA2 plants were used as controls. A randomized complete block design with three replications was applied. The plot size for each treatment was 2.5 m × 3.5 m (8.75 m^2), with a spacing of 20 cm × 20 cm. Then, yield trials in F_6 (wet season 2020; WS20) and F_7 (dry season 2020; DS20) were conducted to evaluate the candidate lines from both the PS and the SSD methods. The yield trial in F_6 was conducted at Tana Grain Polish, Ltd., Phan district, Chiang Rai province (the main production area of japonica rice). Additionally, the F_7 yield trial was conducted in two locations: 1) Tana Grain Polish, Ltd., Phan district, Chiang Rai province; and 2) Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom province (a representative area with higher temperatures), which are located in the northern (19°35′ N, 99°44′ E, 413 m above sea level) and central (14°01′ N, 99°58′ E, 10 m above sea level) parts of Thailand, respectively. The yield trial experimental design and plot size of generations F_6 and F_7 were the same as those for the F_5 generation.

Screening SNP/InDel markers based on Kompetitive Allele Specific PCR (KASPTM) genotyping technology

The screening of breeding lines was conducted in F_5 using SNP/InDel markers, consisting of markers for starch (*wx*^{b}), gelatinization temperature (*SSIIa*), short grain (*GS3*), aroma (*badh2*), blast resistance (*Pi-ta*; TBGI453598), bacterial leaf blight (*Xa21*) and brown plant hopper (*Bph3*), as shown in Table S1. All Kompetitive Allele Specific polymerase chain reaction (KASP) genotyping was performed using the LGC SNP line system following the standard KASP protocols (LGC Group, 2015). The thermal cycles of the PCR assay are shown in Table S2. Finally, the PCR products were analyzed for their genotypes using PHERAstarPlus SNP (LGC; Serial No. 470-0319; Teddington, UK).

Agronomic traits collection

The agronomic traits examined were the days to 100% flowering (DF), plant height (PH), panicle length (PL), number of tillers per plant (NTP), number of panicles per plant (NPP), percentage of seed set (SS), 1,000-grain weight (GW) and grain yield (GY). These traits were determined for the rice plants grown during the field trials. The grain yield in each plot was determined based on the harvested area of 8.75 m^2. The grain moisture was adjusted to 14% and then the yield was extrapolated to units of kilograms per hectare.

Bacterial leaf blight and leaf blast resistance screening

CN1-3, NP3-5, XORE1-1, CR2-4, SK1-2 and XON2-1 isolates were used to screen bacterial leaf blight resistance in the breeding lines and their parents with susceptible (KDML105) and resistant (PYBB-36) check varieties. Resistance reactions were classified as resistant, moderately resistant, moderately susceptible and susceptible as described by International Rice Research Institute (2013).

Seven mixed groups of Thai *Magnaporthe oryzae* were used to screen blast resistance in the breeding lines, their parents and checked varieties, the Sariceltik (susceptible) and JHN (resistance) varieties. The disease scoring was recorded based on a standard system for evaluating damage (International Rice Research Institute, 2013).

Evaluation of grain quality

The dried grains were stored at room temperature for 1 mth prior to the evaluation of the grain quality traits. A sample (300 g) of paddy grains was used from each replicate. The paddy grains were dehulled and polished using a mini polisher. The grain length, grain width and grain length-to-width ratio of the milled rice were recorded to two-decimal-point accuracy using a digital Vernier caliper. The gelatinization temperature and amylose content were evaluated following the procedures described by American Association of Cereal Chemists (2000). The pasting properties of the rice flours were evaluated according to

American Association of Cereal Chemists (2000). Rice flour (3 g) was suspended in water (25 g) in a canister and the viscosity changes were measured using a Rapid Visco Analyzer (Model 4-D; Newport Scientific; Australia).

Analysis of 2AP in rice grains

The brown grains of the breeding lines were analyzed for their 2AP content (measured in parts per million: ppm) with their parents and commercial varieties. After harvest, the paddy grains were dehulled for brown grains and stored at -80 °C before analysis. Subsequently, the brown grains were finely ground in liquid nitrogen. Then samples (each 0.50 g) were separately placed in headspace vials. Static headspace-gas chromatography/mass spectrometry analyses were carried out using chromatography/nitrogen-phosphorus detector (Clarus 680 model) and a gas chromatograph/mass spectrometer (GC/MS; SQ8 model; PerkinElmer Ltd.; USA), a headspace autosampler, a flame ionization detector and a nitrogen-phosphorus detector. A sample headspace was collected through a 3 mL sample loop and automatically transferred to the GC via a heated transfer line. A split/splitless GC injector equipped with a direct 0.2 mm internal diameter glass liner was used. The system operation, data acquisition, data collection and system evaluation were accomplished using the Agilent Chem Station software version A.01.04 and B.01.03 (Agilent Technologies; Germany). The concentration of 2AP was presented in parts per million.

Evaluation of sensory of cooked rice

The rice cooking procedure by Xu et al. (2018) was applied. Whole milled rice samples (500 g, 14% moisture content) were washed three times with distilled water. Then, distilled water was added to the rice samples to achieve an optimum rice-to-water weight ratio (1.0:1.4). The rice was cooked using the preset cooking setting of a rice cooker (Hitachi; model RZ-ZH18; Thailand). Sensory quality evaluation was carried out by seven panelists who had been well trained in the principles and concepts of descriptive sensory analysis. The sensory items all based on scores of 1 to 5 consisted of: smell, appearance, stickiness, softness and taste. A comprehensive assessment was made based on these factors. Using a relative scale, the panelists scored each attribute relative to the reference sample attributes and the overall quality was the sum of the scores for all the attributes.

Statistical analysis

All the data were analyzed using the R program version 3.6.1 (R Core Team, 2014) to test the significance of the agronomic traits and cooking quality results. The data were subjected to analysis of variance and then means were compared using Duncan's multiple range test and significance was considered when $p < 0.05$. The mean of the agronomic and grain quality traits among three groups of genotypes (PS, SSD, parents + controls) was analyzed based on orthogonal contrasts. Principal component (PC) analysis was performed to reduce the complexity of the grain yield data. In addition, the AMMI model was used to analyze the G×E interactions.

Results

Genetic segregations in early generation

The genetic segregations of agronomic traits in the F_2 generation are shown in Fig. 2A–2F. For grain shape segregation, the two parents KH (width = 3.04 mm and length = 6.90 mm) and PTT1 (width = 2.30 mm and length = 9.90 mm) were identified as short and long grain phenotypes, respectively, whereas the paddy grain shapes of their F_2 progenies had ranges of 1.95–3.10 mm for grain width and 6.60–10.40 mm for grain length (Figs. 2E and 2F). In addition, both the grain width and length were segregated based on a normal distribution. Notably, some F_2 progenies showed transgressive segregation in days to flowering and plant height, while most F_2 progenies had a lower seed set than their parents.

