Recent Advances in Population Improvement through RGA under Irrigated Boro Rice Breeding Programme in Bangladesh

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ABSTRACT

Development of new high yielding varieties needs highly accelerated breeding approaches to meet the demand of increased rice production nowadays. To address this issue, Rapid Generation Advance (RGA) of segregating rice population and Line Stage Testing (LST) of fixed breeding lines have been adopted as a routine work in Bangladesh Rice Research Institute. In the present study, we described the evidence of advancing a large number of segregating population and high selection pressure at LST to isolate fixed lines in the Irrigated Boro rice breeding programme. This programme was implemented under development of Favourable Boro Rice and Cold Tolerant Rice. In both programmes, wide variation was observed among the number of population and segregating generation at RGA nursery. A total of 62,269 individual progenies from 64 crosses were advanced through RGA in 2019 whereas 12,122 individual progenies from 45 crosses were advanced in 2020 under the FBR programme. In contrast, 68,531 segregating progenies from 82 crosses and 15,004 progenies from 52 crosses were advanced through RGA under CTR programme during 2019 and 2020, respectively. During LST, selection pressure was imposed for the first time to isolate homozygous lines from a pool of large number of breeding lines. The selection intensity ranged between 0.21-20.1% and 0.6-14.9% for FBR and CTR, respectively. A total of 794 fixed breeding lines having different combinations of favourable alleles of BLB, Blast and different grain quality traits were isolated from 17,633 RGA derived lines. The results obtained from this study suggested that the RGA system has become an effective tool for population improvement in a breeding programme.

Key words: Rapid generation advance, Line Stage Testing, breeding population, trait markers

INTRODUCTION

Rice grows round the year but traditionally there are three rice growing seasons in Bangladesh. Among these seasons, *Boro*, which corresponds to the irrigated ecosystem in the dry season (Afrin *et al.*, 2019) solely occupies around 45% of the rice areas and produces around 55% of rice annually (BBS, 2018). The rice varieties developed and released by the Bangladesh Rice Research Institute (BRRI) for the irrigated ecosystem have made a huge contribution to national food security. Around 70% of the total rice produced in the country in Boro season comes from BRRI varieties. However, only two varieties, BRRI dhan28 and BRRI dhan29 are widely grown covering over 50% of the rice areas in Boro season (Ahmed *et al.*, 2022). These varieties are very old and are showing weakness to different biotic and abiotic stresses. Therefore, development of high yielding farmers' adapted varieties for the irrigated ecosystem is urgently needed.

The rice varieties released so far were developed through the pedigree breeding method, which usually takes longer time (typically 6-8 years) to isolate a superior line from the segregating generations and

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eventually requires 12-14 years to release a new variety. Pedigree based breeding method has intrinsic weakness in improving the quantitatively inherited trait like yield as it favours major effect genes and takes longer time to recycle the progenies in the crosses. Thus, faster improvement of varieties for the favourable ecosystem breaking present yield plateau has been almost impossible through pedigree method. This method allows early generation selection from segregating population, which does not guarantee of producing high yielding progenies in the late generations (Collard *et al.*, 2017). The reason behind this is the continuous decay of linkage disequilibrium among the small effect multiple genes/QTLs conferring yield because of breeders' favour for major genes during early generation selection. Besides, population size is a prime factor for capturing variations of small effect genes/QTLs (Cobb *et al.* 2019a). In pedigree method, although the population size in the early generation (F_2) remains relatively larger (2000 – 3000 progenies), due to selection pressure at successive generations, it makes low chance of capturing the small effects genes/QTLs from the respective crossing population. Moreover, there is a common tendency of the plant breeders to include landrace varieties frequently in the breeding programme to enrich specific traits of current demand, which in turn decays the elite status of the breeding germplasm (Cobb *et al.*, 2019b) and lowers the yield potential. Also, heavily dependence on a few high yielding genetic backgrounds as parents in the breeding programmes has resulted into below average progenies. In contrast, rapid generation advance (RGA), a cross-cutting technology, has come up to speed up the breeding programme further to shorten the breeding cycle (Goulden, 1939, Fuente *et al.*, 2013). In RGA methods, progeny selection is not practiced during advancement of segregating generations; rather successive planting following single seed descent

reduces the time for line fixation. This system not only shortens the cycle time but also provides scope of capturing variations of small effects genes/QTLs for yield (Cobb *et al.*, 2019b). Considering an increased demand for food, climate vulnerability and emerging biotic stresses, rice breeders need to adopt quicker and effective breeding operations to reduce the timeframe required for a complete breeding pipeline (Atlin *et al.*, 2017; Collard *et al.*, 2019).

