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# Shorten rice breeding cycle and developing new promising lines

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## ABSTRACT

Accelerating rice breeding can be accomplished through various approaches, including molecular or conventional techniques. In this regard, some rice breeding programs around the world have changed to use rapid generation advancement (RGA) as a new breeding method. The objectives of this investigation are to establish and implement RGA techniques at the Rice Research & Training Center (RRTC) to develop new, improved, blast disease-resistant, and high-yielding varieties more quickly and at a low cost. Three $F_2$ populations, (Giza177 x Sakha Super300), (IR75589-31 x Giza178), and (Giza179 x IR59673-93-2-3-3-2), were advanced to $F_2$ and evaluated in Lines Stage Trials (LST) and preliminary yield trials (PYT). RGA facilities were installed at the greenhouse of the RRTC, and the $F_2$ plants of each population were harvested individually. One seed from each panicle was used to cultivate the next generation ($F_3$) according to the single seed descent (SSD) method. The RGA technique was successfully implemented at RRTC with simple multipot trays and the maintenance of the greenhouse and screen house. The early generations ($F_2$, $F_3$, $F_4$, and $F_5$) showed good performance in the greenhouse; the narrow space and low fertilizer led to early flowering. promising RGA lines, SK-RGA2-5, SK-RGA2-9, and SK-RGA3-6, revealed grain yields of more than 11.0 t/ha compared with the check varieties, Giza177, Giza178, and Sakha super300, which gave 9.57, 10.17, and 10.50 t/ha, respectively. For grain quality traits, most of the test RGA lines and check varieties have low to medium amylose content, ranging between 17.83 and 23.13%. RGA lines and check varieties gave desirable values for hulling and milling. In general, hulling% ranged between 80.0 and 85.0%, and milling% ranged between 69.43 and 73.0%.

**Keywords:** Rice Breeding, RGA, Single seed descent (SSD), grain yield, Rice blast disease, grain quality.

## INTRODUCTION

Conventional varietal development process needs around 10-12 years to develop new rice varieties. However, a breeding program has a longer breeding cycle for developing variety then farmers will not get early benefit from the new variety (Akhlasur *et al.,* 2019). Adoption of new plant varieties has played a significant role in eradicating global hunger. Previous research has mainly focused on farmer adoption and impact of new crop varieties, although upstream adoption of technologies in plant breeding can generate substantial multiplier effects on downstream impacts (Lenaerts *et al.,* 2022). Accelerated rice breeding can be accomplished through various approaches, including molecular or conventional techniques (Forster *et al.,* 2014). Although molecular breeding methods often lead to improved accuracy, cost or time savings, such methods are technically complex and expensive to set up. Therefore, rapid generation advancement (RGA) is advocated as a feasible alternative for accelerating breeding in public-sector breeding programs in developing countries in the short term (Collard *et al.,* 2017, 2019). RGA is a plant breeding technique first proposed by (Goulden, 1939) to address the problem of poor response to early generation selection due to high levels of heterozygous. The development of new improved and higher yielding varieties will be needed more quickly to meet this demand (Seck *et al.,* 2012). However, most rice breeding programs in the world have not changed in several decades. The development of new higher yielding rice varieties with enhanced disease resistance, tolerance to abiotic stresses, and specific quality characteristics needs to be part of the overall strategy towards food security. The pedigree method is the most widely-used method in rice breeding in Asia, and has been used by the majority of national breeding programs for many decades (Khush and Virk, 2005; Samantara *et al.,* 2022). However, alternative breeding methods such as bulk-population or rapid generation advancement (RGA) may reduce labor and considerable resources, and RGA in particular can accelerate

the development of fixed lines substantially. Another limitation with the pedigree method is that selection usually occurs at a single location up to the $F_6$ or $F_7$ generation, limiting the ability to account for genotype by environment interactions (Bertrand *et al.,* 2019). A major change to the irrigated program was the implementation of single seed descent through RGA as the main breeding method. RGA had been previously used at IRRI for rainfed breeding and for the development of mapping populations. Our new objective was to implement it as main breeding method for the irrigated program on a large scale. Rice in Egypt cultivated only one crop per year during the summer season and the main challenge for the rice breeding program is to grow only one generation per year. It usually takes 10-12 years to develop genetically fixed lines. Hence, it is imperative to use techniques that are cheaper and can shorten the time needed to develop breeding lines, and ultimately, be able to release new varieties after a shorter period. RGA techniques have been developed to accelerate breeding cycles and breeding progress in many crops (Wang *et al.,* 2011, Rizal *et al.,* 2015). One of the simplest and most effective methods to increase genetic gain is to shorten the breeding cycle by adopting quicker breeding methods. Shorter breeding cycles are also preferable in a rapidly changing climate as new recombinants are tested under conditions more similar to future production environments. Observations regarding uniformity were conducted at the $F_6$ stage using panicle rows for seed increase prior to field trials. The use of RGA shifted the emphasis away from visual selection of single plants, which can be greatly influenced by environmental factors and breeder bias. IRRI, a leading global public breeding institute, is currently accelerating its breeding programme through a method named RGA (Atlin*et al.,* 2017; Collard *et al.,* 2019). Utilizing of disease resistance varieties possibly is the maximum cost-effective and consistent method of disease management, maintaining clean environment and stable rice production. Blast disease is the most destructive fungal disease caused by Magnaporthe oryzae Couch (anamorph: *Pyricularia oryzae* Cavara) (Couch and Kohn, 2002). This fungus caused many high yielding varietal breakdown with the high rate of mutation, race shifting and change of dominant specific races on susceptible cultivars as Sakha101 and Sakha104 breakdown (Khush and Jena, 2009; El-Shafey *et al.,* 2015).The objective of this investigation is implemented of (RGA) technique at Rice Research &Training Center (RRTC), Sakha ARC, Egypt. Shorten the rice breeding cycle through RGA to develop new high yielding promising lines and resistance to rice blast.

## MATERIALS AND METHODS

This study was conducted at the greenhouse and the Experimental Farm of Rice Research & Training Center (RRTC), Sakha Kafrelsheikh, Egypt during three years 2020, 2021, and 2022. Three $F_2$ populations, (Giza177 x Sakha Super300), (IR75589-31 x Giza178) and (Giza179 x IR59673-93-2-3-3-2) were advanced to $F_n$ trough rapid generation advancement (RGA) technique according to the protocol developed by IRRI with some modifications, (Bertrand *et al.,* 2017). During the first year (2020), three generations ($F_2$, $F_3$, and $F_4$) were advanced under greenhouse. Second year (2021) $F_5$ generation were advanced at the greenhouse, the ($F_6$) Lines Stage Trials (LST), were evaluated under field conditions. During the third year (2022), the promising lines were evaluated under field conditions. During the second and third year new cycles for different populations were advanced.

