Original Research Article

Improvement of genetic purity of breeder seed by introducing line cultivation method for Myanmar's major rice cultivars

ABSTRACT

Aims: Since the genetic purity of rice seed significantly affects paddy yield and quality, many rice production countries have been attempting to improve seed quality. This study aimed to demonstrate the effectiveness of breeder seed production using line cultivation to ensure traceability of ancestral information by line and individual selection, to improve the genetic purity of breeder seed in Myanmar that has been degraded due to pedigree mixtures.

Study Design: Observational and analytic study design were used to evaluate the effect of the introduction of line cultivation method in practical activities of breeder seed production in Myanmar under the project for improvement of seed purity of breeder seed.

Place and Duration of Study: Department of Agricultural Research at Yezin, Ministry of Agriculture, Livestock and Irrigation, Myanmar, from June 2012 to December 2016.

Methodology: Using nine major rice cultivars in Myanmar, the line cultivation method was evaluated through measurement of the standard deviation and the variance component ratio in heading date, culm length, panicle length, and panicle number from 2012 to 2016. DNA polymorphism analysis by simple sequence repeat (SSR) markers was also conducted using breeder seed of the Sinthukha variety multiplied in 2013, 2014, 2015, and 2016 for the evaluation.

Results: Standard deviations of heading date, culm length, panicle length and panicle number in 2016 were significantly decreased compared to the first year of the introduction of the line cultivation method. Average flowering period among sister lines of all varieties was reduced from 8.25 days in 2013 to 5.25 days in 2016, and the uniformity of heading time among sister lines was improved. The variance component ratio of each trait in 2016 was the highest since 2013. The analysis of breeder seed by SSR markers revealed that the DNA polymorphism ratio of Sinthukha seed in 2016 was lower than that of 2013. This demonstrated that Sinthukha seed in 2016 had improved genetic purity. Consequently, it is considered that other cultivars multiplied by the line cultivation method have improved genetic purity as well.

Conclusion: Line cultivation is an effective method to improve genetic purity and maintain genetic stability of the breeder seed in Myanmar's rice cultivars. Genetically pure breeder seed would improve quality of downstream seed such as certified seed. Consequently, it is expected that productivity and quality of rice will be improved thus income of farmers will increase.

Keywords: Rice selection, Line cultivation, Simple sequence repeats

1. INTRODUCTION

Genetic characteristics of crop varieties are affected by genetic drift, out-crossing, mutation, and artificial mixture with other cultivars or lines [1, 2]. Theoretically, pure line

varieties of self-pollinated crop species are stable over generations. However, practical multiplication of breeder seeds (BS) needs a series of careful controls to preclude contamination of alien genetic elements in each error-prone process.

Rice (*Oryza sativa* L.), a self-pollinated crop species cultivated in Asia and worldwide, is a staple food, providing 31% of all food energy consumed in Asia, 8% in Africa, and 11% in Latin America [3]. Genetic purity of seeds greatly affects paddy yield and quality. Rice plants originating from seed sources with low genetic purity result in an uneven heading, ripening, and grain shape. Mixtures of immature rice, cracked rice, and red rice are also increased. Therefore, many rice exporting countries, like Cambodia, Laos, Myanmar and Vietnam have been attempting to improve seed quality. In Myanmar, genetically pure certified seed (CS) produced with proper quality control is rarely available to farmers and the disseminated effect of using recommended varieties such as high yielding variety (HYV) is concomitantly limited. This is probably one of the main reasons why the yield is stagnating at around 3.8-3.9 tons/ha between 2012 and 2016 [4].

In the Ayeyarwady region, which has the most extensive rice production in Myanmar, most of the seeds had been supplied from seeds exchanged among farmers, so-called farmer's good seeds, or self-saved seeds. This situation is attributed not only to the vulnerability of the agricultural extension systems for CS multiplication and distribution, but also to insufficient quality control of entire seed multiplication systems [5].

The Department of Agricultural Research at Yezin (DAR) currently adopts the panicle line method in BS multiplication. This procedure consists of three parts:

1. selection of several superior lines derived from seeds of single panicles.

2. bulk harvesting of panicles randomly selected from several hills without recording pedigree information.

3. transplanting of seedlings derived from one panicle as one line for maintenance for the following year's BS lines, while the rest of the seeds of bulk panicles are used for foundation seed (FS) multiplication.