The segregating populations grown at a closer spacing in the RGA system are forced to compete with the neighbouring plants for water, air and sunlight. As the principles of nature, this imposed competition leads the plants to flower earlier than the plants grown under standard spacing used for crop cultivation. This makes possible to advance 3-4 generations per year in rice. Segregating generations could be advanced without selection and in the successive generations (Goulden, 1939). Additionally, RGA has been proved as an efficient method to fix transgressive segregation and obtain genetic advance (Snape and Riggs, 1975). However, advancement of segregating population has created immense opportunity to develop and advance large scale population improvement in cereal crops. In recent times, RGA techniques have been adapted in different breeding programmes of different crops, viz, soybeans (Carandang *et al.*, 2006), pigeon pea (Saxena *et al.*, 2019), chickpea (Gaur *et al.*, 2008), sorghum (Rizal *et al.*, 2014), rice (Ohnishi *et al.*, 2011; Tanaka *et al.*, 2016; Collard *et al.*, 2017; Rahman *et al.*, 2019) in developing fixed breeding lines within possible shorter time. More recently, seven generations of oats and triticale (Liu *et al.*, 2016) had been advanced in one year through RGA. Watson *et al.*, 2018 achieved six generations per year in wheat, barley and chickpea.

The use of RGA in the breeding programme is not new, several varieties in different crops have been developed using

this technique (Collard *et al.*, 2017). RGA is now in routine use in many rice breeding programmes in Asia including Bangladesh. Since 2015, BRRI has been using RGA technique in the breeding programmes for different ecosystem including the irrigated rice favourable ecosystems. In this article, we describe our experience and outcomes of using RGA techniques in shortening breeding cycle and isolating potential candidates from early generation multi-location trials of RGA-derived fixed lines.

MATERIALS AND METHODS

Plant materials
Segregating population of different crosses and RGA-derived fixed lines developed by the breeding programme for irrigated ecosystem were used in this study.

Line fixation through RGA techniques
Segregating (F_2 to F_6) populations were grown in the field RGA nursery (Fig 1). A portion of a single panicle having 8-10 seeds was sown at 5 X 5 cm spacing on the raised bed of 50 cm width. A wooden frame was used to make furrows at 5 cm apart on the fertilized and puddled bed to facilitate seeding. Gravel-free powdered garden soil was used to cover the seeds sown in the furrow. Fertilizer management during final land preparation was done with Di-Ammonium Phosphate (DAP), Muriate of Potash, Gypsum and Zinc Sulphate at the rate of 45, 50, 55 and 5.5 kg/ha, respectively. Besides, 50 kg Urea and 25 kg Muriate of Potash were applied at 40-45 days. At maturity, a secondary branch of a single panicle from each hill was harvested and dried. Dried seeds were immediately put into 50°C for 72 hours in oven for breaking the dormancy and proceed for the next cycle of RGA.

Seed amplification of the fixed lines
RGA derived fixed lines were grown in 2.4 m (12 hills) long single-row plots with 20 cm × 20 cm spacing following systematic arrangement design in a trial called Line-Stage Testing (LST). Forty-five-day-old seedlings were transplanted using single seedling per hill. At flowering stage, superior and uniform lines were selected based on days to 50% flowering, plant height, grain type. Lodging tolerance, reaction to major diseases and insects under field condition were also considered for line selection.

Genotyping of RGA derived fixed lines
Genotyping was performed through outsourcing at the Intertek, Australia with the help of International Rice Research Institute (IRRI). A leaf sample of 4-5 cm was collected in glassine bags from a single plant of each of the selected uniform lines of the LST trial and stored at -80°C until it was fridge dried using a lyophylizer machine. Two small, dried leaf discs from each sample were taken into each well of a 96-well plate using a paper punching machine. The 96 well-plates with leaf samples were kept in the oven at 60°C for two days followed by one day cooling period in room temperature before sending to the Intertek, Australia for genotyping. Trait SNP panel designed by the GSL laboratory of IRRI was used for genotyping.