**Soil preparation:** Soil sieving is done to separate fine soil particles from coarse soil particles and to provide a good medium for seed germination. The soil is mixed with basal fertilizer (NPK) to provide nutrients to the germinating seed. Fertilizer rate is 1g per 1kg of soil. Germinated seeds were planted in wet soil using plastic trays (13 row x 20 columns). The maintenance of the greenhouse, including the control of temperature and light system were conducted before the investigation.

**Rapid Generation Advancement (RGA):** The $F_2$ seeds are placed in the drying oven for breaking of dormancy (2 days at 50°C). Direct-dry seeding is done: single seed is placed on each cell in principle following the single seed descent (SSD), but in practice 4 seeds are seeded to ensure 95-100% germination rate and line survival. The minuro trays with seeds are carefully placed inside the plastic box containing soil .Thinning of germinated seedling into 1 plant per cell is done 10 days after seeding (DAS). ,Pruning of plants to one tiller is done continuously if needed. This practice is one of several factors that induce early flowering. Selection of panicles is done to discriminate matured panicles from un-matured; this is done at 70-90 days after sowing depending on the earliness of the population. Those panicles that show physiological maturity (yellowish to golden yellow color) are ready for harvest. Panicles are taken from each plant and carefully stored in paper bags/envelopes to prevent shattering.The panicles from each plant are stored in separate bags/envelopes. Sorting of harvested panicles/seeds is done to properly arrange the seeds for easy storage and retrieval. Drying of the panicles is done to drive out moisture thereby prolonging their lifespan and viability of the seeds. 48 hours of sun drying is sufficient.

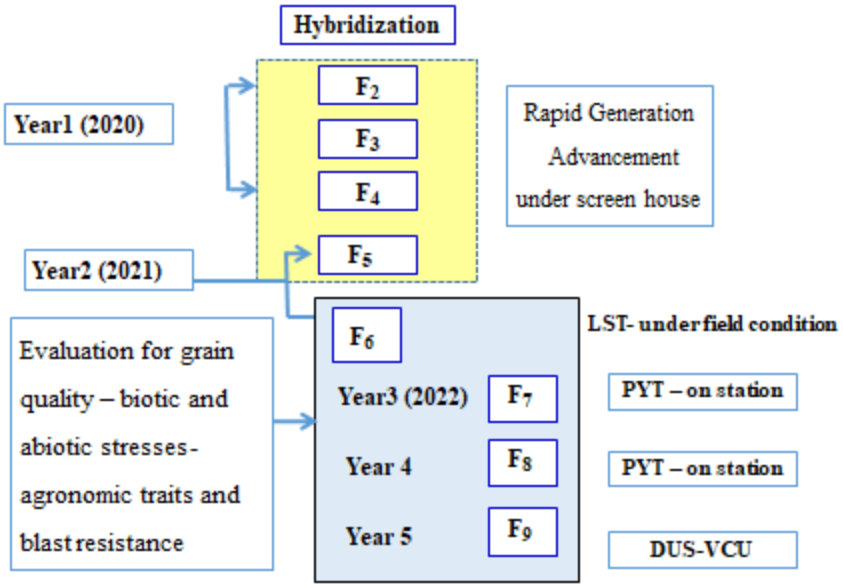


**Fig. 1**. Scheme of shorten rice breeding cycle under Egyptian condition.

The dried seeds are then prepared for the next cycle of advancement the $F_2$, $F_3$, $F_4$ and $F_5$, and then the panicle of $F_5$ threshed per line to be planted as F6 in one row with 5 m long with Giza177 and Giza178 as check variety in Lines Stage Trials (LST). Selection for earliness, grain shape and panicle traits was applied during the different generations. The F6 line and check variety was evaluated in augmented design with applying the recommended culture practice of RRTC. The protocol to advance breeding lines at different generations is proposed in Figure 1: Thirty promising lines ($F_7$) were selected and evaluated in a Randomized Complete Block Design (RCBD) with three replications under field conditions. Genotypes were grown in seven rows five meter long as individual plants 20 x20 cm and all the recommended cultural practices of rice were applied. Eight agronomic characteristics were studied i.e. days to heading (day), plant height (cm), number of panicles plant-1, panicle weight (g), number of spikelets panicles-1, 1000-grain weight (g), spikelet fertility (%) and grain yield (t ha-1). Grain yields were estimated based on the fifth inner rows in the middle of each plot. Ten plants were randomly taken from each plot to estimate the agronomic traits.

**Evaluation of blast disease:**

**Rice blast samples collection, identifications of blast physiological races and Pathogenicity test:** Typical blast lesions on leaves and panicles were collected from infected rice varieties at different locations of rice growing area during 2022 and 2021 seasons. Twenty six isolates of *Pyricularia oryzea* were isolated according to Shabana et al (2013). The isolates were grown and multiplied on banana medium at 28 °C. Using eight international differential varieties (I.D.V.), the isolates identified according to Atkins *et al.* (1967). The plant materials were seeded in plastic trays (30 x 20 x15 cm.). The trays were kept in the greenhouse at 25-30 °C, and fertilized with Urea 46.5% N (5 g/tray). Rice seedlings at 3-4 leaf stage were ready for inoculation by spraying with spore suspension (50 ml) adjusted to 5 x 104 spores/ml. The inoculated seedlings were kept in a moist chamber with at least 90% R.H. and 25-28 oC for 24 hr, and then moved to the greenhouse. Seven to ten days after inoculation, blast reaction was recorded according to the standard evaluation system using 0-9 scale (IRRI, 2013).

**Evaluation of blast under natural field infection:**

Thirty promising RGA lines were used to evaluate its resistance level, in addition the susceptible check Sakha 101 and tolerance check Giza177. Rice materials were naturally exposed to blast infection at the maximum tillering stage at three locations; Sakha, Gemmeza and Zarzora. Forty-five days from sowing, typical lesions of blast were scored, according to standard evaluation system using 0-9 scale (IRRI, 2013) as follows:1-2 = resistant (R), 3 =

moderately resistant (MR), 4-6 = susceptible (S), 7-9 = highly susceptible (HS), Data collection and measurements of all studied traits were recorded according to IRRI standard evaluation system (SES) (IRRI, 2016).