This method leads to a situation where pedigree information is lost in every generation and BS pedigree cannot be traced back. It was also difficult to find and eliminate off-type lines or individuals with this method. Therefore, genetic purity of BS had been vulnerable to contamination with off-type and outcrosses because the contamination with off-type and outcrossed varieties has not been addressed by back-tracing based on past pedigrees. Repeated BS multiplication without pedigree information obscured the concept of pedigree, and the pedigree mixture was accelerated by transplanting multiple seedlings par hill instead of a single seedling.

The variety degradation caused by such a practice was remarkable in Kyawzeya (KZY), Shwewartun (SWT), Ayeyarmin (AYM) and Pawsanyin (PSY) rice varieties, which were released more than 30 years ago. A modern variety, Sinthukha (STK), derived from a cross between Manawthukha (MTK) and IRBB21, was released as a cultivar with insufficient backcross for fixation of the bacterial leaf blight (BLB) resistance gene *Xa21* [6], and DAR currently found lines which are susceptible to BLB.

Line cultivation, on the other hand, ensures traceability of ancestral pedigree information by line, and individual selection may be promoted in BS and FS multiplication [7]. In Madagascar, it was reported that the ratio of off-type plants decreased as the result of the introduction of the line cultivation method for BS multiplication of rice [8, 9].

DNA polymorphisms in the nuclear and cytoplasmic genome ('DNA fingerprinting') has been adopted in the identification of crop varieties in plants and animals. This is because

genetic characteristics based on DNA polymorphisms (such as insertion/deletion polymorphisms or single nucleotide polymorphisms (SNP)) are not influenced by season, year, developmental age, or environmental factors [10, 11]. An appropriate type of marker in the context of the time, cost, type of variety (pure line or cross-pollinated species) is chosen. Identification of crop varieties by DNA marker has been conducted in barley [12], and cotton [13]. Utilization of DNA markers for the registration of varieties according to a distinctness, uniformity, and stability (DUS) assessment promises to reduce time and cost in the evaluation of plant characteristics. This will lead to the adoption of a DNA-based system which is endorsed by the International Union for the Protection of New Varieties of Plants (UPOV). In rice, abundant simple sequence repeats (SSR) markers are reported [14, 15] and it is relatively feasible and cost-efficient to identify registered varieties with a thermal cycler and agarose-gel electrophoresis, both of which are standard in many molecular biology laboratories [16, 17].

This study aimed to demonstrate the effect of the line cultivation method with traceability of ancestral pedigree information, rather than the conventional method. The line cultivation method is expected not only improving genetic purity of BS but also enhancing quality of FS, RS and CS for a stable rice production in Myanmar. BS multiplication was conducted at DAR from 2012 to 2016 and changes in dominant agronomic traits such as heading date (HD), culm length (CL), panicle length (PL) and panicle number (PN) were assessed alongside DNA polymorphisms of BS.

2. MATERIALS AND METHODS

2.1 Plant materials

Nine major rice cultivars in Myanmar were used in this study. Theedatyin (TDY) is the most popular cultivar in the irrigated area of the Southern Delta area in the dry season (DS). The cultivation area of MTK is the largest in Myanmar. STK, which is resistant to bacterial leaf blight (BLB), is derived from a cross between MTK and IRBB21 and carries the BLB resistance gene, *Xa21*. Ministry of Agriculture Livestock and Irrigation (MOALI) has been giving priority to STK for expansion of its sowing area and promoting the replacement of MTK with STK. Sinthwelatt (STL) is a long-grain cultivar and is planted in the Southern Delta zone. KZY and SWT are widely grown in the Northern Delta zone. A long culm cultivar, AYM, was introduced first to the Ayeyarwady Delta in the 1980s, and then extended to the irrigated area of the central dry zones, such as the Sagain and Mandalay regions. A short-grain indigenous cultivar with aroma, PSY, is widely grown in the Ayeyarwady Delta and was introduced to the Shwebo District of the central dry zone several years ago. Hnangar (HK) is also an indigenous variety and is widely grown in the deepwater area of the southern Ayeyarwady Delta [18].

In the seed multiplication system of Myanmar, BS is produced by the DAR. FS and RS are produced by the seed farms under the Department of Agriculture (DOA). CS is produced by CS production farmers under the supervision of the DOA extension officers [5]. BS samples used in this study were collected from DAR. CS samples were collected from CS producers. Rice harvests produced from CS producers from BS purified in this experiment, as well as harvests from their own seeds, were collected for this experiment.

