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Field Rapid Generation Advance: An Effective Technique for Industrial Scale Rice Breeding Program

Abstract:

Conventional varietal development process takes around 10-12 years to develop new rice varieties. However a breeding program has longer breeding cycle for developing variety then farmers will not get early benefit from the new variety. There are several methods in rice breeding. This paper aimed to overview the BRRI developed Field Rapid Generation Advance (FRGA) technique. The pedigree method of breeding was used by BRRI since its inception in 1970. But scientists of modern era need quicker and efficient methods compared to the previous. A transformation in the breeding system is required. Now a day, single seed descent (SSD) method along with FRGA was applied for this purpose. Previously, rice breeders applied RGA technique in the greenhouse and also in trays but not in the field. In the greenhouse, accommodation of large number of crosses with bigger population is troublesome in terms of cost effectiveness, nutrient and pest management. Therefore, greenhouse RGA is not suitable for industrial scale variety development program. A rapid but low cost technique with easier nutrient and pest management is now a long felt demand of the modern breeders. BRRI has developed raised/ flatbed based FRGA technique for managing bigger breeding population (3000-4000 progenies per cross) and rapid cycle breeding which is different from IRRI FRGA system. BRRI has tested the BRRI developed FRGA system and breeders are effectively applying this FRGA technique for industrial scale variety development program.

Key words: Cyclic breeding, FRGA, Line stage test (LST), Single seed descent (SSD)

Introduction:

One of the straightforward techniques to enhance genetic gain is shortening the breeding cycle time (De La Fuente et al., 2013; Atlin et al., 2017; Quddus et al. 2019). Off season progeny advancement is needed for reducing the length of the breeding cycle in rice. Conceptually Rapid Generation Advance (RGA) is a part of breeding procedure where the segregating populations are grown at closer spacing, high temperature and short days to shorten growth duration, thus making possible several generations per year. High temperature is generated due to closer spacing of the population in the field. The duration for advancing from F2 to F5 generally takes about 4 years which can be shortened to 2 years. Goulden (1939) is the first researcher who suggested that hybridization derived segregating generations could rapidly be advanced without selection and in the successive generations one or two progeny per plant can be taken in random. Near homozygous lines can be evaluated in the field only after a short period of hybridization. This method is currently known as Single Seed Descent (SSD). RGA is the most

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appropriate technique for SSD method (Fujimak and Ikehashi 1980).Two hypotheses related to SSD and FRGA need to be satisfied with information. First one is: Why should we practice SSD in rice breeding program avoiding other breeding methods? The second one is: Why FRGA is necessary? There is chance of rejection of high yielding better lines if selection based on yield and other agronomic traits is done in the early generation of segregating population (Kaufmann 1971). Equal opportunity of recombination should be given to each segregating line. Segregating lines should not be discarded before they are fixed. SSD is an efficient method to fix transgressive segregation and obtain genetic advance (Snape and Riggs, 1975). Not only empirical discussion on which method is better for routine rice breeding, but also economic analysis was performed and RGA was considered more advantageous and multiple times cost effective compared to other breeding methods (Collard et al. 2017). Segregating populations are grown using FRGA followed by SSD method to transfer the desirable variations/traits from F2 to F5:6 generation. In IRRI, FRGA was setup through inserting the seedling trays in soil and maintained 104 progenies per tray (Collard et al. 2017). Thus FRGA method that established at BRRI is effective for rapid advancing of larger breeding populations in industrial scale to achieve the target genetic gain (M. A. Rahman, BRRI). Our objective was to discuss the BRRI developed Field Rapid Generation Advance (FRGA) technique for rapid and cyclic breeding.

RGA vs Pedigree method in plant breeding:

Once researcher will know the advantages and disadvantages of FRGA method and its relative advantages over popularly used pedigree method of breeding consequently they might be accepted and applied this FRGA in their own research irrespective of different varietal development pipelines/ areas such as abiotic (salinity, drought, cold, submergence), biotic stresses and favorable environments (Rahman et al. 2017) . A comparison between FRGA and pedigree method is illustrated below (Table 1).