RESULTS

Segregating population in the RGA nursery
The Favourable Boro Rice (FBR) and Cold Tolerant Rice (CTR) Breeding programme of BRRI advances F_2 –F_6 segregating progenies in the Field RGA nurseries with 2.5 generations a year. This breeding programme had advanced a sum of 62,269 progenies from 64 crosses and 68,531 segregating progenies from 82 crosses in 2019 in order to develop breeding lines suitable for favourable and cold prone ecosystem, respectively. Sequentially, in 2020, 12,122 progenies of favourable ecosystem and 15,004 progenies for cold

prone environment were advanced (Table 1). In 2019, majority of the segregating progenies (~ 50%) were under F_2 class in both of the breeding programmes. Under favourable Boro rice (FBR) programme 32,202 F_2 progenies from 26 crosses, 13,303 F_4 progenies from nine crosses, 8,388 F_5 progenies from 12 crosses, and 8,376 F_6 progenies from 17 crosses were advanced in 2019, while in 2020, 3,096 F_2 progenies from 10 crosses, 7,349 F_4 progenies from 26 crosses and 1,777 F_6 progenies from 9 crosses were advanced. In contrast, in the cold tolerant rice (CTR) breeding programme, a total of 29,095 F_2 progenies from 33 crosses, 10,859 F_3 progenies from nine crosses, 10,077 F_4 progenies from five crosses, 12,544 F_5 progenies from 13 crosses, and 5,956 F_6 progenies from 22 crosses in 2019 and 1,048 F_2 progenies from five crosses, 8,066 F_4 progenies from 33 crosses, 3,296 F_5 progenies from nine crosses and 1,994 F_6 progenies from five crosses were advanced in 2020.

Table 1. The number of segregating progenies advanced by field RGA nurseries under FBR and CTR breeding programme in 2019 and 2020.

| programme | Year | | | | | |
| | 2019 | | | 2020 | | |
	Generation	Crosses	Population	Generation	Crosses	Population
FBR	F_2	26	32,202	F_2	10	3,096
	F_4	9	13,303	F_4	26	7,349
	F_5	12	8,388	F_5	-	-
	F_6	17	8,376	F_6	9	1,777
	Total	**64**	**62,269**		**45**	**12,222**
CTR	F_2	33	29,095	F_2	5	1,048
	F_3	9	10,859	F_3	-	-
	F_4	5	10,077	F_4	33	8,666
	F_5	13	12,544	F_5	9	3,296
	F_6	22	5,956	F_6	5	1,994
	Total	**82**	**68,531**		**52**	**15,004**