2.2 Growth condition of plants

Seeds were treated in a fungicidal agent (Homai WP, Nippon Soda Co. Ltd., Japan) and germinated in a seedling bed before being transplanted to the paddy fields about 3 to 4 weeks after sowing (Table 1). The seedlings were transplanted in 24.5 × 30.5 cm spacings in single plants per hill. Nursery, paddy preparation and field management followed the conventional methods of the DAR.

2.3 Line cultivation method

Self-pollinated progenies derived from a single plant were grown as one line. Multiple lines derived from plants belonging to the same lines in the previous year were grown as sister lines. The demand for FS production determined the number of sister lines of each cultivar. Pedigree records were maintained to ensure traceability of ancestral pedigree information. In order to avoid contamination by natural crossing and variety admixture, careful roguing was practiced, and two rows of border plants were discarded, and in the postharvest process, equipment and the working floor were thoroughly cleaned.

The selection was conducted in three steps:

1. group selection of sister lines.

2. selection of single lines within the selected sister lines.

3. selection of individuals within the selected single lines.

The groups of sister lines with high uniformity in agronomic traits such as heading time, plant type and plant height, and visual observations selected a few off-type plants among groups of sister lines. A group of sister lines with low differences in growth stages between lines, and low variations within the lines, was defined as a 'superior' group of sister lines for candidate selection. For single line selection, the line with uniform HD and a low coefficient of variation (CV) in CL, PL, and PN of 20 individuals in a line, was selected. The rest of the lines were bulked together and used for FS multiplication. The procedures are summarized in Fujii *et al*. (2016) [19]. Date of sowing, transplanting and number of lines for each cultivar are shown in Table 1. Regarding STK, the lines susceptible to BLB were discarded after the inoculation test in the dry season of 2012. The inoculation test in STK was conducted in parallel with the line selection in 2013 in order to confirm the BLB resistance of the selected lines.

Table 1. Sowing and transplanting date of breeder seed multiplication at from 2012 to 2016

Variety[1)]	2012/13		2013/14		2014/15		2015/16		2016/17	
	SD[2)]	TD[3)]	SD	TD	SD	TD	SD	TD	SD	TD
Theedatyin (TDY)	14-Jun	11-Jul	29-Jun	25-Jul	16-Jun	8-Jul	8-Jul	29-Jul	22-Jun	15-Jul
Manauthukha (MTK)	14-Jun	11-Jul	14-Jun	9-Jul	16-Jun	8-Jul	9-Jul	31-Jul	23-Jun	16-Jul
Sinthukha (STK)	14-Jun	11-Jul	3-Jul	30-Jul	16-Jun	8-Jul	9-Jul	31-Jul	24-Jun	17-Jul
Sinthwelatt (STL)	14-Jun	11-Jul	19-Jun	14-Jul	17-Jun	8-Jul	24-Jun	15-Jul	21-Jun	14-Jul
Kyawzeya (KZY)	14-Jun	11-Jul	22-Jun	13-Jul	17-Jun	8-Jul	23-Jun	15-Jul	22-Jun	15-Jul
Shwewartun (SWT)	14-Jun	11-Jul	22-Jun	13-Jul	17-Jun	8-Jul	23-Jun	15-Jul	23-Jun	16-Jul
Ayeyarmin (AYM)	14-Jun	11-Jul	1-Jul	27-Jul	18-Jun	8-Jul	9-Jul	31-Jul	23-Jun	16-Jul
Pawsanyin (PSY)	14-Jun	11-Jul	28-Jun	24-Jul	18-Jun	8-Jul	8-Jul	29-Jul	21-Jun	14-Jul
Hnangar (HK)	14-Jun	11-Jul	19-Jun	14-Jul	16-Jun	8-Jul	8-Jul	29-Jul	21-Jun	15-Jul

[1)] Abbreviations of varieties are represented in parenthesis. [2)] SD: Sowing date. [3)] TD: Transplanting date.

2.4 Trait evaluation

Heading is defined as the date when the first panicle is exerted from the leaf sheath of the hill's main culm. Traits for the first heading date, heading date, and the full heading date were defined as days to heading when 10-20%, 40-50%, and more than 90% of plants were heading in a line, respectively. Heading duration was defined as the duration from the first heading date to the full heading date. A trait for CL was defined as the length from the ground to the longest panicle neck in a hill. PL was defined as the length

from the panicle neck to the panicle tip on the longest culm in a hill. PN was defined as the number of panicles, excluding late-heading panicles. For CL, PL, and PN, twenty plants in a line were evaluated. For STK in 2012, HD, CL, PL, and PN of 29 groups were collected since the variation between groups was great. As for the other cultivars in 2012, groups were selected by visual observation, and these data were not taken.