In the F_3 generation, the segregation in the PS method could not be measured because, the number of families was too small. Therefore, genetic segregation of the agronomic traits was examined only for the SSD method. The 200 plants still showed segregation of all traits (Figs. 2G–2L). The paddy grain shapes of their F_3 progenies had ranges of 1.91–3.30 mm for grain width and 6.60–11.5 mm for grain length (Figs. 2K and 2L). In addition, the segregation of days-to-flowering and plant height showed the same results as the F_2 generation. However, the distribution of percentage of seed set of the F_3 populations skewed towards high percentage, indicating that most of the F_3 population showed improved seed fertility compared with the F_2 population (Fig. 2J). Therefore, the F_3 generation was still segregated in all agronomic traits.

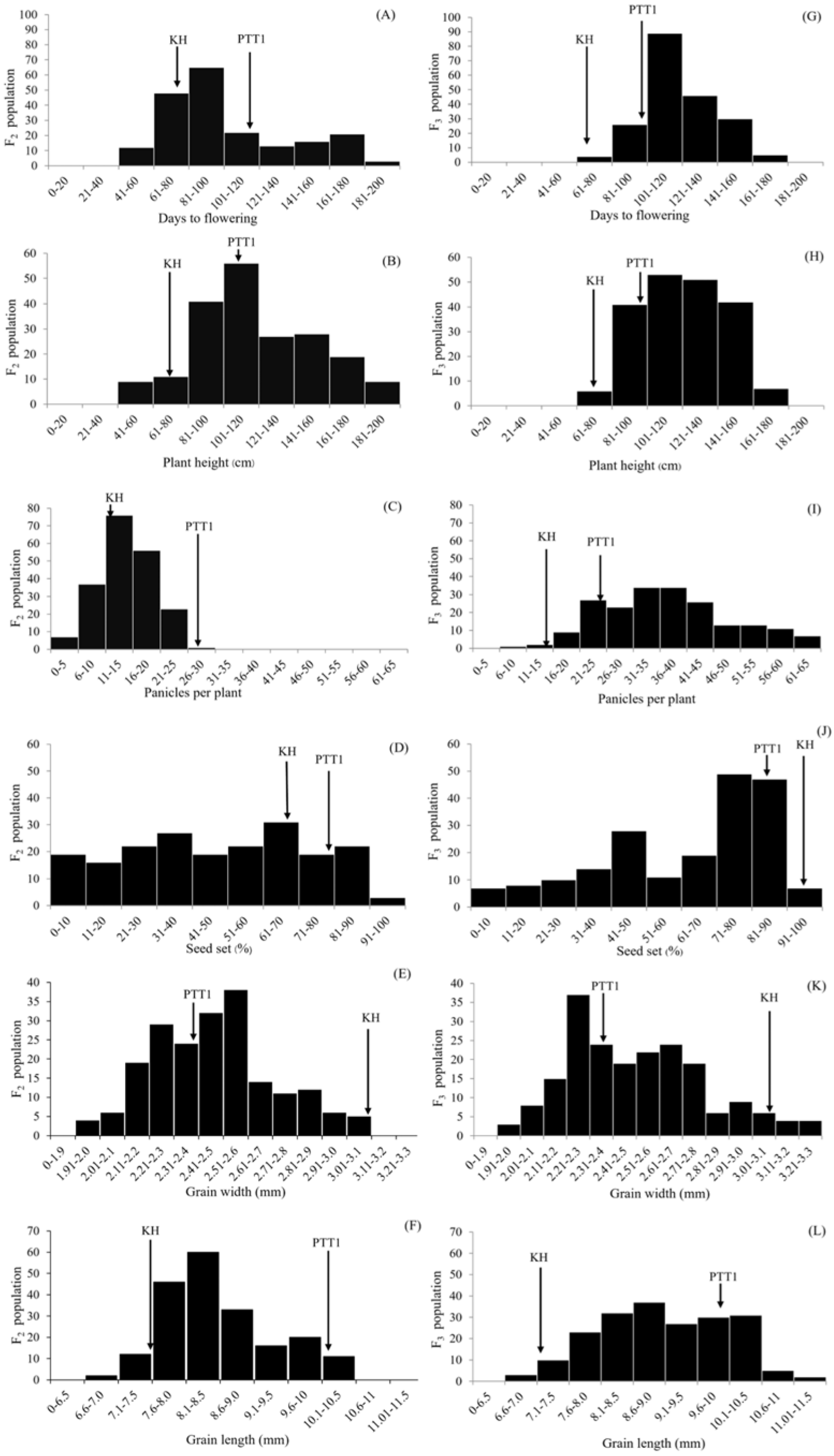

Fig. 2 F_2 segregation (A–F) and F_3 segregation (G–L) of agronomic traits from Koshihikari × Pathumthani1

Preliminary yield trial

The five lines from the PS and SSD methods were selected in the F_4 generation (data not shown) and then evaluated in the preliminary yield trial in the F_5 generation (DS19). Mean comparisons among lines and breeding methods were performed based on the GY, agronomic traits and grain quality. The results showed that the agronomic and grain quality traits were significantly different between breeding methods ($p < 0.05$) and lines ($p < 0.05$), as shown in Tables 1 and 2.

For the PS and SSD methods, days-to-flowering, plant height, seed setting, grain weight, grain yield, amylose content and alkaline test were significantly different (Table 1). The seed set from the PS method was greater than that of the SSD method (87 and 71%, respectively). In addition, the selected lines from the PS method had higher grain yields than those of the SSD method (9.18 and 7.23 t/ha, respectively). However, regarding grain quality, the PS method had a higher amylose content than the SSD method (23.42% and 18.45%, respectively), as shown in Table 1 and the content was also higher than that of the japonica rice commercial varieties. On the other hand, the grain length-to-width ratio of the PS method was close to that of the japonica varieties compared to the SSD method (ratios of 2.08 and 2.24, respectively).

The mean comparison among lines of the days-to-flowering from the nine selected lines (83–95 d) were similar to the japonica varieties KH, DOA1 and DOA2, which were in the range 86–92 d. However, only the line SSD3-5 (108 d) had the same days-to-flowering as the indica variety PTT1 (111 d). In addition, the field results revealed two lines (PS171-31-10 and PS171-36-8) from the PS method that had significantly higher grain yields compared to the other lines and varieties. Furthermore, the best selected line from the PS method (Ps 171-31-10; 12.28 t/ha) produced a significantly higher grain yield than the best line from the SSD method (SSD125-7; 7.87 t/ha), as shown in Table 2.

The grain length-to-width ratios of the two lines PS36-37-9 and PS171-36-8 from the PS method conformed to the japonica standard (ratio < 2). The amylose contents among the selected lines were in the range 12.83–29.02%. The selected lines that conformed to the japonica rice standard (amylose content ≤ 20%) were one line from the PS method and four lines from the SSD method, while the amylose contents of the japonica varieties KH, DOA1 and DOA2 were 17.91%, 18.71% and 19.73%, respectively. The selected lines had high gelatinization temperatures (alkaline score = 5–6) similar to those of KH, DOA1 and DOA2 (Table 2).