Population structure of the segregating progenies

The population structure of the segregating progenies derived from RGA showed wide variations across the segregating population (F_2-F_6) under both FBR and CTR breeding programme. In 2019, the number of progenies per crosses in FBR breeding programme ranged from 442-1912 for F_2, 1325-1745 for F_4, 302-1154 for F_5, and 33-1060 for F_6 (Fig. 1). In CTR, higher number of crosses was in F_2 generation comprising 29,095 progenies with wide range of variation in number of progenies per crosses. The F_2 generations under CTR breeding programme had 182-1889 lines per cross (Fig. 2).
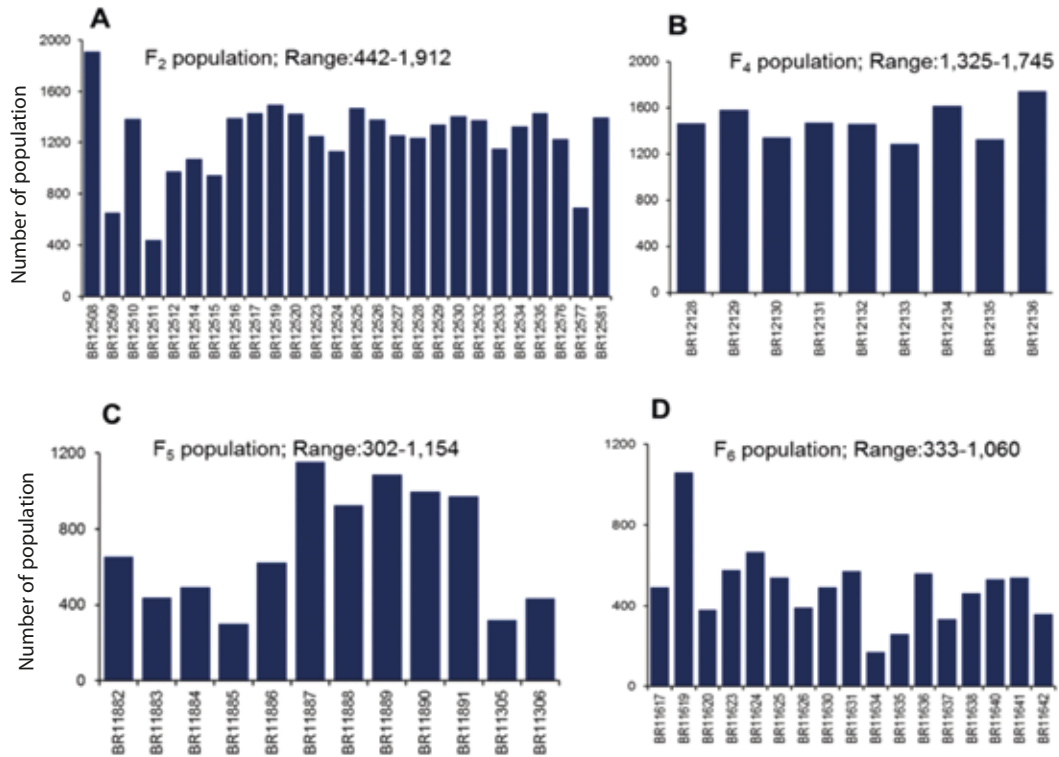
Fig. 1. Histogram showing number of populations produced in F_2 generation (A), F_4 generation (B), F_5 generation (C), and F_6 generation (D) under field RGA nursery of FBR programme during Boro 2019.
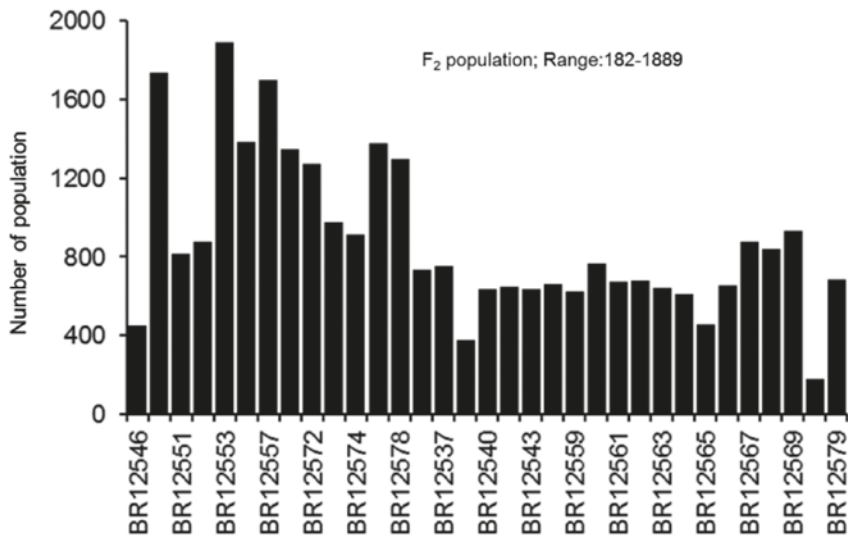

Fig. 2. Bar diagram showing number of population per crosses produced in F_2 generation field RGA nursery of CTR during Boro 2018-19.

In case of F_3-F_6 population, greater variations were observed for each progeny. The number of populations varied from 182-1889 for F_2, 913-1712 for F_3, 1457-2364 for F_4, 553-1386 for F_5, and 29-690 for F_6 (Fig. 3).
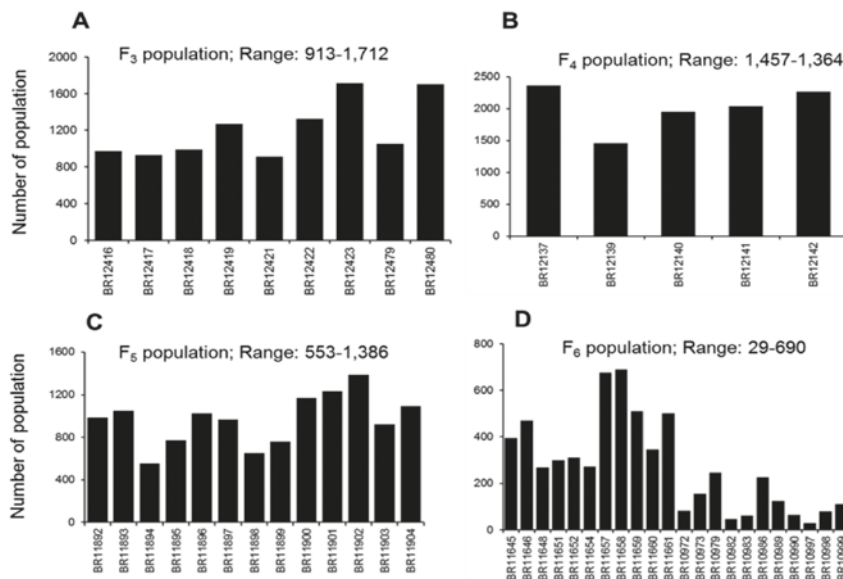

Fig. 3. Histogram showing number of populations produced in F_3 generation (A), F_4 generation (B), F_5 generation (C), and F_6 generation (D) field RGA nursery of CTR during Boro 2018-19.