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Table 1: Comparison between FRGA/RGA and Pedigree method of plant breeding

Criteria	FRGA/RGA	Pedigree
Time required	It takes only 3 years to develop elite fixed lines	It takes 8 years to develop elite lines
Maintaining pedigree records	No need to maintain pedigree records	Pedigree records must be maintained
Selection pressure	Breeder gives selection pressure only after the progenies become genetically fixed	Breeder gives selection pressure on segregating population at early stage
Mode of selection	All plants in the population are taken using single seed decent method and grown in subsequent generation	Only the selected plants are taken and grown in subsequent generation
Accuracy of selection	Since no need to keep pedigree information thus selection accuracy per cross is higher	Selection accuracy per cross is lower
Population structure	We can grow forty lakh (4000000) plants per hectare with (5 cm × 5cm spacing). We can accommodate 12 times higher plant population at the same area in FRGA method compared to the conventional pedigree method.	We can grow only 333334 plants (20cm × 15cm spacing) per hectare.
Probability of genetic gain enhancement	Higher because of large population with target traits based on product profile	Low because of less number of population with less variation in characters of interest.
Population maintenance	Easier way to maintain populations during inbreeding	Comparatively difficult to maintain populations during inbreeding compared to RGA
Influence of natural selection	Natural selection does not influence populations	Natural selection influence populations

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Criteria	FRGA/RGA	Pedigree
Suitability in off season	Well suited to Greenhouse and off season nurseries	Not suitable for off season nurseries
Selection in fixed line stage	Selection based on individual phenotype (uniformity, phenotypic acceptability (PAcp) and grain size and shape) rather than progeny performance	Selection based on progeny performance
Input cost	Lower because of fertilizer, water saving method and minimum post-harvest cost. FRGA was much more (five to 10 times) inexpensive than the pedigree method (Fahim et al., 1998).	Higher since we can't save fertilizer, water and post-harvest cost
Chance of varietal replacement	Higher due to product development based on product profile using speed/rapid breeding thus farmers could get faster benefit from the new product through replacing dominant older one.	Incremental change in the performance of new product/variety could not replace dominant older variety.

A schematic diagram on varietal development process using FRGA with SSD is shown in Fig. 1.