2.5 Data analysis

2.5.1 Standard deviation

Agronomic traits of HD, CL, PL and PN were evaluated by average (Av.) and standard deviation (SD). The effectiveness of the line cultivation method in each year was evaluated by comparing the SD of the first year with that of the year concerned using the *F*-test.

2.5.2 Variance component

We assumed that the linear model for phenotypic value y_{ijk} of a k-th plant in j-th sister line of the i-th cultivar would be

$$y_{ijk} = \mu + g_i + s_{ij} + e_{ijk}$$

where μ is a population mean, g_i is the effect of cultivar i and s_{ij} is the effect of j-th sister line of the i-th cultivar, and e_{ijk} is an error. The total phenotypic variance σ_P^2 was partitioned to the variance component of cultivar means $\sigma_{cultivar}^2$ and sister lines means within a cultivar $\sigma_{sisters}^2$ by estimation as

$$\sigma_P^2 = \frac{\sum_i \sum_j \sum_k \left(y_{ijk} - y_{...}\right)^2}{n-1}$$

$$\sigma_{sisters}^2 = \frac{\sum_i \sum_j \left(y_{ij\cdot} - y_{i\cdot\cdot}\right)^2}{(n_s - 1)}$$

$$\sigma_{cultivar}^2 = \frac{\sum_i (y_{i\cdot\cdot} - y_{...})^2}{n_c - 1}$$

where n, n_s, and n_c represent numbers of plants, sister lines and cultivars. Dots ($\cdot$) represent means in the dotted index. This is a nested analysis of variance (ANOVA) to divide total phenotypic variance to three variance components of cultivars (genotypes), sister lines within cultivars, and residuals. These parameters were calculated as *aov()* function for the ANOVA in statistical software R [20]. Because the data had unbalanced numbers of sister lines by cultivars and traits, *p*-values were not estimated. The effectiveness of the line cultivation method in each year was evaluated by variance component ratio (VCR) in total phenotypic variance as $\sigma_{cultivar}^2/\sigma_P^2$ representing for cultivar means and $\sigma_{sisters}^2/\sigma_P^2$ representing for means of sister lines within a cultivar. The $\sigma_{cultivar}^2/\sigma_P^2$ is equivalent to broad-sense heritability. Since HD data of individual plants were not available due to the methodology of data collection, only σ_{sister}^2 was not estimated for HD.

2.6. DNA marker analysis

BS samples for DNA marker analysis were randomly collected from BS seeds of STK for FS multiplication. Single, unhulled rice seeds were put into each of 96-well deep-well plates with two metal beads and 150 µl of extraction buffer. The extraction buffer contained 100 mM of Tris-HCl (pH 8.0), 100 mM of NaCl and 4.5 unit of amylase. The

rice grains were briefly crushed with a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) at 1,900 rpm, twice, for 30 seconds to promote extraction of genomic DNA from the aurone layer and embryo. After adding 150 µl of extraction buffer, the samples were incubated for an hour at 60 °C for digestion of starch by amylase. After centrifugation at 2000 rpm for 5 min, 50 µl of supernatant and 50 µl of isopropyl alcohol were mixed in new 96-well plates to extract the DNA pellet by centrifugation at 3000 rpm for 10 min. The DNA pellets were dissolved in 100 µl of 0.1x TE buffer. Simple sequence repeat (SSR) markers were used for molecular polymorphism analysis. Polymerase chain reaction (PCR) were performed in a 15 µL reaction mixture containing 50 mM KCl, 10 mM Tris·HCl (pH 9.0), 1.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM primer, 0.75 units of *Taq* polymerase (Takara, Otsu, Japan), and 5 µl of template DNA solution in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). PCR conditions were 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. Amplified fragments of DNA after PCR were electrophoresed on a 4% agarose gel and then photographed with UV trans-illuminator (Printgraph, ATTO, Tokyo, Japan). Ninety-three SSR markers were used for a whole-genome survey to detect polymorphisms of STK in 2014 [21], and four markers were selected (*RM3340*, *RM1248*, *RM8121*, and *RM6925)* for polymorphism analysis of other STK seeds.

3. RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Agronomic trait analysis

As regard to the BS lines of 2012, ten moderate hills were selected from each of the ten superior lines of BS which were multiplied by the conventional method of DAR in 2011. These ten lines originated from bulk panicles without ancestral pedigree records. From 2012, the introduction of the line cultivation method was started, with line selection and individual selection with traceability of ancestral pedigree information. First, 20-30 moderate individuals were selected from each panicle line as the BS lines of the next season and HD, CL, PL and PN of these individuals were measured in nine cultivars from 2012 to 2016 (Tables 2-5). Since the SD is a parameter independent of the average in a normal distribution, SD was shown in a comparison of genetic purity of cultivars over these years. According to the advancement of generations, the SD of HD (Table 2), CL (Table 3), PL (Table 4) and PN (Table 5) tended to improve by cultivar from the first year when trait evaluation was started. Compared with the first year, the SD of all agronomic traits in 2016 was lower for all cultivars except for STL and SWT. The results of the *F*-test also showed that the SD of all agronomic traits decreased significantly between the initial year and the latter generations (Table 6).

Table 2. Standard deviation of heading date in breeder seeds from 2012 to 2016 in wet season.

Year	Item	Variety[1)]							
		TDY	MYK	STK	STL	KZY	SWT	AYM	PSY
2012	AV. (day)			106.4					
	SD.			1.64					
	df			156					
2013	AV. (day)	94.9	101.9	103.7	101.3	115.3	122.4	117.4	121.6
	SD.	1.30	1.73	1.56	0.61	0.90	0.83	1.64	1.14
	df	10	7	19	13	14	14	14	4
2014	AV. (day)	85.8	98.1	99.1	95.9	103.9	109.1	110.1	107.9
	SD.	1.18	1.29	1.18	1.09	0.94	0.81	1.29	1.22
	df	15	15	15	15	11	15	15	14
2015	AV. (day)	81.9	95.3	96.3	95.5	114.0	117.9	112.4	109.4
	SD.	0.79	0.86 *	0.89 **	0.89	0.76	0.99	1.15	1.24
	df	11	15	17	15	7	7	15	11
2016	AV. (day)	87.1	96.4	97.8	96.4	109.5	116.8	115.1	112.9
	SD.	0.64 *	0.52 **	0.73 **	0.76	0.53	0.92	0.77 **	0.57 *
	df	12	9	12	13	9	9	13	9

[1)] **, **, and *** represent significant difference of standard deviation from one in the first year at 5%, 1%, and 0,1%, respectively.*

Table 3. Standard deviation of culm length in breeder seeds from 2012 to 2016 in wet season.

Year	Item	Variety[1)]																			
		TDY		MYK		STK		STL		KZY		SWT		AYM		PSY		HK			
2012	AV. (cm)					95.1															
	SD.					4.71															
	df					289															
2013	AV. (cm)	78.7		94.6		93.4		109.1		112.5		122.4		136.4		137.0		148.2			
	SD.	5.30		6.15		4.03		5.79		6.50		6.17		9.37		7.55		11.86			
	df	99		119		79		79		79		79		59		59		78			
2014	AV. (cm)	87.1		97.7		106.5		114.7		118.6		126.3		151.7		159.7		149.9			
	SD.	3.30	**	3.88	***	3.67	**	3.51	***	3.79	***	6.81		10.77		4.63	**	7.60	***		
	df	39		59		79		59		59		59		79		39		59			
2015	AV. (cm)	73.6		94.6		93.2		110.5		126.9		132.9		151.1		126.0		137.8			
	SD.	3.17	***	2.94	***	2.49	***	2.47	***	4.60	**	5.73		7.94		6.57		4.08	***		
	df	59		79		79		39		79		59		79		79		79			
2016	AV. (cm)	73.1		91.4		98.4		115.2		114.4		129.2		159.1		118.0		144.4			
	SD.	3.81	**	3.20	***	3.19	***	5.57		5.12	*	3.86	***	4.56	***	3.72	***	5.40	***		
	df	79		59		79		79		79		79		79		59		79			

*[1)] *, **, and *** represent significant difference of standard deviation from one in the first year at 5%, 1%, and 0,1%, respectively.*

Table 4. Standard deviation of panicle length in breeder seeds from 2012 to 2016 in wet season.