Table 1 Means and F values of orthogonal contrasts for agronomic and grain quality traits evaluated in pedigree selection (PS), single seed descent (SSD), parents (P) and control (C) varieties in F_5 generation in dry season 2019

Agronomic and grain quality trait	PS versus (P, C)		F test
	Mean PS	Mean (P, C)	
DF (d)	88	94	**
PH (cm)	94	100	**
PL (cm)	22.14	22.14	ns
NTP	16	19	**
NPP	16	18	*
SS (%)	87	82	*
GW (g)	24.1	29.5	**
GY (t/ha)	9.18	6.44	**
W:L	2.08	2.23	*
AC (%)	23.42	18.64	**
AK (1–7)	5	6	*
Agronomic and grain quality trait	SSD versus (P, C)		F test
	Mean SSD	Mean (P,C)	
DF (days)	97	94	*
PH (cm)	101	100	ns
PL (cm)	21.83	22.14	ns
NTP	17	19	*
NPP	16	18	*
SS (%)	71	82	**
GW (g)	27.2	29.5	**
GY (t/ha)	7.23	6.44	**
W:L	2.24	2.23	ns
AC (%)	18.45	18.64	ns
AK (1–7)	6	6	ns
Agronomic and grain quality trait	PS versus SSD		F test
	Mean PS	Mean SSD	
DF (days)	88	97	**
PH (cm)	94	101	**
PL (cm)	22.14	21.83	ns
NTP	16	17	ns
NPP	16	16	ns
SS (%)	87	71	**
GW (g)	24.1	27.2	**
GY (t/ha)	9.18	7.23	**
W:L	2.08	2.24	ns
AC (%)	23.42	18.45	**
AK (1–7)	5	6	*

DF = days-to-flowering; PH = plant height; PL= panicle length; NTP = number of tillers per plant; NPP = number of panicles per plant; SS = seed set; GW = 1,000-grain
weight; GY = grain yield; W:L = grain width-to-length ratio; AC = amylose content; AK = alkaline test.
*, ** = significant at $p < 0.05$ and $p < 0.01$, respectively.

Table 2 Agronomic traits and grain quality of breeding lines compared to their parents (PTT1 and KH) and commercial varieties (DOA1 and DOA2) as control in F_5 generation in dry season 2019

Line/Variety	DF (d)	PH (cm)	PL (cm)	NTP	NPP	SS (%)	GW (g)	GY (t/ha)	W:L	AC (%)	AK (1–7)
PS36-7-2	$88^{cd1/}$	89^{ef}	24.37^{bc}	19^{a-c}	19^{ab}	83^{bc}	23.3^{f-h}	9.30^{ab}	$2.2\ 1^{cd}$	29.02^{a}	4^{c}
PS36-37-9	89^{cd}	84f	20.43^{cd}	14^{e}	12^{f}	86^{a-c}	24.0^{fg}	6.83^{c-e}	1.95^{ef}	20.6^{cde}	5^{b}
PS171-26-1	95^{b}	108^{bc}	25.67^{ab}	15^{c-e}	15^{b-f}	89^{ab}	22.1^{h}	6.66^{c-e}	2.04^{de}	25.94^{b}	5^{b}
PS171-31-10	83^{e}	89^{ef}	22.33^{bc}	14^{cd}	14^{c-f}	92^{a}	28.1^{c}	12.28^{a}	2.20^{cd}	19.66^{def}	5^{b}
PS171-36-8	87^{de}	101^{cd}	17.90^{e}	20^{ab}	20^{ab}	87^{ab}	23.0^{gh}	10.83^{ab}	1.97^{e}	21.88^{c}	5^{b}
PS mean	88	94	22.14	16	16	87	24.1	9.18	2.08	23.42	5
SSD3-5	108^{a}	123^{a}	23.43^{bc}	15^{c-d}	14^{de}	77^{cd}	30.0^{b}	5.86^{de}	2.24^{cd}	28.38^{a}	5^{b}
SSD12-8	92^{bc}	85^{f}	23.47^{bc}	14^{de}	14^{de}	88^{ab}	25.2^{ef}	5.58^{bc}	2.53^{b}	14.93^{h}	5^{b}
SSD35-10	86^{de}	101^{b-d}	21.00^{c-e}	14^{c-e}	14^{de}	62^{ef}	24.6^{e-g}	7.33^{c-e}	2.05^{de}	12.83^{i}	6^{a}
SSD125-7	95^{b}	113^{ab}	24.20^{bc}	18^{b-e}	18^{bc}	77^{cd}	27.4^{cd}	7.87^{bc}	2.07^{de}	17.186^{g}	5^{b}
SSD129-6	88^{cd}	82^{f}	17.07^{e}	22^{ab}	18^{bc}	51^{f}	29.0^{bc}	6.50^{cde}	2.29^{c}	18.88^{efg}	6^{a}
SSD mean	97	101	21.83	17	16	71	27.2	7.23	2.24	18.45	6
PTT1	111^{a}	107^{bc}	29.23^{a}	24^{a}	24^{a}	90^{a}	26.1^{de}	5.35^{e}	3.43^{a}	18.23^{b}	6^{a}
KH	87^{cd}	91^{d-f}	18.20^{de}	14^{e}	13^{ef}	80^{bc}	29.0^{bc}	5.41^{de}	1.72^{g}	17.91^{fg}	6^{a}
Parent mean	99	99	23.72	19	18	85	27.5	5.38	2.58	19.36	5
DOA1	90^{bc}	104^{bc}	20.67^{cd}	20^{ab}	20^{ab}	74^{de}	30.3^{b}	8.00^{bc}	2.04^{de}	18.71^{efg}	6^{a}
DOA2	86^{de}	99^{c-e}	20.47^{cd}	19^{ab}	18^{bc}	84^{bc}	32.4^{a}	7.00^{cd}	1.76^{fg}	19.73^{def}	5^{b}
Control mean	93	102	20.57	20	19	79	31.4	7.50	1.90	20.23	6
F test	**	**	**	**	**	**	**	**	**	**	**
%CV	3.35	6.46	11.6	19.90	19.74	8.52	4.23	18.17	5.56	5.44	2e-16

PS = pedigree selection; SSD = single seed descent; P = parent varieties; CV = coefficient of variation; DF = days-to-flowering; PH = plant height; PL= panicle length; NTP = number of tillers per plant; NPP = number of panicles per plant; SS = seed set; GW = 1,000-grain weight; GY = grain yield; W:L = grain width-to-length ratio; AC = amylose content; AK = alkaline test; CV = coefficient of variation.
Different lowercase superscripts in same column indicate significant differences ($p < 0.05$).
*, ** = significant at $p < 0.05$ and $p < 0.01$, respectively

The DNA marker was clarified in five lines from each method with their parents. The results showed that the markers for short grain (*GS3*), bacterial leaf blight (*Xa21*) and leaf blast (*Pi-ta*) were detected in all 10 lines, while the marker for brown plant hopper (*Bph3*) was not detected in any of the lines. Of the quality traits, wx^b was detected in only one line derived from the PS method (PS171-31-10), which was similar to KH, while wx^b was detected in the four lines of the SSD method (SSD12-8, SSD35-10, SSD125-7, SSD129-6). In contrast, gelatinization temperature (*SSIIa*) traits similar to those of KH were detected in most samples based on the PS method except for PS171-26-1 and two lines of the SSD method (SSD3-5, SSD35-10). Notably, the aromatic gene (*badh2*) was detected in the three lines from the SSD method (SSD12-8, SSD125-7, SSD129-6), which was similar to PTT1; however, it was not found in any line from the PS method (Table 3). Thus, when considering all traits (grain yield, agronomic traits, grain quality and DNA markers), the two lines PS171-31-10 (harboring *GS3*, wx^b, *SSIIa*, *Xa21* and *Pi-ta*) from the PS method and SSD125-7 (harboring *GS3*, wx^b, *SSIIa*, *badh2*, *Xa21* and *Pi-ta*) from the SSD method were used in the yield trials in F_6 and F_7.