**Table1.** Rice blast isolates form different governorates, Egyptian rice cultivars and their race identification

| No | Governorate | District | Rice cultivar | No | Governorate | District | Rice cultivar |
|---|---|---|---|---|---|---|---|
| 1 | Kafrelsheikh | Sakha | Sakha 108 | 14 | Sharqia | Zagazig | Sakah104 |
| 2 | Kafrelsheikh | Kafrelsheikh | Sakha104 | 15 | Gharbia | Shabsher Elhessa | Sakah101 |
| 3 | Sharquia | Zagazeg | Sakah101 | 16 | Beheira | Itaie Elbarood | Sakah101 |
| 4 | Gharbia | Gemmzia | Sakah101 | 17 | Gharbia | Samanood | Sakha108 |
| 5 | Beheira | Shobarkheet | Sakha108 | 18 | Kafrelsheikh | Sakha | unknown |
| 6 | Damietta | KafrSaad | Sakah101 | 19 | Sharqia | Zagazig | Sakah101 |
| 7 | Kafrelsheikh | Alhamra | Sakah101 | 20 | Dakahlia | Meet ghamr | Sakah101 |
| 8 | Kafrelsheikh | Alagozeen | Sakha104 | 21 | Dakahlia | ELmansoura | Sakha101 |
| 9 | Dakahlia | ELmansoura | Sakha104 | 22 | Kafrelsheikh | Sakha | Pi No-4 |
| 10 | Kafrelsheikh | Elryad | Sakha104 | 23 | Kafrelsheikh | Sakha | Sakha108 |
| 11 | Beheira | Zarzora | Sakah101 | 24 | Kafrelsheikh | Sakha | unknown |
| 12 | Kafrelsheikh | Qallin | Sakah101 | 25 | Kafrelsheikh | Fowa | Sakha104 |
| 13 | Kafrelsheikh | Elmrabeen | Sakah101 | 26 | Sharqia | Zagazig | Sakah101 |

**Statistical Analysis:** The data were subjected to analysis of variance (Steel *et al.,* 1996) to determine the significant differences among genotypes for all the characters evaluated by the IRRISTAT program for pooled data. A combined analysis of variance for the two years was carried out for the yield and nailed components. The data were analyzed using Gene's program. Cluster and principal component analysis were performed.

## RESULTS

Early generation stage: Rice breeding cycle was shortened by using the implementation of Rapid Generation Advance (RGA) in the three rice populations. The $F_2$ plants of each population were harvested individually, one seed from each panicle was used to cultivate the next generation ($F_3$) according to the single seed descent (SSD) method. F3 were advanced to F4 by using the same methods. Figure 2: show different stages of RGA starting from soil preparation up to harvest. The seed germination ranged between 90-95% without any significant differences between the three populations. In general the $F_5$ lines showed good germination % (97%) and high growth rate for all populations. The $F_5$ lines of exhibited more uniformity, short stature the plant height (30-60 cm), panicle weight ranged between 0.5 -2.5 gr and number of grains per panicle ranged between 8.0 - 75.0 grains. Most of F5 plant revealed one panicle per plant, only a few plants gave two panicles per plant. Selection of panicles is done to discriminate matured panicles from un-matured. This is done at 70-90 DAS depending on the earliness of the population.

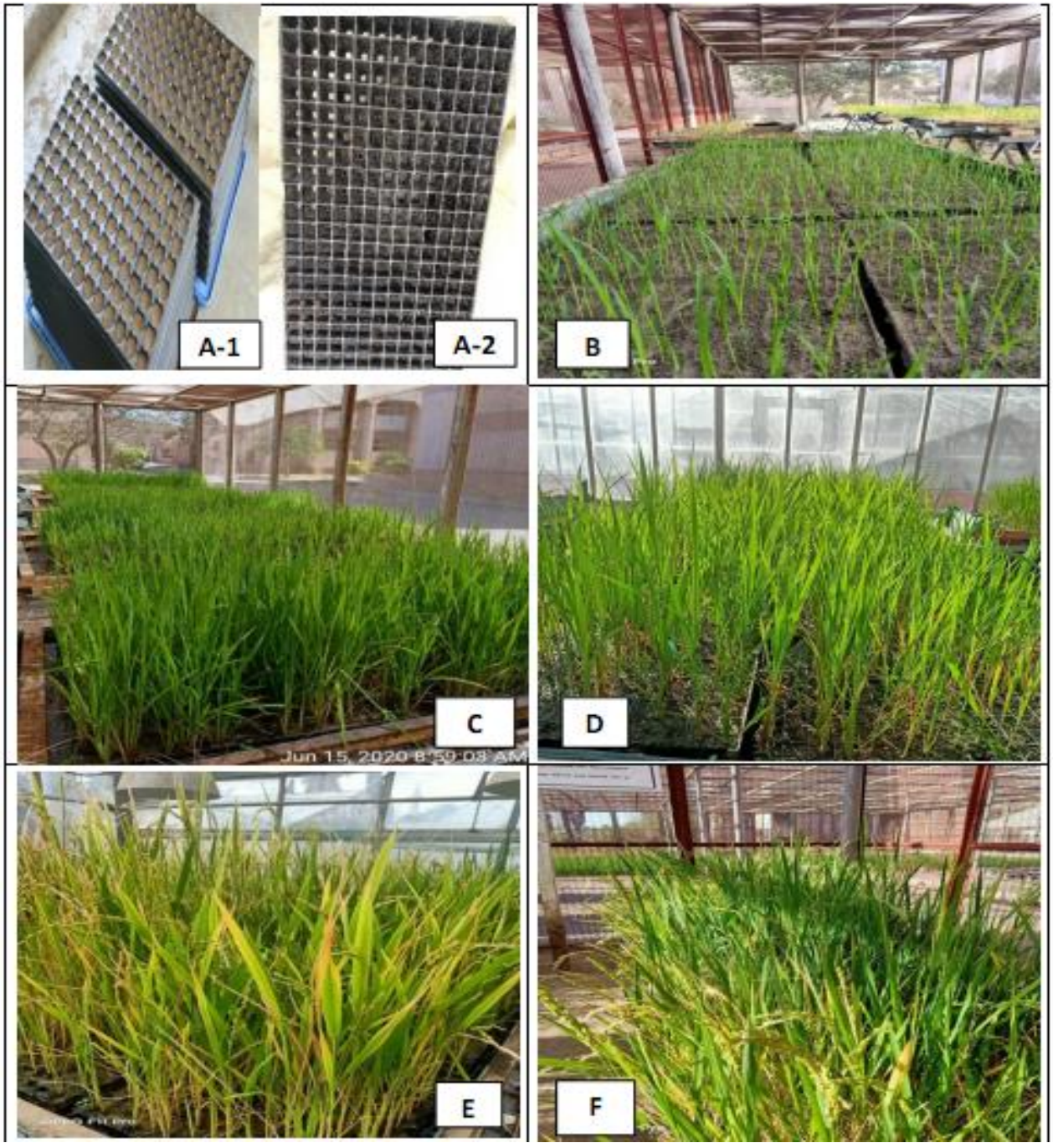


**Fig. 2.** Overview of rapid generation advance (RGA) system. (A-1) seedling trays 8 rows × 13 columns enclosed in plastic trays. A-2) seedling trays 13 rows × 20 columns, (B) Seedling stage, (C) vegetative stage under screen house. D) Vegetative stage under screen house (E) Filling stage (F) maturity stage.