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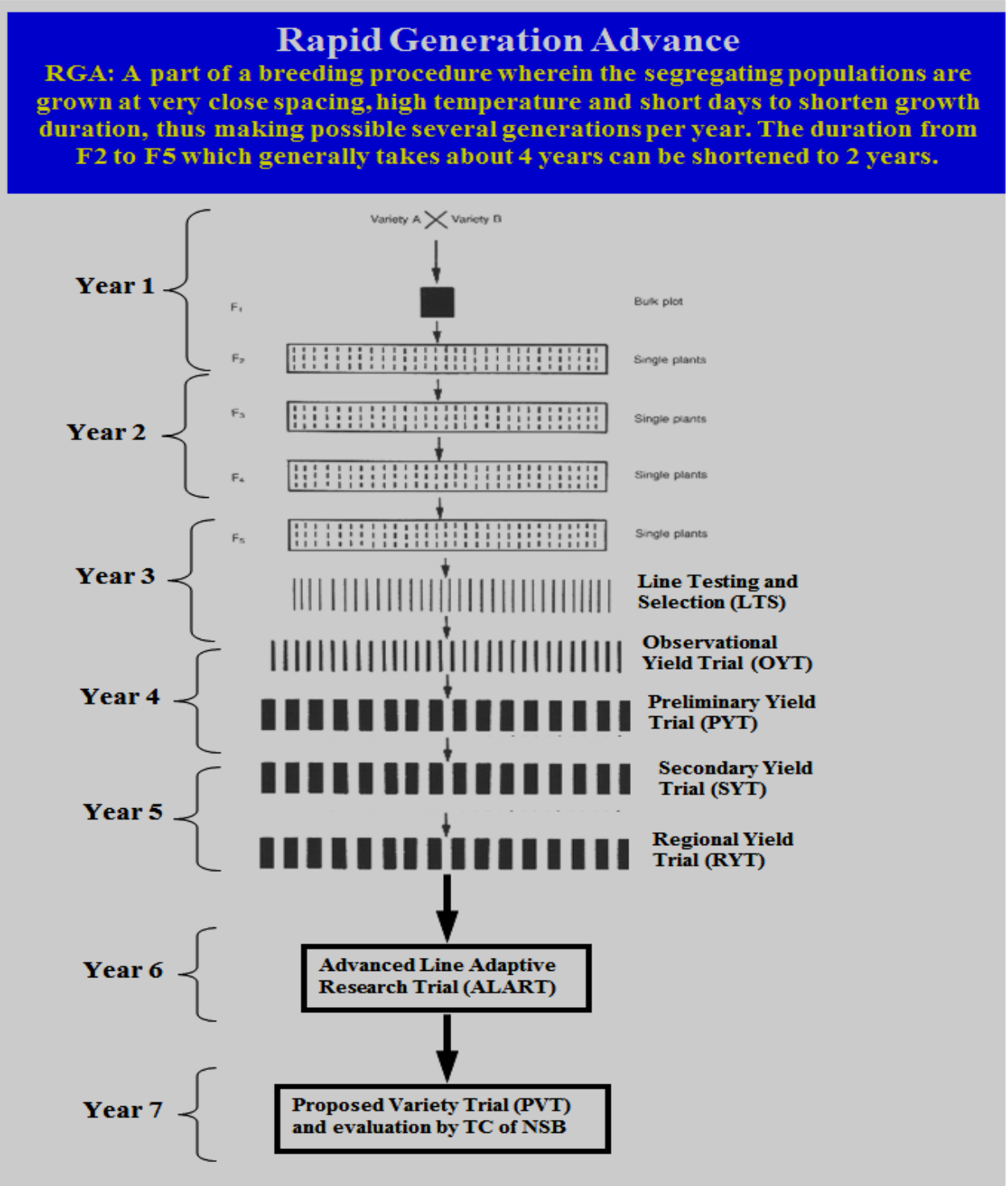

Fig. 1. Rice variety development and release procedure using RGA/FRGA and SSD

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Classification of RGA method:

We can broadly categorize RGA systems in two types.

1) Screen house RGA: Progenies from segregating generations are grown inside the screen house (Uses seedling tray or minuro tray or wooden boxes or metal trays)
   Vergara et al. 1982 discussed RGA technique using wooden boxes and metal tray and Collard et al. 2017 discussed RGA in seedling tray.
2) Field RGA (FRGA): Field RGA also can be divided into two categories:

A) Seedling tray FRGA:
   i) Sowed seeds densely in the seedling trays directly but not sown into the soil of the field area (Fahim et al. 1998).
   ii) Seedling trays are inserted directly into the soil (Collard et al. 2017).

B) Raised or flat bed FRGA: Segregating populations were grown in the field in raised or flat beds

Bangladesh Rice Research Institute shows the pioneering role in the development and application of raised bed Field RGA technique. In this paper, raised bed FRGA is empirically discussed.

In our experience, screen house RGA is suitable for highly photosensitive crosses where we can control the photoperiod. Screen house RGA population faces microclimatic problems and pest outbreak risks while Field RGA is free from those problems. In terms of cost effectiveness, Field RGA is more suitable for photo-insensitive or weakly photosensitive crosses than the screen house RGA. Among the FRGA techniques raised or flatbed FRGA is the most cost effective. In seedling tray FRGA, plastic trays are used to restrict the root growth, but it also makes intercultural operations, fertilizer and pest management difficult in the field. Restricted and weak root might cause the rice plants to lodge during strong wind. Raised bed FRGA technique does not put restriction on the root growth of the rice plant. In BRRI, FRGA population did not lodge during strong wind while many yield trial population lodged. With (5cm × 5cm) spacing, raised bed FRGA population does not produce so many tiller. Though the border plants produce a few tiller; plants in the middle portion of the bed remain tillerless (only mother tiller remains) or produce only one to three tiller.