Table 3 SNP/Indel marker information on the breeding lines identified in F_5 generation

Line/Variety	*GS3* (C:C)	wx^b (T:T)	*SSIIa* (GC:GC)	*badh2* (TATAT:TATAT)	*Bph3* (G:G)	*Xa21* (G:G)	Pi-ta (T:T)
PS36-7-2	C:C	G:G	GC:GC	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
PS36-37-9	C:C	G:G	GC:GC	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
PS171-26-1	C:C	G:G	TT:TT	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
PS171-31-10	C:C	T:T	GC:GC	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
PS171-36-8	C:C	G:G	GC:GC	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
SSD3-5	C:C	G:G	GC:GC	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
SSD12-8	C:C	T:T	TT:TT	TATAT:TATAT	A:A	G:G	T:T
SSD35-10	C:C	T:T	GC:GC	TATAT:AAAAGATTATGGC	A:A	G:G	T:T
SSD125-7	C:C	T:T	TT:TT	TATAT:TATAT	A:A	G:G	T:T
SSD129-6	C:C	T:T	TT:TT	TATAT:TATAT	A:A	G:G	T:T
KH	C:C	T:T	GC:GC	AAAAGATTATGGC:AAAAGATTATGGC	A:A	G:G	T:T
PTT1	A:A	G:G	TT:TT	TATAT:TATAT	G:G	G:G	T:T

PS = pedigree selection; SSD = single seed descent; KH = Koshihikari; PTT1 = Pathum Thani1; A = adenine; T = thymine; C = cytosine; G = guanine.

Yield trial validation

The air temperature during the yield trial in F_6 and F_7 showed that interval of mean day/night temperature in Chiang Rai, WS20 (F_6), Chiang Rai, DS20 (F_7) and Nakhon Pathom, DS20 (F_7) was 3–4 °C. However, the mean day/night temperature values in both provinces in DS20 were lower than that in WS20. The mean day/night temperature and the maximum/minimum temperature in WS20 were more stable throughout the growing season than those in DS20. In addition, the interval of maximum/minimum temperature in WS20 was 8 °C, while those in Chiang Rai and Nakhon Pathom in DS20 were 14 °C and 9 °C, respectively (Fig. S1).

The grain yield, agronomic traits and grain quality of the two candidate lines (PS171-31-10, SSD125-7) in F_6 and F_7 as well as the controlled varieties are shown in Table 4.

Table 4 Agronomic traits and grain quality of candidate lines (PS171-31-10 and SSD125-7) compared with their parents (Koshihikari and Pathumthani1) and commercial varieties (DOA1 and DOA2) in yield trial (F_6 and F_7)

Trait	Season	Location	Generation	PS171-31-10	SSD125-7	DOA1	DOA2	KH	PTT1	Mean	F test	%CV
DF (d)	WS20	CR	F_6	$93^{b1/A2/}$	79^{cA}	75^{cdB}	73^{cdeB}	67^{eB}	108^{aB}	81	**	5.45
	DS20	CR	F_7	95^{bA}	76^{dA}	89^{cA}	85^{cdA}	88^{cA}	117^{aA}	92	**	1.95
	DS20	NKP	F_7	91^{bAB}	69^{dB}	76^{cB}	75^{cB}	66^{deB}	117^{aA}	82	**	1.53
	Mean			93	75	80	78	74	114			
PH (cm)	WS20	CR	F_6	115.0^{aA}	108.7^{bA}	89.3^{dB}	107.3^{bA}	66.2^{eC}	105.7^{bcA}	98.7	**	3.48
	DS20	CR	F_7	84.5^{cB}	$90.4b^{cB}$	99.1^{aA}	97.6^{aB}	94.7^{abA}	101.7^{aBC}	94.7	**	4.11
	DS20	NKP	F_7	87.8^{bB}	80.7^{cC}	86.1^{bcB}	88.2^{bC}	87.4^{bB}	102.0^{aB}	88.7	**	3.92
	Mean			96.8	93.3	91.5	97.7	82.8	103.1			
NTP	WS20	CR	F_6	29^{abA}	34^{aA}	23^{bcA}	23^{bcA}	23^{bcA}	24^{bcA}	25	**	14.83
	DS20	CR	F_7	17^{bB}	19^{abB}	23^{aA}	20^{abA}	20^{abA}	23^{aA}	20	**	15.30
	DS20	NKP	F_7	14^{cB}	15^{cBC}	17^{bB}	14^{cB}	16^{bcB}	25^{aA}	17	**	15.00
	Mean			20	23	21	19	20	24			
NPP	WS20	CR	F_6	29^{bA}	34^{aA}	20^{cA}	20^{cA}	21^{cA}	22^{cA}	24	**	19.46
	DS20	CR	F_7	16^{bB}	17^{abB}	20^{aA}	18^{abA}	17^{abB}	20^{aAB}	18	**	11.75
	DS20	NKP	F_7	13^{bB}	13^{bBC}	13^{bB}	12^{bB}	13^{bC}	18^{aB}	14	**	19.76
	Mean			19	21	15	14	14	20			
PL (cm)	WS20	CR	F_6	22.4^{bA}	23.1^{bA}	21.6^{bA}	21.8^{bA}	17.2^{cA}	21.6^{aAB}	22.5	**	7.64
	DS20	CR	F_7	22.4^{bA}	22.0^{bA}	20.1^{cA}	20.5^{cA}	19.9^{cA}	25.5^{aA}	21.7	**	5.27
	DS20	NKP	F_7	23.3^{aA}	21.0^{abA}	19.6^{bA}	19.5^{bA}	18.6^{bA}	23.6^{aA}	21.0	**	8.29
	Mean			22.7	22	20.4	20.6	18.6	23.6			
SS (%)	WS20	CR	F_6	89.0^{aA}	82.7^{bA}	75.5^{cA}	82.7^{bA}	87.8^{abA}	81.55^{bcA}	83.2	**	4.68
	DS20	CR	F_7	67.7^{bcB}	70.5^{aB}	68.5^{bB}	70.2^{abB}	68.4^{bB}	72.0^{aB}	69.6	**	8.11
	DS20	NKP	F_7	67.9^{bB}	69.8^{abB}	67.5^{bB}	71.5^{aB}	66.2^{cB}	71.3^{aB}	69.3	**	7.14
	Mean			74.9	79.9	88.0	90.4	92.4	75.0			
GW (g)	WS20	CR	F_6	28.8^{cdA}	29.7^{cA}	30.0^{bcA}	32.4^{aA}	31.7^{abA}	27.6^{dA}	29.2	**	3.82
	DS20	CR	F_7	26.4^{abA}	22.4^{bB}	29.0^{aA}	28.6^{aAB}	28.3^{aAB}	26.8^{abA}	28.3	**	5.48
	DS20	NKP	F_7	23.8^{bB}	22.4^{bB}	30.5^{aA}	31.6^{aA}	30.2^{aA}	22.9^{bB}	26.1	**	4.99
	Mean			26.3	25.7	29.8	31.1	30.1	25.8			
GY (t/ha)	WS20	CR	F_6	8.83^{aA}	8.01^{abA}	8.32^{bcA}	8.25^{bcA}	6.42^{cdA}	5.88^{dB}	7.62	**	14.17
	DS20	CR	F_7	4.36^{cB}	4.05^{cB}	5.23^{bB}	5.65^{bB}	4.00^{cB}	6.93^{aA}	5.04	**	6.86
	DS20	NKP	F_7	2.01^{cC}	2.12^{dC}	2.15^{cC}	3.13^{bC}	2.47^{cC}	4.58^{aC}	2.74	**	9.30
	Mean			5.07	4.43	5.23	5.68	4.30	5.80			
L:W ratio	WS20	CR	F_6	2.00^{bA}	1.49^{dBC}	2.19^{bA}	1.80^{cA}	1.98^{bcA}	3.45^{aA}	2.17	**	3.82
	DS20	CR	F_7	1.97^{bA}	1.76^{cB}	1.66^{cB}	1.60^{cA}	1.70^{cB}	3.47^{aA}	2.03	**	8.79
	DS20	NKP	F_7	1.98^{bA}	2.18^{bA}	1.99^{bA}	1.60^{cA}	1.69^{cB}	3.23^{aA}	2.12	**	8.39
	Mean			1.98	1.81	1.95	1.67	1.79	3.38			
AL (%)	WS20	CR	F_6	15.88^{cAB}	17.89^{bA}	19.15^{aA}	17.68^{bA}	15.48^{cB}	18.24^{bA}	17.39	**	2.57
	DS20	CR	F_7	16.58^{bcA}	17.68^{bA}	19.52^{aA}	17.88^{bA}	15.58^{cB}	18.52^{abA}	17.63	**	3.85
	DS20	NKP	F_7	17.85^{bA}	18.21^{abA}	19.88^{aA}	18.68^{abA}	17.56^{bA}	19.55^{aA}	18.62	**	3.82
	Mean			16.77	17.92	19.52	18.08	16.20	18.76			
AK (1–7)	WS20	CR	F_6	5	6	6	5	6	6	6	ns	2e-16
	DS20	CR	F_7	5	5	6	5	5	6	5	ns	2e-12
	DS20	NKP	F_7	5	5	6	5	5	6	5	ns	2e-15