**Lines Stage Trials (LST):** the F6 performance of 170 lines was presented in figure 3: Transgressive segregation was observed for all studied traits and some RGA lines outperformed check varieties for grain yield and other agronomic traits.

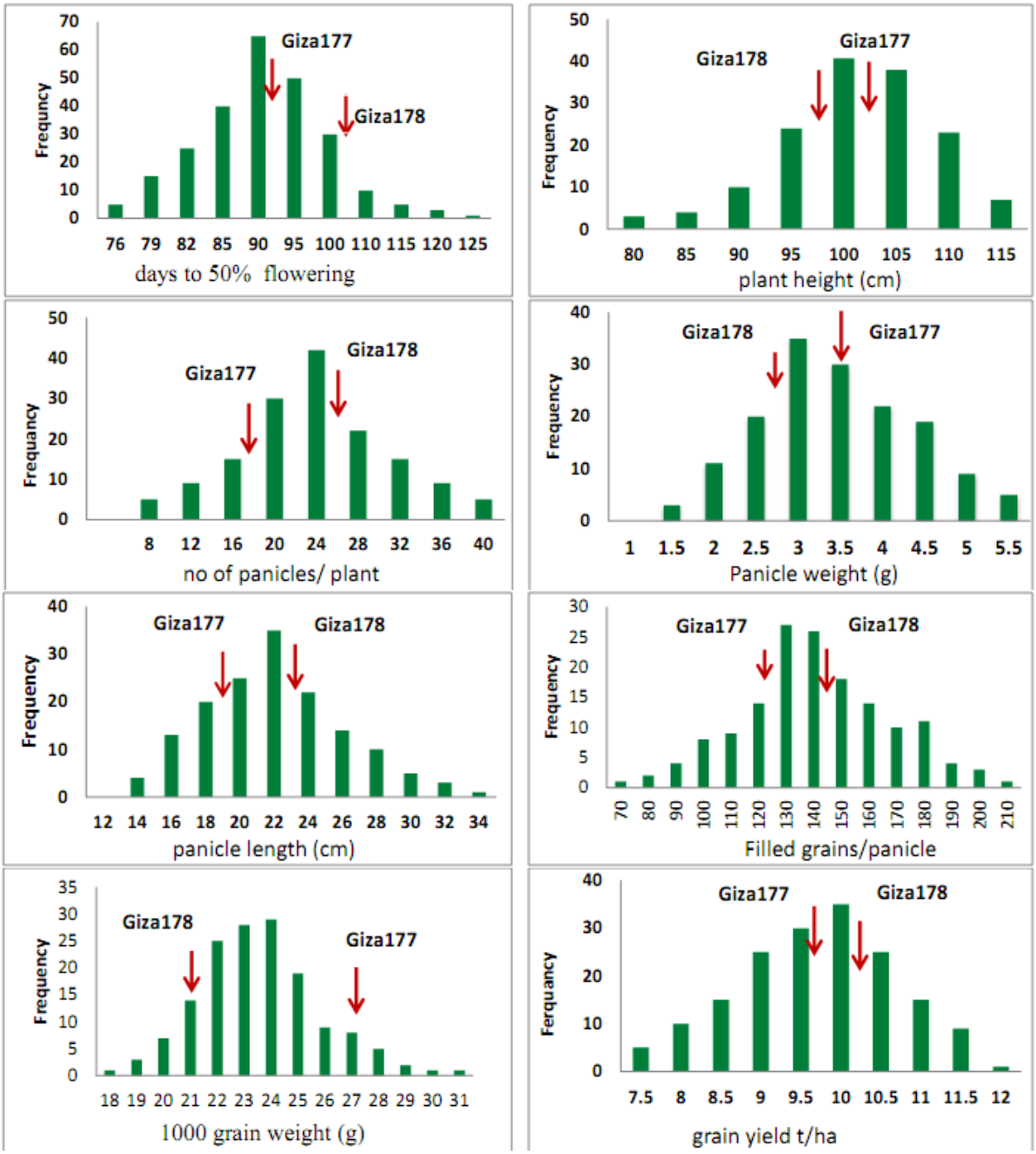


**Figure 3.** Histogram of grain yield and agronomic traits of 170 RGA lines F6.

The number of days to 50% flowering exhibited different values tacking the normal distribution, ranging between 76 to 125 days. The beak values were between 85 and 95 days, most of the F6 lines were earlier than the two checks Giza177 (91 days) and Giza178 (102 days). Regarding plant height it ranged between 80 and 115 (cm) and the beak values of the promising lines ranged between 95 and 110 (cm) compared with Giza177 (103 cm) and Giza178 (97 cm). Number of the panicle per plant showed wide variation among their values ranged between 8 and 40 panicle per plant, and followed the normal distribution. The majority of number of RGA lines had high number of panicle per plant ranged between 20 and 28 compared with Giza177 (17) and Giza178 (26 panicles).

Concerning panicle length and panicle weight, wide variations were observed among the two traits for RGA lines. Panicle weight ranged from 1.5 to 5.5 g, the majority of RGA lines ranged between 2.5 and 4.5 g, compared with the check varieties Giza177 (3.5 g) and Giza178 (2.8 gr). Panicle length of RGA lines ranged between 14 to 34 cm, compared with the check varieties Giza177 (27 cm) and Giza178 (21 cm). Number of filled grains per panicle of RAG lines ranged between 70 to 210 grain per panicle compared with the check varieties Giza177 (127) and Giza178 (145). The 1000 grain weight of RGA ranged between 18 to 31 g, compared with Giza177 (27 g) and Giza178 (21 g). Grain yield of RGA ranged between 7.5 and 12 t/ha, more than 30 promising RGA lines yielded 10.5 t/ha compared with Giza177 (9.7 t/ha) and Giza178 (10.2 t/ha).

**Preliminary yield Trial (PYT**): The analysis of variance 30 RGA genotypes with three checks is shown in three replications are presented in Table 2. Highly significant and highly differences were observed among the genotypes for all studied traits except amylose contact% and hulling%.