In 2020, the number of progenies per population varied from 152-492 for F_2, 160-450 for F_4, 122-345 for F_6 under FBR breeding programme (Fig. 4). On the contrary, the number of progenies per population in CTR breeding programme ranged from 108-292 for F_2, 195-436 for F_4, 192-1004 for F_5, and 167-1010 for F_6 (Fig. 5).
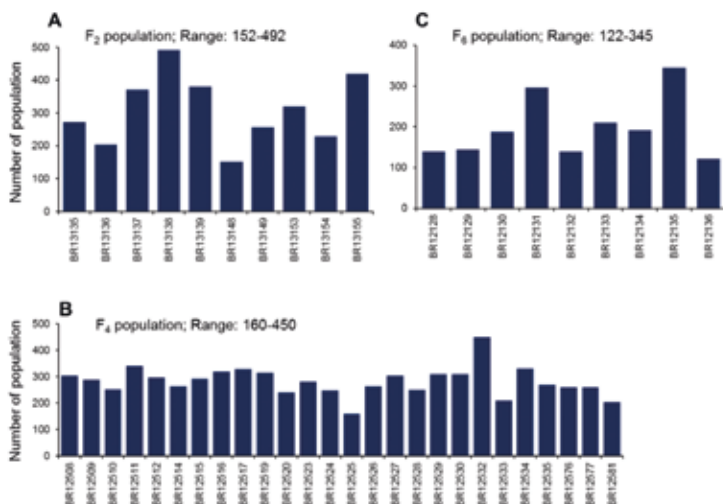

Fig. 4. Histogram showing number of populations produced in F_2 generation (A), F_4 generation (B), F_6 generation (C), field RGA nursery of FBR during Boro 2020.
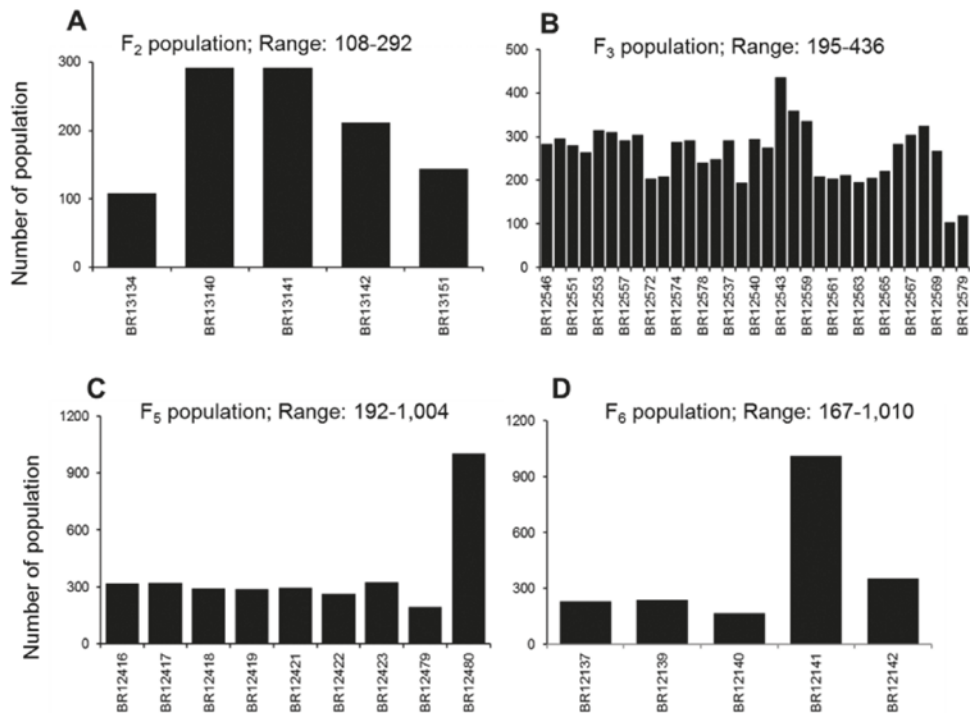
Fig. 5. Histogram showing number of populations produced in F_2 generation (A), F_4 generation (B), F_5 generation (C), and F_6 generation (D) field RGA nursery of CTR during Boro 2020.