DF = days-to-flowering; PH = plant height; PL= panicle length; NTP = number of tillers per plant; NPP = number of panicles per plant; SS = seed set; GW = 1,000-grain weight; GY = grain yield; W:L = grain width-to-length ratio; AC = amylose content, AK = alkaline test.

DS = dry season 2020; WS = wet season 2020; CR = Chiang Rai province; NKP = Nakhon Pathom province; CV = coefficient of variation.

Different lowercase superscript in each row (agronomic traits, cooking quality) are significantly different ($p < 0.05$).

Different capital superscripts in each column (genotype) between seasons are significantly different ($p < 0.05$).

*, ** = significant at $p < 0.05$ and $p < 0.01$, respectively.

In addition, the plant type, paddy grains, milled grains and cooked rice of these lines or varieties are shown in Figs. 3A and 3B. The yield and agronomic traits were significantly ($p < 0.05$) different among the seasons, location and among lines or varieties in each season. In the F_6 generation, the days-to-flowering value of PS171-31-1 (93 d) was intermediate between the japonica (67 d) and indica (108 d) parent varieties, while SSD125-7 (79 d) was not significantly different from the DOA1 (75 d) and DOA2 (73 d). In addition, the japonica (KH) and indica (PTT1) parents had the shortest and longest days-to-flowering. The plant height of PS171-31-1 (115.0 cm) was the highest, while that of SSD125-7 (108.7 cm) was not significant different with the value of DOA2 (107.3 cm) but was greater than that of DOA1 (89.3 cm) and KH (66.2 cm). In addition, the number of tillers per plant, number of panicles per plant and grain yield of the two lines were higher than those of the japonica parent but lower than those of the indica parent. However, the grain yields of PS171-31-10 (8.83 t/ha) and SSD125-7 (8.01 t/ha) were not significantly different from those of DOA1 (8.32 t/ha) and DOA2 (8.25 t/ha) (Table 4).

In the F_7 generation, the yield trial was conducted at two locations (Chiang Rai and Nakhon Pathom) in DS20. The results showed that most agronomic traits were slightly different between the two locations except for grain yield (Table 4). The grain yield in Chiang Rai was higher than that in Nakhon Pathom in every line or variety. The grain yields of the two candidate lines in Chiang Rai were not significantly different from those of Koshihikari but were lower than those of DOA1, DOA2 and PTT1. This result was consistent with that in Nakhon Pathom except for SSD125-7, which had the lowest grain yield (Table 2).

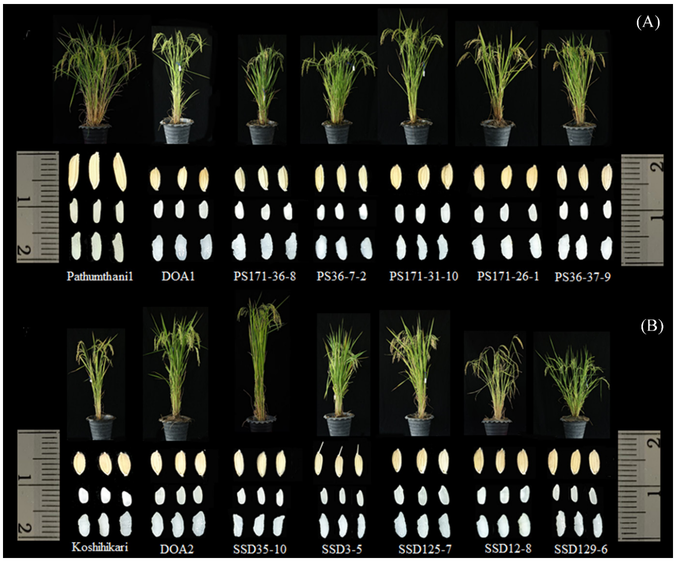

Fig. 3 Plant type, paddy grain, milled grain and cooked grain of five breeding lines: (A) pedigree selection (PS); (B) single seed descent (SSD) in F_5 generation compared with their parents (Pathumthani1 and Koshihikari) and commercial varieties (DOA1 and DOA2), where scale is in millimeters

Stability of grain yield

The yield trial in F_6 (WS20) performed better than F_7 (DS20) for both locations. The plant height, number of tillers per plant, number of panicles per plant, seed set and grain yield in WS20 were much higher than those in DS20. However, the days-to-flowering, panicle length and grain weight were slightly different between the two seasons. The biplot graph between PCA1 and PCA2 of the grain yield in Fig. 4A indicated that DOA2 was also highly stable, while PS171-31-10 and SSD125-7 had high grain yields only in WS20 in Chiang Rai. Thus, the two candidate lines may be suitable to grow in the rainy season, especially in Chiang Rai province. In addition, the grain yield from Nakhon Pathom was lower than that from Chiang Rai province in every line or variety. The genotype and genotype × environment biplot of grain yield stability is shown in Fig. 4B. The relationship between the mean grain yield and PC1 indicated that DOA1 and DOA2 had the highest stability (PC1 = -0.17 and 0.12, respectively) and higher yields than PS171-31-10 and SSD125-7. However, PS171-31-10 had greater stability and grain yield than SSD125-7. When considering the season, the mean grain yield in WS20 in Chiang Rai was the highest while that in DS20 in Nakhon Pathom was the lowest. Thus, in term of grain yield, PS171-31-10 had more grain yield and was more stable than SSD125-7. In addition, the rainy season in Chiang Rai province was suitable for both PS171-31-10 and SSD125-7 rather than the dry season.