**Table 2.** analysis of variance of grain yield and agronomic traits of promising 30 RGA lines.

| **SOV** | **df** | **DAF 50%** | **plant height** | **Tillers No** | **panicle length** | **panicle weight** | **spikelets fertility%** |
|---|---|---|---|---|---|---|---|
| **Replications** | 2 | 0.13 | 7.87 | 2.37 | 1.07 | 0.16 | 7.15 |
| **Treatments** | 32 | 145.01** | 250.82** | 23.30** | 8.70** | 1.47** | 44.47** |
| **Error** | 64 | 0.4 | 3.38 | 2.83 | 0.77 | 0.24 | 6.21 |
| **Total** | 98 | | | | | | |
| **SOV** | DF | Filled grain | 1000 GW | yield | Amylose | Hulling | Milling |
| **Replications** | 2 | 175.26 | 0.5 | 1.31 | 83.34 | 664839.9 | 0.99 |
| **Treatments** | 32 | 665.39** | 10.22** | 1.07** | 40.76ns | 665123.88ns | 2.30* |
| **Error** | 64 | 100.96 | 0.44 | 0.17 | 37.67 | 665872.5 | 1.32 |
| **Total** | 98 | | | | | | |

*and ** are significant and highly significant at 0.05 and 0.01 probability

**Performance of promising RGA lines:** Performance of grain yield, agronomic traits and grain quality of 30 promising RGA lines in replicated trials are presented in Table 3 and 4. Regarding the number of days to 50% flowering, most of the RGA lines were early maturing of between 83.33 and 97.33 days, compared with the check varieties, Giza177, Giza178 and Sakha Super300, 91.0, 105.0 and 123.33 days respectively. The RGA line showed wide variation of the plant height, the promising RGA lines, SK-RGA1-6, SK-RGA2-1 and SK-RGA2-6 were the shortest plant height, 90.80, 95.7 and 94.2 cm respectively. The plant height of check varieties, Giza177, Giza178 and Sakha Super300 were 105.7, 97.67 and 112.67 cm respectively. The RGA lines showed high differences in the number of panicles per plant, SK-RGA1-6, SK-RGA2-8 and Sk-RGA2-9 recorded the highest values of, 25.0, 25.2 and 26.4 respectively. Number of panicles per plant for the check varieties, Giza177, Giza178 and Sakha Super300 were 17.0, 25.0 and 18.0 respectively. Concerning panicle length (cm) and panicle weight (g), most of the RGA lines showed high values compared with the check varieties. The RGA lines, SK-RGA1-3, SK-RGA-1-13, SK-RGA2-5 and SK-RGA3-6 recorded longest and heaviest panicle, (25.52 cm and 5.63 gr), (26.97cm and 5.67 gr), (27.97 cm and 5.83 gr) and 27.0 cm and 6.23) respectively. Spikelets fertility% of the RGA lines ranged between 82.2 and 96.47% while the check varieties Giza177, Giza178 and Sakha Super300 exhibited high spikelets fertility%, 90.47, 91.17 and 92.60 respectively. Grain yield and grain quality traits are presented in Table 3. Wide differences were observed among the RGA lines and check varieties. Number of filled grains per panicle of RGA lines ranged between 131.07 and 192.37, compared with the check varieties, Giza177, Giza178 and Sakha super300, giving 140.5, 175.3 and 170.47 grain respectively. RGA lines, SK-RGA1-12, SK-RGA1-14 and SK-RGA2-4, exhibited heaviest 1000 grain weight of, 30.0, 30.0 and 30.33 (g) respectively. In the same time the three check varieties Giza177, Giza178 and Sakha super300 give, 27.67, 21.67 and 26.10 (g). Grain yield of RGA lines varied between 7.23 and 11.87 t/ha,

promising RGA lines, SK-RGA2-5, SK-RGA2-9 and SK-RGA3-6 revealed grain yield more than 11.0 t/ha compared with the check varieties, Giza177, Giza178 and Sakha super300, give 9.57, 10.17 and 10.50 t/ha respectively.

**Table 3.** Agronomic and panicle traits of 30 promising RGA lines and the three checks

| Population | **RGA line** | **DAF 50% (day)** | **Plant height (cm)** | **Panicle/ plant** | **panicles length (cm)** | **panicle weight (g)** | **spikelets fertility%** |
|---|---|---|---|---|---|---|---|
| Giza177 x Sakha super300 | SK -RGA 1-1 | 88.33 | 106.07 | 21.60 | 23.67 | 4.30 | 91.97 |
| | SK -RGA 1-2 | 89.33 | 108.17 | 23.20 | 23.63 | 4.73 | 90.73 |
| | SK -RGA 1-3 | 88.33 | 109.20 | 22.33 | 25.53 | 5.63 | 91.27 |
| | SK -RGA 1-4 | 91.33 | 101.40 | 18.47 | 22.80 | 4.60 | 93.30 |
| | SK -RGA 1-5 | 86.67 | 122.00 | 18.00 | 24.87 | 5.87 | 92.93 |
| | SK -RGA 1-6 | 89.67 | 90.80 | 25.00 | 20.27 | 3.93 | 93.13 |
| | SK -RGA 1-7 | 89.33 | 114.80 | 16.47 | 20.80 | 3.83 | 95.93 |
| | SK -RGA 1-8 | 87.67 | 131.00 | 20.53 | 21.03 | 5.40 | 93.60 |
| | SK -RGA 1-9 | 91.67 | 99.20 | 20.60 | 21.83 | 4.40 | 84.13 |
| | SK -RGA 1-10 | 92.33 | 98.00 | 19.27 | 20.53 | 4.17 | 95.73 |
| | SK -RGA 1-11 | 91.00 | 123.80 | 18.73 | 24.73 | 4.77 | 96.47 |
| | SK -RGA 1-12 | 86.67 | 110.27 | 22.80 | 20.60 | 5.03 | 88.73 |
| | SK -RGA 1-13 | 91.67 | 114.07 | 18.00 | 26.97 | 5.67 | 86.67 |
| | SK -RGA 1-14 | 87.33 | 106.00 | 20.40 | 23.23 | 3.27 | 94.93 |
| | SK -RGA 1-15 | 90.33 | 99.20 | 19.40 | 21.57 | 4.17 | 89.10 |
| IR75589-31 x Giza178 | SK -RGA 2-1 | 90.00 | 95.07 | 19.00 | 21.07 | 4.23 | 85.80 |
| | SK -RGA 2-2 | 89.33 | 98.40 | 21.47 | 21.37 | 4.47 | 82.20 |
| | SK -RGA 2-3 | 97.33 | 103.00 | 22.00 | 21.17 | 4.37 | 82.90 |
| | SK -RGA 2-4 | 90.67 | 103.27 | 18.53 | 21.23 | 3.57 | 89.90 |
| | SK -RGA 2-5 | 89.33 | 105.40 | 24.93 | 27.97 | 5.83 | 86.53 |
| | SK -RGA 2-6 | 89.33 | 94.20 | 21.20 | 19.53 | 2.27 | 89.20 |
| | SK -RGA 2-7 | 97.33 | 111.00 | 20.20 | 20.10 | 5.47 | 94.80 |
| | SK -RGA 2-8 | 92.33 | 109.80 | 25.20 | 26.53 | 5.10 | 92.50 |
| | SK -RGA 2-9 | 92.33 | 113.60 | 26.40 | 22.87 | 5.43 | 92.80 |
| Giza179 x IR59673-93-2-3-3-2 | SK -RGA 3-1 | 88.67 | 104.27 | 18.80 | 23.97 | 4.03 | 93.90 |
| | SK -RGA 3-2 | 86.67 | 99.00 | 18.80 | 21.63 | 4.87 | 94.10 |
| | SK -RGA 3-3 | 86.33 | 111.73 | 19.27 | 23.23 | 4.70 | 88.00 |
| | SK -RGA 3-4 | 86.67 | 123.20 | 19.20 | 23.90 | 4.57 | 87.57 |
| | SK -RGA 3-5 | 93.33 | 106.27 | 17.80 | 22.60 | 4.27 | 86.40 |
| | SK -RGA 3-6 | 83.33 | 109.73 | 19.60 | 27.00 | 6.23 | 86.83 |
| Giza177 | CK1 | 91.33 | 105.07 | 17.20 | 21.53 | 3.70 | 90.47 |
| Giza178 | CK2 | 105.00 | 97.67 | 25.50 | 23.83 | 3.57 | 91.17 |
| Sakha Super 300 | CK3 | 123.33 | 112.67 | 18.00 | 22.63 | 4.37 | 92.60 |
| LSD 5%<br>1% | | 1.61<br>1.85 | 2.43<br>2.91 | 2.14<br>2.65 | 1.56<br>2.12 | 0.38<br>0.67 | 4.17<br>5.21 |