Development and selection of fixed lines in Line Stage testing (LST)

LST is a kind of seed amplification stage of F5:6 of F6:7 fixed lines for the next step of field evaluation for yield potential. In this stage, homogeneous lines are isolated. In FBR breeding programme, a total of 8,791 lines were evaluated (Table 2). In each cross, the number of populations ranged from 137-496 lines. Large variation was observed in flowering, plant height and grain size and shape. Final selection was made based on genotyping profiles for BLB, Blast and BPH resistance, and different grain quality traits. Initially progeny selection was performed based on visual phenotypic performance, which followed screening against the presence of favourable alleles for the target key traits.

In FBR breeding programme, 1,959 homogeneous lines were initially identified. The genotypic profiles of these lines showed that majority of the lines (88.5%) had favourable alleles for high amylose specific marker *wx-A*, *wx-10* and *wx-b*. Out of initially selected lines, 493 and 441 lines had favorable alleles of blast resistant genes *Pb1* and *Pi-ta* (Fig. 6). In addition, 147 lines had favourable allele for BB resistance gene *xa5*. Finally, 406 fixed lines from 23 cross-families were selected for yield evaluation. The number of selected lines per cross ranged from 1 to 86 lines. The selection intensity also varied from 0.21-20.1% across the tested population in LST (Table 2).

Table 2. Selection Summary of RGA derived breeding population in LST under FBR.

| Cross name	Developed	Selected	Selection intensity (%)
BR11617	432	9	2.1
BR11619	489	3	0.6
BR11623	493	8	1.6
BR11624	496	3	0.6
BR11626	377	3	0.8
BR11630	475	30	6.3
BR11631	496	21	4.2
BR11634	137	5	3.6
BR11635	231	7	3.0
BR11636	488	23	4.7
BR11637	305	51	16.7
BR11638	427	86	20.1
BR11640	493	61	12.4
BR11641	486	1	0.2
BR11882	376	2	0.5
BR11884	318	5	1.6
BR11885	296	2	0.7
BR11886	365	5	1.4
BR11887	387	21	5.4
BR11888	368	8	2.2
BR11889	333	13	3.9
BR11890	167	16	9.6
BR11891	356	23	6.5
Total	8,791	406	-
Range	137-496	1-86	0.21-20.1
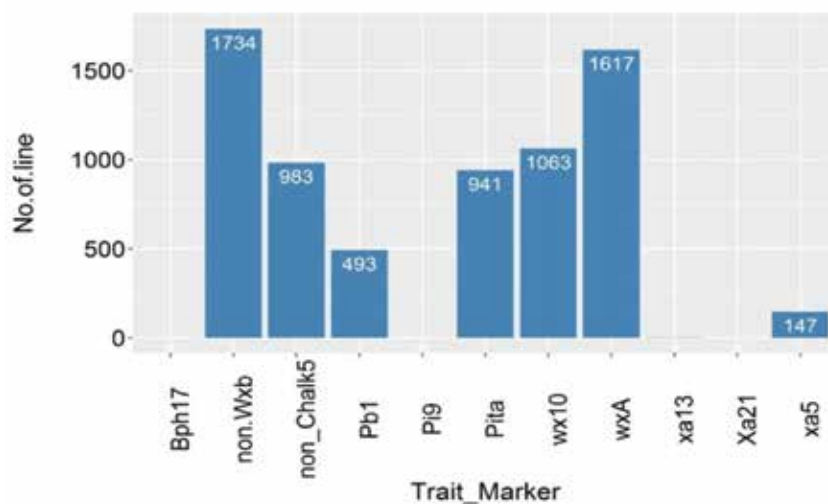
Fig. 6. Frequency of trait makers showing favourable alleles for different key target traits among 1,940 LST lines under FBR breeding programme.

Under CTR, 8,842 RGA derived lines comprising 76 – 567 lines per cross-family were evaluated in LST (Table 3). Large variation was observed in flowering, plant height and grain size and shape. The genotypic profiles showed that among the initially selected 1780 lines, the favourable alleles for Blast resistant genes, *Pita* and *Pb1* were present in 25.2% and 48.0% lines. Moreover, 88.5% lines had favourable alleles for high amylose content, 2.5% for BPH resistance and only 1.0% for BLB resistant gene *xa5* (Fig. 7). Finally, 388 lines were selected based on the presence of favourable alleles for target traits and acceptable visual appearance (Table 3). The number of selected lines ranged between 1 to 49 lines per cross. The selection intensity also varied from 0.60-14.9% across the tested population in LST (Table 3).

Table 3. Selection Summary of RGA derived breeding population in LST under CTR.