Grain quality

The grain quality (grain size and amylose content) differed slightly for WS20 and the two locations in DS20. The grain size of the candidate lines and japonica varieties was identified as short grain based on the length-to-width ratio of milled grain. The amylose content of all lines or varieties was less than 20%, which was recognized as soft rice when cooked. In addition, the gelatinized temperature based on the alkali spreading value was not significantly different for WS20 and DS20 and they were classified as having low cooking times (Table 4).

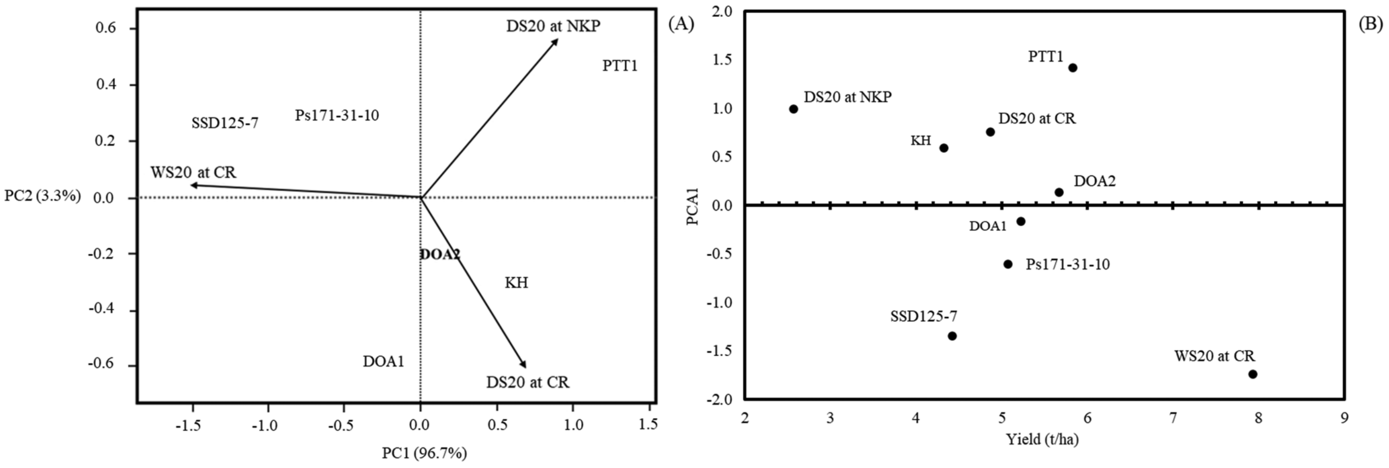

Fig. 4 Biplot graph of principal component (PC) analysis PC1 score *versus* PC2 score for: (A) grain yields of two candidate lines (PS171-31-10 and SSD125-7) and control varieties (DOA1 and DOA2); (B) biplot graph of PC1 score *versus* mean grain yield of two candidate lines and control varieties, where DS20 = dry season 2020; WS20 = wet season 2020; CR = Chiang Rai; NKP = Nakhon Pathom; KH = Koshihikari; PTT1 = Pathum Thani1; SSD = single seed descent; PS = pedigree selection

The pasting temperature, peak viscosity, breakdown, final viscosity, setback, peak time and peak temperature of the rice flour samples were significantly different among the lines or varieties (Table 5). DOA2 had the highest peak viscosity (1122.0 cp), followed by PTT1 (882.5 cp) and DOA1 (864.0 cp), while KH (610.0 cp) and SSD125-7 (421.0 cp) have relatively low peak viscosities compared to the other rice lines or varieties. The final viscosity values showed the same tendency as for peak viscosity. In addition, the setback results showed that PTT1 and DOA2 had higher recovery than the other lines or varieties, while KH had the lowest setback value. In summary, PTT1 and DOA2 had a harder texture than the other rice lines or varieties when cooked. In addition, KH had the softest texture, followed by PS171-31-10 and SSD125-7.

Evaluation sensory of cooked rice and 2AP content

The sensory test evaluated only milled grain derived from Chiang Rai in DS20. The results revealed that the highest overall scores were for PS171-31-10 (37.58 overall score), the second highest was for SSD125-7 (35.37 overall score), while DOA1, DOA2 and KH were grouped as the third highest sensory scores (Figs. 5A). Therefore, the two breeding lines were assigned to this breeding program in terms of cooking quality.

The 2AP content of the two candidate lines and their parents was analyzed based on brown grains derived from Chiang Rai and Nakhon Pathom provinces in DS20. SSD125-7, which received the *badh2* gene from the indica parent (PTT1), had 2AP contents of 0.60 ppm and 0.40 ppm in Chiang Rai and

Table 5 Mean (± SD) of rice flour properties of two candidate lines (PS171-31-10 and SSD125-7) compared with their parents (Koshihikari and Pathumthani1) and control varieties (DOA1 and DOA2)

Line/Variety	Pasting temperature (°C)	Viscosity (Rapid Visco Analyzer units)				Peak time (min)	Peak temperature (°C)
		Peak viscosity	Breakdown	Final viscosity	Setback		
PS171-31-10	83.58±0.60^{b}	457.5±4.9^{d}	126.0±5.6^{cd}	614.5±4.9^{d}	157.0±0.0^{bc}	5.84±0.05^{c}	76.08±1.05^{a}
SSD125-7	84.00±0.07^{b}	421.0±4.2^{d}	84.5±0.7^{d}	571.5±9.6^{d}	150.5±6.3^{bc}	6.00±0.00^{bc}	78.76±0.98^{a}
DOA1	76.70±0.00^{c}	864.0±8.4^{b}	178.5±2.1^{bc}	1057.5±6.3^{b}	193.5±2.1^{ab}	6.20±0.09^{ab}	69.79±0.58^{b}
DOA2	75.50±0.63^{c}	1122.0±9.8^{a}	215.0±3.6^{ab}	1.55.5±6.3^{a}	233.5±6.2^{a}	6.37±0.23^{a}	67.25±1.25^{bc}
Koshihikari	74.65±0.63^{c}	610.5±9.9^{b}	163.5±3.5^{bc}	723.5±7.5^{c}	113.0±5.6^{c}	5.96±0.05^{bc}	65.15±0.75^{c}
Pathumthani1	88.00±0.00^{a}	882.5±9.1^{b}	259.0±7.0^{a}	1131.5±2.1^{b}	249.0±7.5^{a}	5.96±0.05^{bc}	68.51±1.89^{b}
F test ($p < 0.05$)	**	**	**	**	**	*	*
CV%	6.87	6.87	4.03	6.87	6.87±	2.57	3.25

CV = coefficient of variation.