Year	Item	Variety[1)]								
		TDY	MYK	STK	STL	KZY	SWT	AYM	PSY	HK
2012	AV. (cm)			25.4						
	SD.			1.73						
	df			289						
2013	AV. (cm)	26.2	25.6	25.6	32.5	28.7	30.2	25.6	26.1	29.4
	SD.	1.82	1.51	1.61	1.90	4.54	2.31	2.16	2.23	2.41
	df	99	119	79	79	79	79	59	59	78
2014	AV. (cm)	26.3	25.4	27.0	32.3	32.9	32.1	27.2	27.0	29.4
	SD.	1.64	1.01 **	1.15 ***	1.52	1.43 ***	2.03	1.61 *	1.91	1.60 **
	df	39	59	79	59	59	59	79	39	59
2015	AV. (cm)	27.1	24.9	25.9	33.9	30.2	28.3	25.9	26.3	28.84
	SD.	1.59	1.13 **	1.36 **	1.19 **	1.55 ***	1.49 ***	1.26 ***	1.19 ***	1.54 ***
	df	59	79	79	39	79	59	79	79	79
2016	AV. (cm)	25.8	24.0	25.3	32.3	29.8	30.2	27.3	26.8	29.84
	SD.	1.62	1.42	1.43 *	2.73	1.63 ***	1.58 ***	1.66 *	1.37 ***	1.73 **
	df	79	59	77	79	79	79	79	59	79

*1) *, **, and *** represent significant difference of standard deviation from one in the first year at 5%, 1%, and 0,1%, respectively.*

Table 5. Standard deviation of panicle number in breeder seeds from 2012 to 2016 in wet season.

Year	Item	Variety[1)]												
		TDY		MYK		STK	STL		KZY	SWT	AYM	PSY		HK	
2012	AV.					13.1									
	SD.					3.59									
	df					289									
2013	AV.	18.5		17.2		18.2	13.2		13.4	11.7	14.2	16.1		18.7	
	SD.	4.70		6.08		5.26	3.74		3.06	2.86	3.53	4.69		5.75	
	df	99		119		79	79		79	79	59	59		78	
2014	AV.	17.7		16.3		14.3	14.4		13.9	13.1	15.8	15.6		16.5	
	SD.	3.96		3.35	***	3.47	3.03		3.07	2.89	4.04	2.95	**	4.20	*
	df	39		59		79	59		59	59	79	39		59	
2015	AV.	16.5		17.9		16.8	14.7		15.8	14.7	17.8	14.2		15.6	
	SD.	3.09	***	3.43	***	3.52	2.42	**	3.50	3.83	3.87	3.02	***	3.52	***
	df	59		79		79	39		79	59	79	79		79	
2016	AV.	15.9		18.9		19.0	14.5		13.8	14.3	16.7	13.3		19.9	
	SD.	2.77	***	3.62	***	3.70	2.87	*	2.41	* 3.08	3.02	2.62	***	3.92	***
	df	79		59		79	79		79	79	79	59		79	

*[1)] *, **, and *** represent significant difference of standard deviation from one in the first year at 5%, 1%, and 0,1%, respectively.*

Next, total phenotypic variances were divided to each of variance component of cultivars (genotypes), sister lines within cultivars, and residuals, to identify which of variance components contributed decrease of total phenotypic variance of HD, CL, PL, and PN. The VCR of cultivar means was confirmed to be higher in order of HD, CL, PL, and PN among the nine cultivars used in this study (Tables 2-5). The VCR of cultivars also revealed that genetic purity gradually improved from 2012 to 2016. At the same time, VCR of sister line means (decreased and was lowest in 2016.) was assumed to be zero as expected where given sister lines of one cultivar shared an identical population mean. Since rice is a self-pollinated species, genetic characteristics of variety has not been affected by genetic drift in pure line. Therefore, we conclude that improvement of VCR of cultivars was caused by decrease of VCR sister lines due to success of removal of contamination/cross-pollination.

The HD showed the highest among the measurements, suggesting that the genetic component of variance of HD contributed predominantly to phenotypic variance among the traits and was the most reliable measure for evaluating genetic purity of BS seeds. The value of HD and CL was highest in 2016.

Table 6. Percentage of the variance component in the total variance of agronomic traits in nine rice cultivars from 2013 to 2016 in wet season

Agronomic trait	Variance component	Variance component ratio of total variance (%)			
		2013	2014	2015	2016
Heading date	Cultivar	98.4	98.1	99.3	99.6
Culm length	Cultivar	97.3	90.2	99.5	99.6
	Sister lines	2.6	9.8	0.5	0.3
Panicle length	Cultivar	95.2	93.7	94.5	96.5
	Sister lines	4.0	6.1	5.0	2.9
Panicle number	Cultivar	86.1	82.8	82.6	92.8
	Sister lines	11.5	12.8	13.5	5.4