Cross name	Developed	Selected	Selection Intensity (%)
BR11645	300	6	2.0
BR11646	433	21	4.8
BR11648	241	36	14.9
BR11651	250	8	3.2
BR11652	267	23	8.6
BR11654	258	8	3.1
BR11657	464	6	1.3
BR11658	567	30	5.3
BR11659	434	49	11.3
BR11660	329	26	7.9
BR11661	471	28	5.9
BR10972	85	5	5.9
BR10973	150	1	0.7
BR10979	272	8	2.9
BR10989	139	3	2.2
BR10998	76	3	3.9
BR10999	100	4	4.0
BR11892	357	5	1.4
BR11894	389	8	2.1
BR11895	354	13	3.7
BR11896	321	38	11.8
BR11897	304	6	2.0
BR11898	254	3	1.2
BR11899	368	5	1.4
BR11900	351	13	3.7
BR11901	347	2	0.6
BR11902	326	9	2.8
BR11903	330	11	3.3
BR11904	305	10	3.3
Total	8,842	388	-
Range	76-567	1-49	0.6 - 14.9

DISCUSSION

Higher number of segregating populations in RGA nursery

BRRI had been using pedigree method for breeding rice since its establishment in 1970. Although, using this breeding method, many varieties of different crops have been developed in many countries; it showed its weakness in identifying superior genotypes aiming to breaking current level of yield ceiling. On the contrary, single seed decent (SSD) method using RGA techniques have shown promise in this regard (Collard *et al.*, 2017). The SSD-RGA not only produces superior progenies but also shorten breeding cycle significantly, which in turn brings a frame-shift change enhancing genetic gain in the breeding population. Very recently, BRRI has adopted this breeding method (Rahman *et al.*, 2019) targeting accelerated genetic gain in different rice breeding programmes including boro rice for favourable and cold prone environments. For a successful breeding programme, existence of wide variations is pre-requisite. RGA method is capable of producing large number of fixed lines from segregating population rendering huge variations between them. In the present study, we also observed wide variation in visual agronomic performances and genotyping profile for key target traits in the LST class fixed lines developed through SSD-RGA techniques.

Firstly, under FBR programme, 62,269 individual progenies from 64 crosses and 12,122 individual progenies from 45 crosses were advanced through RGA in 2019 and 2020, respectively. In contrast, 68,531 progenies from 82 crosses and 15,004 progenies from 52 crosses were advanced under CTR in 2019 and 2020, respectively. These observations clearly indicate that RGA system was capable to produce a large number of segregating progenies (F_2-F_6) in the RGA nurseries. Thus, larger variations

can be conserved from elite-by-elite crosses. Use of RGA system for advancing segregating population has been reported in the Philippines, Thailand, India, Bangladesh and Japan (Eunus *et al.*, 1980; Vergara *et al.*, 1982; Das, 2013; Janwan *et al.*, 2013; Manigbas and Lambio, 2015; Maruyama, 1989; Rahman *et al.*, 2019).

In this study, we observed remarkable differences in the distribution of each segregating progenies in both of the breeding programmes. The ranges of progenies were always higher for F_2 population and always lower for F_6 population. For instance, the number of lines per cross in F_2 population ranged between 442-1912 in FBR 2019 whereas it ranged from 33-1060 in F_6 population in the same year. In 2019 under CTR breeding programme, the F_2 population had 182-1,889 progenies per cross whereas 29-690 for F_6 population under CTR programme. In 2020, there were less variation among number of progenies for each cross under both FBR and CTR programme. In principle, SSD-RGA should produce almost equal number of progenies from F_2 generation to F_6 generation. The reason behind the comparatively a smaller number of progenies of late generations in both the breeding programme might be due to higher rate of mortality or poor recovery rate during RGA process because of inadequate training to the personnel concerned for handling RGA activities and unavailability of optimized RGA protocol. In recent days, situation has been remarkably improved in realizing large number of progenies per cross at each generation. However, only 400 LST lines is good enough for yield evaluation to isolate breeding lines of 2-sd yield advantage over the population mean with combinations of favourable alleles of couple of target traits. With the progression of the breeding programmes in last few years, the breeders in BRRI have enriched their knowledge level in handling RGA population. Now,

initial breeding population size in F_2 generation has been lowered to 500 to conserve available variation from the elite-by-elite crosses.