Different lowercase superscripts in each column are significantly different ($p < 0.05$).

*, ** = significant at $p < 0.05$ and $p < 0.01$, respectively.

Nakhon Pathom, respectively. However, the 2AP content in PTT1 was higher than that of SSD125-7 in Chiang Rai (1.45 ppm) and Nakhon Pathom (0.65 ppm). In contrast, PS171-31-10, DOA1, DOA2 and KH had no 2AP detected in their rice grains (Fig. 5B).

Evaluation of biotic stress resistance

Bacterial leaf blight and blast resistance were evaluated in the yield trial with WS20 (F_6). DNA markers for bacterial leaf blight resistance (*Xa21*) and blast resistance (*Pi-ta*) were detected in PS171-31-10 and SSD125-7. These lines were screened for their reactions to bacterial leaf blight and blast resistance. Surprisingly, both lines, together with KH, were identified as susceptible to bacterial leaf blight (lesion length >9.1 cm) in every strain. However, PTT1 (indica parent) was identified as moderately resistant to XONS2 and NP3-5 and susceptible to the other strains (Fig. 5C). Therefore, only the *Xa21* gene did not confer resistance to bacterial leaf blight. Although only the *Pi-ta* gene was observed, PS171-31-10, SSD125-7 and their parents were identified as resistant to leaf blast in mixed strains 1–6 and moderately resistant in mixed strain 7 (Fig. 5D).

Discussion

In this research, intersubspecific crossing between japonica and indica was performed, and the consumable product in the F_2 generation tended to show quantitative segregation among all agronomic traits, while transgressive segregation was also found in all agronomic traits. It could be suggested that the epistasis, overdominance and complementary action of additive genes controlled these traits (Mohammadi et al., 2013; Bassuony et al., 2022). In addition, Wang et al. (2014) suggested that agronomic traits were controlled by a large number of genes, directly or indirectly affecting the performance of agronomic traits. Furthermore, Ponce et al. (2020) reported that the segregation of grain size was controlled by quantitative genetics that combined grain length and grain width. However, the *GRAIN SIZE3* (*GS3*) gene can be used as the major quantitative trait loci (QTL) to identify grain length differences between indica and japonica types (Fan et al., 2006; Saichompoo et al., 2021). In the current study, *GS3* could be detected in selected short-grain lines in F_5. Thus, *GS3* could be used as the DNA marker for grain size in the breeding program.

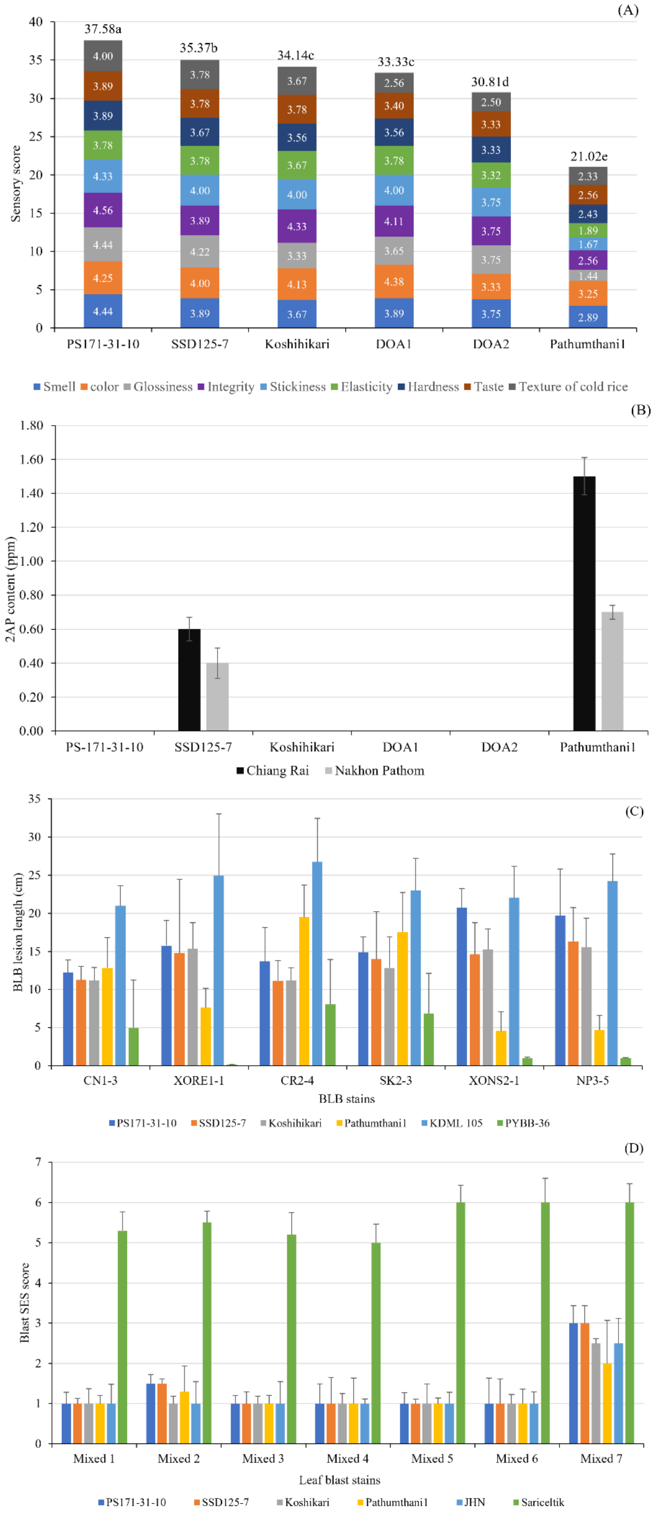

Fig. 5 Performance of the candidate lines (F_7) derived from Koshihikari x Pathumthani1 (PS171-31-10 and SSD125-7) compared with their parents and commercial varieties in dry season 2020: (A) sensory tests; (B) 2AP content; (C) resistance to six strains of bacterial leaf blight (BLB); (D) resistance to seven mixed strain groups of blast, where ppm = parts per million and error bars indicate SD.

Notably, the F_2 segregation of seed set varied in the range 0–100%, while the parent varieties had seed sets of 66% and 78% in KH and PTT1, respectively. These corroborated the results of Liu et al. (1996), who found that the spikelet fertility of intersubspecific progenies varied widely among the crosses from almost completely sterile to fully fertile. Negrao et al. (2008) reported that gene introgression from indica to japonica rice had problems of high sterility and poor plant type, with some linkage drag in the progenies. Approximately 50 loci for interspecific fertility have been identified in rice, including loci that cause female gamete abortion and those that induce pollen sterility (Ouyang et al., 2009). Finally, the selection of short-grain characteristics along with cooking quality can be performed objectively using either of the PS or SSD methods. However, the current results suggested that the spikelet sterility in F_2 was a result of natural selection that improved the spikelet fertility in the F_3 progenies and the distribution of seed set in F_3 from the SSD method was skewed to the positive. In addition, the seed set of selected lines from F_4 to F_7 was identified as spikelet fertility.