**Table 4.** Grain yield and grain quality traits of 30 promising RGA lines and the three checks

| Population | RGA line | Filled grain/ panicle | 1000 grain weight (g) | Grain yield t/ha | Amylose content % | Hulling % | Milling % |
|---|---|---|---|---|---|---|---|
| Giza177 x Sakha super300 | SK -RGA 1-1 | 153.53 | 26.33 | 10.40 | 19.77 | 85.23 | 72.43 |
| | SK -RGA 1-2 | 162.83 | 28.67 | 10.33 | 20.97 | 83.90 | 72.43 |
| | SK -RGA 1-3 | 188.57 | 26.67 | 10.90 | 20.00 | 83.10 | 71.00 |
| | SK -RGA 1-4 | 157.50 | 28.33 | 10.17 | 20.30 | 84.10 | 71.67 |
| | SK -RGA 1-5 | 149.87 | 29.00 | 10.50 | 22.00 | 83.77 | 71.67 |
| | SK -RGA 1-6 | 131.47 | 24.67 | 7.53 | 18.33 | 84.10 | 71.33 |
| | SK -RGA 1-7 | 157.83 | 24.67 | 10.33 | 21.17 | 84.10 | 71.33 |
| | SK -RGA 1-8 | 186.03 | 27.67 | 10.03 | 22.50 | 83.43 | 72.10 |
| | SK -RGA 1-9 | 144.97 | 27.33 | 9.17 | 22.23 | 84.10 | 72.10 |
| | SK -RGA 1-10 | 135.03 | 24.67 | 8.57 | 21.60 | 81.67 | 72.10 |
| | SK -RGA 1-11 | 151.73 | 29.00 | 9.90 | 17.83 | 83.77 | 72.10 |
| | SK -RGA 1-12 | 164.50 | 30.00 | 10.13 | 21.63 | 84.10 | 72.10 |
| | SK -RGA 1-13 | 163.63 | 28.33 | 10.87 | 21.30 | 82.57 | 70.57 |
| | SK -RGA 1-14 | 131.30 | 30.00 | 9.87 | 21.30 | 82.00 | 71.00 |
| | SK -RGA 1-15 | 156.37 | 28.67 | 10.17 | 20.77 | 82.10 | 72.10 |
| IR75589-31 x Giza178 | SK -RGA 2-1 | 141.00 | 29.33 | 9.93 | 22.97 | 80.00 | 72.10 |
| | SK -RGA 2-2 | 145.50 | 28.00 | 9.20 | 21.47 | 81.00 | 70.90 |
| | SK -RGA 2-3 | 144.03 | 27.67 | 10.47 | 19.17 | 82.10 | 72.10 |
| | SK -RGA 2-4 | 144.37 | 30.33 | 10.23 | 18.27 | 82.10 | 71.00 |
| | SK -RGA 2-5 | 171.73 | 29.33 | 11.13 | 18.10 | 81.43 | 72.10 |
| | SK -RGA 2-6 | 131.07 | 23.67 | 7.23 | 20.70 | 82.33 | 69.43 |
| | SK -RGA 2-7 | 181.10 | 28.33 | 10.27 | 22.43 | 83.10 | 72.10 |
| | SK -RGA 2-8 | 178.00 | 28.00 | 10.87 | 21.50 | 82.10 | 72.10 |
| | SK -RGA 2-9 | 192.37 | 29.33 | 11.87 | 19.30 | 80.00 | 71.00 |
| Giza179 x IR59673-93-2-3-3-2 | SK -RGA 3-1 | 138.90 | 28.33 | 10.00 | 21.33 | 81.00 | 71.00 |
| | SK -RGA 3-2 | 156.27 | 27.50 | 10.27 | 21.90 | 80.90 | 71.00 |
| | SK -RGA 3-3 | 150.27 | 29.17 | 9.17 | 20.10 | 81.00 | 72.10 |
| | SK -RGA 3-4 | 159.13 | 27.53 | 9.97 | 23.13 | 81.00 | 72.77 |
| | SK -RGA 3-5 | 154.00 | 26.50 | 10.33 | 21.87 | 80.67 | 72.10 |
| | SK -RGA 3-6 | 187.80 | 28.00 | 11.47 | 19.17 | 81.00 | 71.00 |
| Giza177 | CK1 | 140.50 | 27.67 | 9.57 | 20.23 | 83.43 | 71.77 |
| Giza178 | CK2 | 175.30 | 21.67 | 10.17 | 17.83 | 82.10 | 72.00 |
| Sakha Super 300 | CK3 | 170.47 | 26.10 | 10.50 | 20.33 | 84.00 | 73.77 |
| LSD 1% | | 5.18 | 1.23 | 0.35 | 1.15 | 1.34 | 0.23 |
| 5% | | 6.13 | 1.69 | 0.62 | 1.45 | 1.80 | 0.41 |

**Grain quality traits:** Most of the test of RGA lines and check varieties gave low and medium amylose content ranging between 17.83 and 23.13%. RGA lines and check varieties give desirable values of hulling and milling%. In general hulling% ranged between 80.0 and 85.23% and milling% ranged between 69.43 and 72.77%.

Reaction to blast disease: The 30 promising RGA lines were tested in blast nursery at Sakha (Kafrelsheikh governorate), Gemmiza (Garbia governorate) and Zarzoura (ELbeharia governorate) to test the reaction of these genotypes to blast. The data in Table 5 indicated that most of the promising lines and Giza177 (tolerance check) were resistant (with score R) in the three locations. Only eight RGA lines show moderate resistance (MR), under the field conditions. While Sakha101 (susceptible check) was susceptible by blast in the field under the same three locations conditions.