Selection of promising fixed lines in LST

In the population improvement through the RGA system, no selection pressure is imposed on segregating progenies (F_2-F_6). Thus, the LST trial of RGA derived fixed lines has been considered as an important step for seed increase and allowing selection of highly heritable traits. This is the very first step of selection for plant type, disease resistance or other desired traits (Collard *et al.*, 2017, Rahman *et al.*, 2019). At the present study, RGA derived fixed lines were grown in single row consisting of only 10-12 hills. In our experience, this was sufficient to isolate better lines with uniformity in flowering time, grain size and shapes. Under FBR programme, 8,791 lines were transplanted in the field with at least 137 lines for each cross. Considering desirable agronomic characters and presence of favourable alleles for BLB, Blast and grain quality traits, a sum of 406 promising breeding lines were selected from LST trial of FBR. On the other hand, out of 8,842 lines grown with at least 76 lines for each cross under CTR programme, 388 promising breeding lines were selected for further evaluation in replicated field trial. The selection intensity in these breeding programmes varied from 0.21 – 20.1% to 0.60-14.9%. These results indicated that at least 100 RGA derived fixed lines were sufficient to produce possible variations in LST trial. During first selection of RGA derived lines, there were advantages in isolating desired lines based on a panicle-row rather than selecting single plants. We quickly discarded at least 20-30% of the inferior lines with obvious defects by visual observation. Taken together, 794 fixed breeding lines were isolated from 17,633 RGA derived lines under FBR and CTR programme for irrigated Boro rice development. In a separate study of salinity tolerance breeding programme, around 3000-4000 plants per cross was maintained in F_2 to ensure around 400 LST lines per cross. At LST, 2882 elite fixed lines were tested in T. Aman 2018-19. Selection was applied to isolate progeny rows on the basis of our desired traits like homogeneity, growth duration, grain type, disease tolerance, and phenotypic acceptability (Rahman *et al.*, 2019).

Likewise, in the RGA population of IRRI, several 100 lines per population were maintained in fixed line evaluation which is required to increase the likelihood of identifying transgressive segregants (Collard *et al.*, 2017, Snape and Riggs, 1975). Uses of advanced molecular markers have shown great promise in developing high yielding modern varieties with specific trait benefits. Using gene/QTLs specific markers and relevant donor parents in the breeding programme powered by RGA technique could generate future varieties with significant improvement in genetic gain. In LST trial, genotyping was done to figure out the favourable alleles for all desired traits in the breeding population of FBR and CTR. However, SNP-based trait genotyping has showed remarkable efficiency in identifying genetically important lines with multiple stress tolerance including seedling stress salt tolerance, blast resistance and gall midge resistance (Debsharma *et al.*, 2022). In the breeding population designed for irrigated Boro rice, trait marker profiles were considered mainly for amylose, BLB, Blast and BPH resistance. In the LST of FBR, selected 441 lines had blast resistant genes *Pb1* and *Pi-ta*, 147 lines had favourable allele for BB resistance gene *xa5*, while in CTR breeding programme, 88.5% lines had favourable alleles for high amylose content, 2.5% for BPH resistance and only 1.0% for BLB resistant gene *xa5*. These results indicated that the RGA breeding method had better advantage of integrating marker

assisted selection than the phenotyping in reduced population sizes after selecting comparably superior lines. Additionally, shortcomings of present trait improvement programme such as lack of favourable allele for *Xa21* (bacterial leaf blight resistance) and *Pi9* (blast resistance) can also be addressed by deploying MAS under forward breeding strategy in the LST class fixed lines.

CONCLUSIONS

The aim of establishing RGA facilities included production of large number of segregating population in each cross, conservation of possible genetic variation developed from elite-by-elite crosses and increase of genetic gain from newly developed RGA derived fixed lines. This study revealed that present RGA system of FBR and CTR programme was able to fulfill the objectives of adopting RGA techniques as a routine activity in the breeding programme for producing large number of segregating progenies (F_2-F_6) for both the programmes. Further, it also confirmed that LST trial of RGA derived fixed lines was able to capture wider variation among fixed lines with wide distribution of multiple traits. Thus, selection was made after possessing high selection pressure in a large set of breeding population. In the future, the rate of genetic gain and trait improvement could be accelerated through the careful implementation of modern breeding techniques including RGA.

AUTHOR CONTRIBUTIONS

M M E Ahmed, W Afrin, M Y Khan and P S Biswas performed the experiments for rapid generation advance of segregating generations and managed the Lines Stage Testing experiments. M M E Ahmed and W Afrin prepared the leaf samples of selected LST population for trait genotyping through SNP markers. M M E Ahmed, P S Biswas, and MRA Sarker wrote the draft manuscript. P S Biswas and K M Iftekharuddaula revised the final version of the manuscript. All authors read and approved the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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