As for HD, the SD of all cultivars significantly decreased from the first year and was less than 1.0 in 2016, except for STL, KZY, and SWT with a SD of less than 1.0 from the first year (Table 2). The VCR of cultivars was 98.4% in 2013 and increased to 99.6% in 2016 (Table 6). In the sister lines of all cultivars, the number of days required for 10% heading of the earliest heading line to 90% heading of the slowest heading line decreased from 8.25 days in 2013 to 5.25 days in 2016 on average, and the uniformity of heading time became higher in all cultivars. In particular, that of MTK was greatly reduced by five days from 2013 to 2016 (Fig.1). Regarding CL (Table 3), the SD for all cultivars decreased significantly in 2014 except for SWT and AYM. In 2015, the SD of TDY, MTK, STK, STL and HK was the lowest. In 2016, the SD of all cultivars decreased compared to that of the first year and showed significant differences across all cultivars, except for STL. The VCR of cultivars increased from 97.3% in 2013 to 99.6% in 2016, and VCR of sister lines decreased from 2.6% in 2013 to 0.3% in 2016 (Table 6). With regard to PL (Table 4), the SD of MTK, STK and KZY in 2014 and SD of STL, SWT, AYM, PSY and HK in 2015 was the lowest with significant differences compared to that of the initial year. In 2016, except

for STL, the SD was lower for all cultivars compared to that of the initial year and showed significant differences for all cultivars except for TDY, MTK and STL. The VCR of cultivars in 2016 was 96.5%, which was higher than that in 2013 of 95.2%, and the VCR of sister lines decreased from 4.0% in 2013 to 2.9% in 2016 (Table 6). As for PN (Table 5), the SD gradually decreased from 2014, and in 2016 all cultivars (except for STK and AYM) showed significant differences compared to the first year. The VCR of cultivars increased from 86.1% in 2013 to 92.8% in 2016, and the VCR of sister lines decreased from 11.5% in 2013 to 5.4% in 2016 (Table 6).

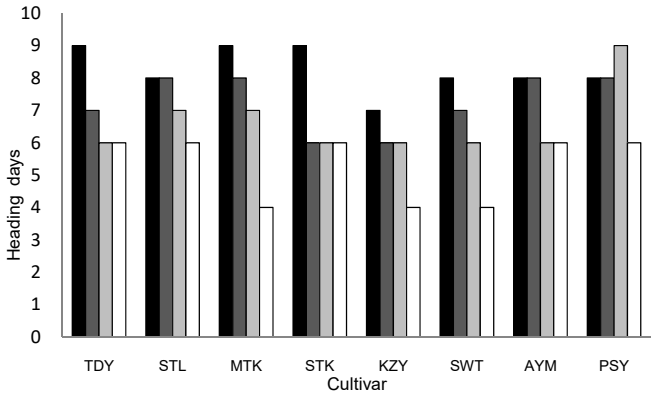

Fig. 1. Transition of the days required from 10% heading to 90% heading

3.1.2 DNA marker analysis

The whole genomic region of the 96 breeder seeds of STK harvested in 2014 was surveyed for DNA polymorphisms at 93 SSR markers (Table 7). No grain with off-type polymorphic bands was found, suggesting that the genetic purity of STK BS was likely to be high. Next, genetic purity of BS in 2013, 2015, and 2016, and CS in 2014, 2015, and 2016, seeds harvested from paddies grown from CS in 2016, and seeds harvested from paddies grown from non-CS in 2016, was investigated using the four markers *RM3340*, *RM1248*, *RM8121*, and *RM6925*. Seeds with off-type DNA polymorphisms were found at a rate of 0.42%, 1.67%, and 0.00% in 2013 BS, 2015 BS, and 2016 BS, respectively. The seeds with off-type polymorphisms were found in 2014 CS, 2015 CS, and 2016 CS. Seeds harvested from paddies grown from CS and non-CS in 2016 showed 1.19% and 7.5% off-type grains.

Table 7. Detection of off-type polymorphism in Sinthukha breeder seed and certified seed from 2013 to 2016 using DNA marker

Seed class	Rate of off-type bands by year[1)]			
	2013	2014	2015	2016
Breeder seed (BS)	0.42 (1/240)	0.00 (0/96)	1.67 (8/480)	0.00 (0/120)
Certified seed (CS)	–	2.08 (12/576)	2.18 (8/367)	2.24 (7/312)
Paddy grown from certified seeds	–	–	–	1.19 (2/168)

Paddy grown from non CS	–	–	–	7.50 (9/120)

[1)] Digits in parenthesis represents the number of grain showing off-type band per number of grain tested.