**Table 5.** Evaluation of promising RGA lines in natural blast nursery at three locations.

| No | RGA lines | Locations | | | No | RGA lines | Locations | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sakha | Gemmiza | Zarzoura | | | Sakha | Gemmiza | Zarzoura |
| 1 | SK -RGA 1-1 | R | R | R | 17 | SK -RGA 2-2 | R | R | R |
| 2 | SK -RGA 1-2 | R | R | R | 18 | SK -RGA 2-3 | R | R | R |
| 3 | SK -RGA 1-3 | R | R | R | 19 | SK -RGA 2-4 | R | R | R |
| 4 | SK -RGA 1-4 | R | R | R | 20 | SK -RGA 2-5 | R | R | R |
| 5 | SK -RGA 1-5 | R | R | R | 21 | SK -RGA 2-6 | R | R | R |
| 6 | SK -RGA 1-6 | R | R | R | 22 | SK -RGA 2-7 | R | R | R |
| 7 | SK -RGA 1-7 | R | R | R | 23 | SK -RGA 2-8 | R | R | R |
| 8 | SK -RGA 1-8 | MR | R | MR | 24 | SK -RGA 2-9 | MR | MR | MR |
| 9 | SK -RGA 1-9 | R | R | R | 25 | SK -RGA 3-1 | MR | MR | MR |
| 10 | SK -RGA 1-10 | MR | MR | R | 26 | SK -RGA 3-2 | MR | MR | MR |
| 11 | SK -RGA 1-11 | MR | R | MR | 27 | SK -RGA 3-3 | R | R | R |
| 12 | SK -RGA 1-12 | R | R | R | 28 | SK -RGA 3-4 | MR | MR | MR |
| 13 | SK -RGA 1-13 | R | R | R | 29 | SK -RGA 3-5 | MR | MR | MR |
| 14 | SK -RGA 1-14 | R | R | R | 30 | SK -RGA 3-6 | R | R | R |
| 15 | SK -RGA 1-15 | R | R | R | Giza177 (Resistance check) | | R | R | R |
| 16 | SK -RGA 2-1 | R | R | R | Sakha101 (susceptible check) | | HS | S | S |

R = Resistant, MR = moderately resistant (S)= susceptible (S), (HS)= highly susceptible

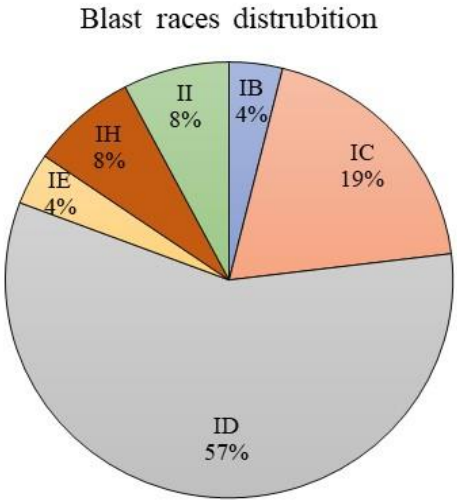


**Figure 4**: Distribution of different blast race groups on the International Differential Varieties under artificial inoculation with 26.

**Table 6.** Promising lines reaction under artificial inoculation against 27rice blast races.

| Genotypes | Isolate no./ace identification/ action | | | | | | | | | | | | | | | | | | | | | | | | | | resistance % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | |
| | IC-11 | IE-3 | ID-15 | ID-15 | IC-11 | ID-15 | IC-31 | ID-11 | IH-1 | IC-8 | 1D-15 | IH-1 | IB-3 | ID-15 | ID-12 | ID-13 | IC-1 | ID-9 | ID-12 | ID-11 | ID-15 | ID-8 | II | ID-3 | II | ID-8 | |
| SK -RGA 1-1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-2 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-3 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-4 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-5 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-6 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-7 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-8 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | MR | R | R | R | R | R | R | R | R | 96 |
| SK -RGA 1-9 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-10 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | MR | R | R | R | R | R | S | R | R | 96 |
| SK -RGA 1-11 | R | R | R | R | R | MR | R | R | R | R | R | R | R | R | R | R | R | MR | R | R | R | R | R | R | R | R | 96 |
| SK -RGA 1-12 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-13 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-14 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 1-15 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-1 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-2 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-3 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-4 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-5 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-6 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-7 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | MR | R | R | R | R | R | S | R | R | 96 |
| SK -RGA 2-8 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 2-9 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | MR | R | R | R | R | R | MR | R | R | 96 |
| SK -RGA 3-1 | R | R | R | R | R | S | R | S | R | R | R | MR | HS | S | R | S | R | R | MR | R | S | R | R | R | R | S | 66.7 |
| SK -RGA 3-2 | MR | R | R | R | R | MR | R | S | R | R | R | R | S | R | MR | S | R | R | R | R | R | R | R | R | R | R | 77.8 |
| SK -RGA 3-3 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| SK -RGA 3-4 | MR | R | S | MR | MR | HS | HS | R | R | S | R | HS | HS | MR | R | S | R | HS | R | R | S | R | S | MR | MR | R | 40.7 |
| SK -RGA 3-5 | MR | R | R | MR | R | MR | R | R | R | R | R | R | S | MR | MR | R | R | HS | R | R | R | R | R | MR | R | MR | 74.1 |
| SK -RGA 3-6 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |
| S101 | S | R | MR | S | R | HS | MR | R | R | R | MR | MR | S | MR | MR | S | MR | R | HS | R | MR | R | S | R | R | HS | 67 |
| Giza177 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | 100 |

R = resistant, MR = moderately resistant (S)= susceptible (S), (HS)= highly susceptible

Race identification of rice blast populations under greenhouse conditions: According to the reaction of different isolates on the international differential varieties, the isolates were identified to race level. Where, 26 isolates were identified to seventeen races (Table 6) and were categorized in six race-groups; 4% race belonging to group IB, 19% races to group IC, 58% races as most common to ID group from the total isolates, 4% race to group IE, 8% race to group IH, 8% race races II, as a virulent group (Fig 4). Race identification and monitoring play an important role to determine race shifting, whereas, current data indicated that race group ID represent the most common races follow by group IC. Data in Table 6 showed that twenty-one promising rice RGA were resistant and nine were susceptible to highly susceptible for P. Oryzea under greenhouse conditions.