Raised bed FRGA at BRRI:

Segregating populations were grown in the field in raised beds (9 m × 1.25 m) with very close spacing (5 cm × 5 cm) and low fertilizer (half of recommended dose) to shorten growth duration (**Fig. 2).** In the 1st year (season 1), a cross between variety A and variety B was performed. True F1 plants were confirmed by observing phenotypic characters, bulked and grown in the RGA nursery(1st year, season 2). Around 3000-4000 randomly selected F2 seeds were sown in raised bed at high density. Raising healthy seedlings was achieved by using a lower seed rate (50 gm^{-2}) than the usual practice. Older seedlings (21 day-old, salinity programme; 36 days-old, insect resistance programme) were transplanted

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after pre-screening. Growth duration became seven days shorter when older seedlings (36 days) were transplanted than 21-day-old seedlings in the FRGA field. Because seedlings remain in the seedbed more than five weeks with closer spacing and higher temperature (microclimatic variation) that may trigger early flowering compared with younger (21-days old) seedling. A spacing of 5 cm × 5 cm was used to accommodate a high density of seedlings compared to the traditional pedigree method. Therefore we accommodated 12 times more plants in the same area using FRGA compared to the conventional pedigree method. We grew over 4,000,000 plants per hectare compared to whereas in the pedigree method we can grow only >300,000 plants (20 cm × 15 cm spacing) per hectare. Using FRGA, fertilizers were not applied by top dressing during the cultivation, which reduced input costs for every generation. Although the plants showed nitrogen deficiency symptoms, sufficient panicles from each plant were produced. In line stage testing (LST), 1-2 rows were grown from single panicle of fixed lines derived from F6 or F7 generation.

Fertilizer and other management in FRGA:

Fertilizers @ 60:9.5:30:10:1.8 kg NPKSZn/ha (130-48.5-60-55-5.5 kg/ha Urea-TSP-MoP-Gypsum-$ZnSO_4$) were used with split application of N at 15, 30, 50 days after transplanting (DAT). Total amount of P K S were applied at the time of final land preparation**.** Polythene fence was used to prevent the plants from rat damage (Fig. 4a). Polythene sheets were also used during cold season for seed germination (Fig. 4b).Two types of water controlling practices were followed: (1) minimum irrigation as required; or (2) standing water. For the first method, it was important not to over-stressing plants. The water level in the field was lower than field capacity but irrigation was applied before cracks were visible on the soil, which saved irrigation water. Using the second method, particularly during cooler season, 5-7 cm standing water was kept for a couple of weeks just after recovery after transplanting and in this way it was possible to suppress weeds and also to reduce mortality due to cold. Weeding was done when necessary using hand tools (Fig. 3). Clipping was done one time and just after flowering. All the tillers were removed except the main tiller to make harvesting easier. Clipping was subsequently avoided and during harvesting, in which only one panicle was collected from each plant which was then uprooted. This reduced costs and saved more time. Panicles were harvested when they contained more than 50% mature seed. No pedigree records were kept, which saved considerable labour. The field condition during vegetative, flowering and maturity stage is given in Fig. 5. In the second year, F3 and F4 generations were grown in the FRGA nursery using similar way. However seedling mortality in FRGA nursery was higher during the *T. aman* (wet season) because of higher temperature and humid weather aggravating sheath blight and sheath rot diseases. We had sown each panicle separately in the seedbed. Only one healthy seedling per panicle was transplanted in the main FRGA bed (Fig. 2). Necessary control measures were taken for biotic stress management as well.