3.2 Discussion

In Myanmar, BS multiplication of rice has been practiced for many years using the panicle line method derived from bulked panicles. In this method, all panicles are used for the next BS multiplication without pedigree information. The individuals derived from a single plant are grown as a group of plants (a line), but ancestral pedigree information is lost and each individual cannot be traced back to its ancestry. Therefore, it has been suggested that the detection and exclusion of off-type plants and populations derived from cross-pollinated plants are difficult. Consequently, genetic purity of BS is not able to be maintained at a sufficiently high level.

In this study, the line cultivation method, which ensures traceability of ancestral pedigree information by line and the individual selection was introduced to BS multiplication of rice in Myanmar. Genetic purity of nine major rice cultivars was investigated by evaluating agronomic traits and DNA polymorphisms. The line cultivation method is commonly used for self-pollinating crops such as rice, and progeny in each generation can have their ancestry traced back for several generations. The advantage of this method is that populations or pedigrees exhibiting trait segregation due to possible outcrossing of the ancestral pedigree, or contamination with other varieties can be identified. A series of methods were suggested to be suitable for maintaining and improving the genetic purity of BS.

Ikeda *et al*. [7] recommended the introduction of the line cultivation method for BS multiplication to maintain genetic purity on the basis of agronomic studies on FS purification of NERICA cultivars in Benin. Application of the line cultivation method to BS multiplication in Madagascar resulted in heading duration from the beginning to the end of panicle appearance being shortened, and the coefficient of variation in some agronomic traits and off-type rate were decreased [7, 8].

To assess the effects of the introduction of the line cultivation method, the SD of HD, CL, PL, and PN in all cultivars was evaluated and were found to be lower in 2016 compared to the first year, except for HD of STL and SWT, PL of STL, and PN of STK (Tables 2-5). Among the sister lines in all cultivars, heading duration decreased by about three days from 2013 to 2016 (Fig. 1). These data suggest that the uniformity of HD increased in all cultivars and genetic fixation of the agronomic traits in all cultivars was increased. The reason why the SD of HD in STL and SWT did not decrease in 2016 compared with 2013 was that the SD of these cultivars was low enough (at 1.0 or less) as of 2013. The VCR of cultivars (which is equivalent to broad-sense heritability) was also evaluated in HD, CL, PL, and PN during 2013–2016 after introduction of the line cultivation method with record traceability. The VCR of cultivars in 2016 became higher in order of HD, CL, PL, and PN. The VCR of cultivars in HD and CL increased from 2013 to 2016, and both of them were 99.6% in 2016 (Table 6). The VCR for HD and CL in cultivars was higher than that in PL and PN, as has been observed previously [22, 23, 24, 25]. HD is a quantitative character with high heritability controlled by some major genes [22, 23, 24, 25, 26, 27, 28]. This suggests that the observation of HD is the most effective measure of detecting genetic purity. CL is known to be a highly inheritable character [22, 23, 24, 25]. The results of our study also showed the same VCR for cultivars in terms of HD (Table 6). Semi-dwarf genes were introduced to several short culm cultivars [29, 30]. Heritability of PL is comparatively high, while PN is not a stable character and its heritability is lower than PL [23, 24, 25]. The present study confirms these observations (Table 6). No polymorphism was detected in BS by the DNA analysis of STK in 2014 and 2016 (Table 7). Several polymorphisms were detected in the BS of 2015. This can be attributed to the BS samples in this study being randomly collected from the bulk BS for FS multiplication. The observed polymorphisms were possibly caused by genetic

segregation at unfixed loci during fixation, or by mixing different varieties during the threshing and drying process. Since the line cultivation method was also applied to BS of eight other cultivars, the results of the DNA polymorphism analysis suggest that genetic purity of the other cultivars could be as high as STK in 2016.

4. CONCLUSION

The results demonstrate that the line cultivation method that ensures traceability of ancestral pedigree information by line and individual selection is effective in improving and maintaining the genetic purity of BS in Myanmar. Since the DAR has implemented the line cultivation method with all cultivars, it can be expected that the BS quality of all cultivars will be improved. The genetically pure BS will also enhance the quality of FS, RS and CS. Rice production using CS with high genetic purity can be expected to bring higher income to farmers because of the improvement of productivity and quality. Further, seed producer will be able to enjoy higher profit from CS of good reputation.

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