When the F_2 and F_3-SSD populations were compared, no significant difference was found regarding the distribution of subspecies characteristics in the two populations. This was the same result as Wang et al. (2014), who suggested that SSD could maintain genotype frequencies between generations. After that, the five breeding lines in F_5 from each method were selected to evaluate the performance of the breeding results. The PS method was superior to the SSD method in terms of the mean grain yield and seed set. However, the mean days-to-flowering, plant height and 1,000-grain weight from the SSD method were higher than those from the PS method. In addition, the number of tillers/plant, number of panicles/plant and length of the panicle were not significantly different between the PS and SSD methods. The results were similar to those from another study, in which the PS method was reported to be superior to the SSD method in terms of grain yield in the F_4 and F_5 generations in rice (Kanbar et al., 2011).

In addition, Lungu et al. (1987) successfully used the PS method in early generations to increase the grain yield in spring wheat. However, the current research achieved successful individual selection from the PS method for grain yield in early generations, which was contrary to this other report. In addition, Collard et al. (2017) suggested that it was more appropriate to compare the best lines produced from each of the different methods together rather than to compare specific population means because one of the ultimate goals of breeding is to identify transgressive segregants. Therefore, in the current study, the three selected lines from the PS method had higher grain yields relative to the mean values of both the PS and SSD methods.

In the F_5 generation, *wx^b* from KH, which is the major factor for good cooking quality in terms of the Japanese rice standard (Kobayashi et al., 2018), was detected in four selected lines from the SSD method, while these markers were less frequently detected in selected lines from the PS method. Owing to the fact that the F_2 selection in the PS method was mainly focused on short grain by phenotyping and then only 9 of 200 plants were identified as short grain, the gene recombination between grain size (*GS3*) and flour type (*wx^b*) may not be enough because the F_2 population was too small due to spikelet sterility. This was consistent with Lalić et al. (2008), who suggested that the desired genes might disappear if selection is focused on key traits from the PS method. In contrast to the SSD method, the selection was started at F_4, so there was an opportunity to find every desired gene using MAS.

Finally, PS171-31-10 (harboring *GS3, wx^b, SSIIa, Xa21* and *Pi-ta*) and SSD125-7 (harboring *GS3, wx^b, badh2, Xa21* and *Pi-ta*) were selected for yield trials, flour properties and sensory tests with their parents and control varieties in the F_7 generation. The yield trials showed that the phenotypes of the two candidate lines, for days-to-flowering, plant height and grain shape, were more similar to the japonica parent and control varieties, while, in contrast, grain weight was similar to the indica parent. Therefore, the selection objective that focuses on short grain also exposes some traits introgressed from japonica rice, especially early flowering. Kim et al. (2018) indicated that the functional *Hd1* alleles of temperate japonica induced early flowering in tropical regions. This may suggest that the genotype of both candidate lines may still have *Hd1* alleles, like japonica varieties. In addition, the grain yields of the two candidate lines along with the japonica parent and control varieties in Chiang Rai (high altitude) were higher than that of Nakhon Pathom (low altitude). Thus, the genotype of the two candidate lines, like that of the japonica parent and control varieties, could adapt to the environment at higher latitudes or higher altitudes in the northern part of Thailand, rather than the lower altitude areas in the central part of Thailand.

The results of the flour properties and sensory test showed that two candidate lines were significantly different from KH, DOA1 and DOA2, although these lines or varieties had the same *wx^b* gene. According to Juliano et al. (1993), the *wx^b* gene resulted in a moderate amylose content in rice grains. However, other *Wx* alleles have also been reported in japonica, including *Wx^{la}* and *Wx^{mq}* with low amylose content (Zhou et al., 2020).

Thus, it can be suggested that the differences in flour properties and sensory testing among the lines or varieties in the current experiment were also caused by the expression of other *Wx* alleles besides *wx*^{b}. In addition, the sensory test of PS171-31-10 produced the highest score, although the *badh2* gene was not detected in this line. This finding may suggest that consumers of Japanese food may be unfamiliar with aroma in rice (Hori et al., 1992; Calingacion et al., 2014). However, SSD125-7 in which *badh2* gene was detected may be accepted in countries where consumers prefer aromatic rice, such as Thailand. Furthermore, the *SSIIa* genes that are involved in amylopectin chain elongation also control the eating quality of cooked rice grains by altering starch characteristics, such as amylose content, gelatinization temperature and gel consistency (Umemoto, 2018). Thus, *SSIIa* is another key factor that influences eating quality (Sun et al., 2005). In this breeding program, *SSIIa* was found in PS171-31-10 but not in SSD125-7 which well agreed with the higher sensory score of PS171-31-10 than for SSD125-7. However, KH (japonica parent), which is a variety with high cooking quality in temperate regions, had the third highest sensory score, although it had both *wx*^{b} and *SSIIa* genes. It is possible that when temperate japonica is grown in hotter climates, such as tropical regions, the cooking quality and palatability suffer (Chun et al., 2015; Zhao et al., 2017). Therefore, the present study provided additional evidence for the effect of climatic conditions on cooking quality (Li et al., 2016). From the aforementioned characteristics of agronomic traits, grain shape, presence of *wx*^{b} and *SSIIa* and response to geography, it can be inferred that the genetic background of the two candidate lines was closer to that of the japonica parent (KH) than that of the indica parent (PTT1).

Considering bacterial leaf blight resistance, it was surprising that both candidate lines, together with KH, that contain the *Xa21* gene were identified as susceptible to six Thai pathotypes, although in the past, this gene was reported to provide broad-spectrum resistance against the Xoo races (Das and Rao, 2015). Therefore, the *Xa21* gene cannot be used as a single gene for BLB resistance in Thai rice breeding programs. Yugander et al. (2017) reported that single resistance genes cannot provide broad-spectrum resistance against the prevalent pathotypes. In addition, Ancheta et al. (2021) reported that *xa5* genes remained broad-spectrum resistant to bacterial leaf blight in almost all pathotypes in Thailand. Therefore, the *Xa21* gene may not be suitable for establishing resistance to pathotype in Thailand; thus, combining more than one resistance gene should be more effective to achieve broad bacterial leaf blight resistance.

Conclusion

The two promising lines generated from PS (PS171-31-10) and SSD (SSD125-7) combined with MAS methods improved the short-grain type and had similar potential for grain yield and cooking quality, compared to japonica commercial varieties. However, some target genes were missing in the selected lines based on the PS method because the selection procedure which focused on short grain was initiated in an early generation (F_2). On the contrary, selection based on the SSD method began at later generation (F_4) and thus was more likely to keep the desired genes better than the PS method. In contrast, the grain yield potential of the PS method was better than that of the SSD method due to the potential for phenotype selection from an early generation. However, the method chosen by various breeders will depend on their preferences and other factors, such as the genetic diversity of the parents, number of progenies, agronomic traits and the budget of the breeding program.

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