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Fig.2: Seedlings transplanted directly in the raised bed with closer spacing

Fig. 3: Weeding at the early vegetative stage

Fig. 4a: Use of polythene fence to prevent rat infestation

Fig.4b: Special use of polythene for seed germination during cold season

Fig.5: Flowering and Maturity stage

Fig. Segregating population was grown in Field RGA method at west byed in BRRI

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We harvested one panicle per plant from all 3000-4000 plants per cross which ensured the representation of progeny from every plant. In the 3rd year, we will have F5 generation and single panicle per plant was harvested following the same procedure described above. After F5-F6, LST trials were done where almost all the plants were homozygous. In this stage progeny rows will be selected on the basis of our desired traits like homogeneity, growth duration, grain type, disease tolerance, and phenotypic acceptability. In BRRI, 400 LST lines per cross is targeted to achieve two standard deviation yield advantage. In the 4th year, observational yield trial (OYT) and preliminary yield trial (PYT) will be carried out followed by other trials (e.g. advanced yield trial (AYT), regional yield trial (RYT), advanced line adaptive research trial (ALART) and proposed variety trial (PVT) following the normal testing procedure. Currently a large number of F2:3 populations consisting of 160,075 progenies from breeding program for *boro*, cold, salinity and submergence tolerance, disease resistance are being advanced using FRGA at BRRI (Table 2a). Moreover, FGRA provides an opportunity to manage bigger breeding population at BRRI to achieve the target of increasing genetic gain.

Table 2a. FRGA activities at BRRI, Gazipur, 2016-17

Program and Ecosystem	No. of crosses	No. of progeny lines
Breeding for favorable *boro*	42	43,207
Breeding for cold tolerance	31	28,127
Breeding for salinity tolerance	23	47,225
Breeding for submergence tolerance	13	17,476
Breeding for disease resistance	12	24,040
Total	121	160,075

We have a complete pipeline of FRGA population from F2 to F5 generation in salinity tolerant breeding program and we have to conduct LST in every year in every season. We have FRGA derived elite fixed lines to conduct yield trials in each season. We have conducted LST using 2882 elite fixed lines in salinity tolerant breeding program, T. aman, 2018-19 and generation advancement at Boro, **2017-18** (Table 2b).

Table 2b. Segregating population in Field RGA, Salinity, Boro, 2017-18

Generation	No. of cross	Progenies transplanted	Progenies harvested
F2	9	24,645	19,600 (F3)
F3	11	14,750	5,966 (F4)
F4	12	10,150	9,535 (F5)
F5	11	3,492	2,882 (F6: LSTinT. Aman)
Total	43	53,037	37,983

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Modification of raised bed FRGA:

Currently, one seed per panicle is germinated and grown in a seedbed. Then 20 to 45 days old seedlings are transplanted in a raised bed in the main field depending on season. Germination failure causes reduction in the segregating population size. Therefore, initiatives were taken to sow 1) half of the panicle per plant and 2) one tertiary branch of the panicle/rachis per plant in the main field instead of one seed(panicle dropped method). Both the technique requires several times more land area compared to single seedling transplanting. There is a simulation regarding the test population size in Line Stage Trial (LST). We need only 400 recombinant inbred lines (RIL) from a single cross to harness two standard deviation yield advantage. If the initial F2 population size is 3000 then there is no concern about the germination failure at all. The present procedure of raised bed FRGA in BRRI is still has great reproducibility to continue this method. However, current FRGA technique is in continuous improvement process to achieving the best performance by using this FRGA method.

Conclusions:

Day by day breeding methods are modernized to fulfill the growing demands of the plant breeders. Now a day, breeders want to get better elite lines quickly to replace the old cultivars. In this paper, we have an empirical discussion on the fruitfulness of Single Seed Descent (SSD) and raised bed Field RGA method. To the best of our knowledge, this is the first attempt to classify RGA methods and describe FRGA method in a new way. Application of BRRI developed raised bed FRGA technique in the modified Single Seed Descent (SSD) method is more efficient than conventional pedigree method. It is the most inexpensive and easy method to handle among all the RGA techniques as well. This will boost up the breeding pipeline and reduce the time of the breeding cycle. This paper will help the researchers to apply the BRRI developed raised bed or flatbed FRGA technique in their own breeding program for speed breeding.

Future research scope:

FRGA with SSD method will be more effective for breeders if we could advance more than three generations per year. Multiple agronomic managements may also be tested and applied to reduce the life cycle length of rice plants to accomplish more generation in a year.

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