## DISCUSSION

The Preliminary results of this investigation confirmed the successful implementation of RGA at RRTC, with simple plastic materials and the maintenance of the greenhouse and screen house. This new breeding scheme will help rice breeders to get three generations per year and improve the genetic grain (Cobb *et al.,* 2019; Atlin and Econopouly, 2022). The International Rice Research Institute (IRRI), Transformed Rice Breeding program through a five year project" funded by the Bill and Melinda Gates Foundation. Changes were implemented with the specific objectives to increase the rate of genetic gain for yield and improve the effectiveness and efficiency of breeding operations. The three populations used in this study were selected to meet the main objectives of the rice breeding program at RRTC. The parents Giza178 and Giza179 are high yielding and tolerant to salinity but the improvement of the grain quality and grain shape are very important. Also, Sakha Super300 high yielding variety but photoperiod sensitive and late maturity for this reason crossed with early maturity Giza177. There is an urgent need to double the production amount to feed more than 9 billion people in this world by 2050 (Ray *et al.,* 2013; Arbelaez *et al.,* 2015: Saraswathipura *et al.,* 2022). The early generations ($F_2$, $F_3$, $F_4$, and $F_5$) showed good performance at the greenhouse the narrow space and low fertilizer which lead the plant for early flowering (Kanbar *et al.,* 2011). The LST trials was an important stage for seed increase and selection of highly heritable traits using short rows, because there is no previous selection for plant type, disease resistance or other traits. Large phenotypic variation was observed within RGA populations observed at this stage, which has been a concern of rice breeders. There were advantages for select lines based on a panicle-row compared to selecting single plants (Bertrand *et al.,* 2017). The new RGA lines showed good performances, early flowering, high yielding, good grain quality and tolerance to blast disease. More evaluation for the new promising RGA lines is needed in advance trials under different biotic and abiotic stresses. Rapid breeding cycles, high selection accuracy, selection of parents with high breeding value, and high selection intensity are the key drivers of genetic gain for such traits (Cobb *et al.,* 2019). The RGA breeding method is highly suitable for integrating MAS because screening can be performed during line fixation and be used to reduce population sizes (Pindel *et al.,* 2015). The advantages of RGA as a breeding method include the technical simplicity, requirement of less field, and labor resources as well as funds Also the speed of the method if off-season or quicker generation advancement can be achieved (Poehlman and Sleper, 1995; Stoskopf *et al.,* 1993). Compared to the pedigree method, record keeping is not required for RGA.

Usually, a seed increase step in the field is required to produce sufficient seed for yield testing in plots. Given future challenges related to climate change and the inadequacy of current crop yield trajectories to nourish the world's population by 2050, accelerated crop improvements are critical (Bailey-Serres *et al.,* 2019). This will require quick action to develop new varieties tolerant to biotic and abiotic stresses. This can be achieved through RGA to develop new varieties in a short time. Race identification and monitoring for rice blast disease play an important role to determine race shifting and the reaction of tested promising RGA lines. whereas, current data indicated the 30 promising lines were resistance under natural infection while under artificial inoculation they arrived to twenty-one promising rice RGA were resistant and nine were susceptible for *P. oryzea*. Concerning the race identification, the race group ID represent the most common races follow by group IC. Race identification and shifting are in agreement with (EL-Shafey *et al.,* 2015; Awadallah *et al.,* 2021). Whereas, physiological races play an important role for breakdown the new cultivars and promising lines especially when increasing the growing area of one or two cultivars. Many investigators studied the physiological races of the fungus at different rice-growing areas and the role of physiological races to breakdown the new promising lines. Awadallah *et al.* (2021) who showed the distribution of races with different rice entries and locations and this new physiological race was associated with breakdown for new rice genotypes.

## CONCLUSION:

Adoption of new plant varieties has played a significant role in eradicating global hunger. Accelerated rice breeding can be accomplished through various approaches, including molecular or conventional techniques. RGA technique was successfully implemented at RRTC, with simple multipot trays and maintenance of the greenhouse and screen house. The early generations (F2, F3, F4, and F5) showed good performance at the greenhouse; the narrow space and low fertilizer lead the plants for early flowering. promising RGA lines, SK-RGA2-5, SK-RGA2-9 and SK-RGA3-6 revealed grain yield more than 11.0 t/ha compared with the check varieties, Giza177, Giza178 and Sakha super300, give 9.57, 10.17 and 10.50 t/ha respectively.

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# تقصير دورة تربية الأرز وإستنباط سلالات مبشرة

رأفت النمكى[1] ، صابر صديق[1] ، أسامة البدوي[1] ، إيمان بليح[1] ، سعيد سلطان[1] ، مرفت عوض الله[1] ، عبدالعزيز طاحون[2] ، أحمد سمير طه[1].
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يمكن تسريع طرق تربية الأرز من محاور مختلفة تشمل البيتكنولوجى والطرق التقليدية. في هذا الصدد تم تغيير بعض برامج تربية الأرز في العالم لاستخدام الطرق السريعة المتقدمة كطريقة تربية جديدة. تتمثل أهداف هذا البحث في تأسيس وإستخدام هذه الطرق والتقنيات في برنامج التربية بمركز البحوث والتدريب فى الأرز وذلك لإستنباط أصناف جديدة محسنة وعالية المحصول ومقاومه لمرض اللفحه بطرق سريعة ومنخفضة التكلفة. إستخدم فى هذة الدراسة ثلاث عشائر من الجيل الثانى F2) ) وهى جيزة 177 × سخا سوبر300) و x (IR75589-31 جيزة 178) و (جيزة 79 x IR59673-93-2-3-3-2) تم تطويرها الى الجيل الخامس بالصوبة ثم تقييمها في تجارب PYT) - LST) بالحقل. تم حصاد نباتات F2 من كل عشيرة على حدة ، واستخدمت بذرة واحدة من كل سنبلة لزراعة الجيل التالي (F3) وفقًا لطريقة . SSD أكدت الدراسة نجاح إستخدام RGA في مركز البحوث والتدريب فى الأرز ، باستخدام صوانى بلاستيكية مقسمة وعمل صيانة للصوب السلكية والزجاجية . أظهرت الأجيال المبكرة F2 و F3 و F4 و F5 نموا جيدا حيث أن المساحة الضيقة والأسمدة المنخفضة دفعت النباتات إلى الإزهار المبكر. أعطت السلالات الجديدة RGA 5 SK-RGA2-9، SK-RGA2-9و SK-RGA3-6 المستنبطة بطريقة RGA محصول حبوب أكثر من 11.0 طن / هكتار مقارنة بأصناف المقارنة ، جيزة 177 ، جيزة 178 وسخا 300 ، أعطت 9.57 و 10.17 و 10.50 طن. / هكتار على التوالي. بالنسبة لصفات جودة الحبوب ، كانت أيضا معظم السلات الجديدة منخفضة ومتوسطة الأميلوز حيث تراوحت النسبة بين 17.83 و 23.13٪. أيضا تراوحت نسبة التقشير بين 80.0 و 85.0٪ والتبيض 69.43 و 73.77٪.

**الكلمات المفتاحية:** تربية الأرز ، محصول الحبوب ، مرض لفحة الأرز ، جودة الحبوب.