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RAPID GENERATION ADVANCE OF RICE AT THE INTERNATIONAL RICE RESEARCH INSTITUTE

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RAPID GENERATION ADVANCE OF RICE AT THE INTERNATIONAL RICE RESEARCH INSTITUTE[1]

ABSTRACT

A short generation rice cycle helps rice breeders who work with photoperiod-sensitive crosses where only one generation is possible per year because flowering is delayed if rice is planted off-season. Short generation is also necessary when breeding rice for low-temperature areas where only one crop can be grown each year. The rapid generation advance (RGA) method shortens rice plant growth duration by using high temperature-short day treatments to make three generations possible each year. The duration from F_2 to F_5, which generally takes 4 years, can be reduced to 2 years.

Besides shortening crop duration, RGA allows many crosses to be processed in limited space and time. Environment can be controlled, and problems such as bad weather, rats, and other disease and insect pests are minimized. Also, limited selection and screening is possible using RGA.

RGA was suggested by Goulden in 1939. Japanese rice breeders began using it in 1958. By 1977 31% of the rice areas in Japan grew cultivars bred using RGA (Nakane 1979). In 1976 Ikehashi began using RGA for photoperiod-sensitive rice breeding at IRRI (Ikehashi 1977). By 1980, IRRI RGA facilities were in full operation.

RGA can help shorten breeding periods at national programs in rainfed wetland areas where photoperiod sensitivity is important. Few new photoperiod-sensitive rice varieties have been named in the last 20 years, yet they are grown on more than 20 million hectares. Breeding for photoperiod-sensitive varieties has been neglected because more time is needed to develop them than is needed for photoperiod-insensitive varieties like the new, high-yielding breeds.

In photoperiod-sensitive rice breeding, selection for maturity date is site-specific. Strong specificity limits international testing and breeding material exchange because selection for a specific environment may result in discarding varieties best suited for other areas. RGA solves these difficulties. Through RGA, fixed bulk materials from IRRI are sent to different sites where primary selections, especially flowering dates, are made.

This report describes cultural methods we have evolved to obtain optimum growth, shortest possible growth duration, and maximum use of space. Various characters that can be screened during RGA and the general breeding procedure are discussed.

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RAPID GENERATION ADVANCE OF RICE AT THE INTERNATIONAL RICE RESEARCH INSTITUTE

Rapid generation advance (RGA) is not a total breeding method in itself but a breeding procedure where segregating populations are raised in an environment using close spacing, high temperature, and short days to minimize growth duration and make several generations per year possible.

RGA preserves the potential variability of each cross so genetically fixed materials can be rigorously selected under local conditions. However, certain characters can be selected during RGA. After RGA, the F_5 to F_6 lines are almost homozygous. Trait selection is reliable at this stage, even if low heritability traits such as elongation ability, which causes problems if selected at an early generation, are desired.

Because the special RGA environment does not allow researchers to visually assess plant population, potential performance of crosses cannot be observed. To overcome this problem, scientists may plant a sample of the reserve F_2 seed and monitor traits essential to the breeding objective. Care should be taken when selecting parents for the crosses and the breeding objective should be well defined. Taking notes on individual plants is not advisable, this defeats one of the major RGA advantages.

Standard steps taken before and during RGA are:

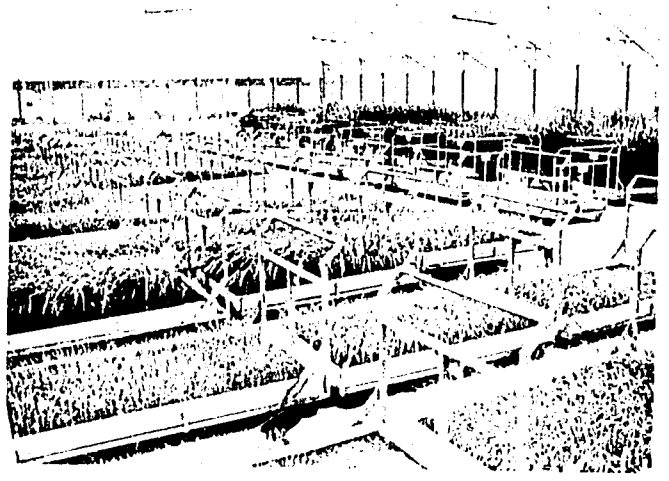
1. Hybridization. Specific breeding objectives are determined and the parents to be used selected based on adaptability to the area and specific traits needed to improve the variety for that area. The promising donor or parent may be identified through field observation, published literature, or the IRRI germplasm data bank. A local scientist can make the cross or IRRI can be requested to make specific crosses (Fig. 1). The cross may be simple or complex, depending on the objectives and donor availability.

2. Production of F_2 seeds. About 100 g of F_2 seeds are needed for the first RGA planting. However, if the procedure is strictly followed, 300 g reserve seed will be needed for other purposes (see Table 1). To produce this amount, grow at least 20 F_1 plants at ordinary spacing. Also grow the parents of the crosses as F_1 generation references.

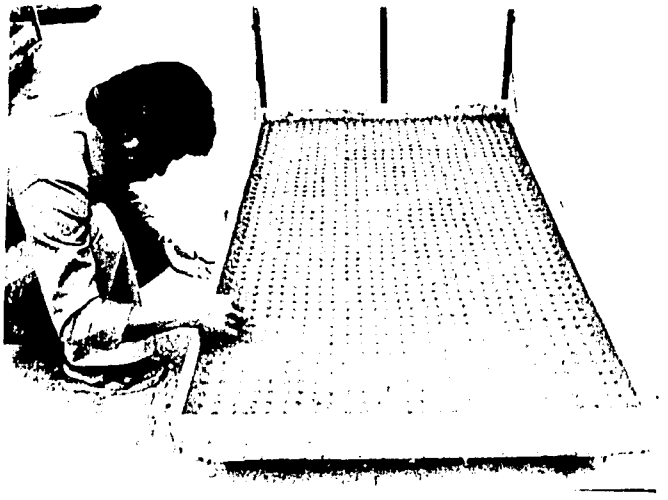
3. F_2 planting. When a cross is submitted for RGA at IRRI (Plant Physiology Department), a RGA number is assigned and information about the cross is listed (see Table 2 for the form).

   Generally, 1,000 to 1,500 F_2 plants are grown for each cross. At least 3,000 seeds are needed. Before beginning the process, seed dormancy must be broken. To break dormancy, fresh seeds are dried for 2 to 3 days and subjected to 50°C for 5 days.

   Seeds are soaked for 24 h, then incubated for 24 h. Wooden boxes or metal trays are prepared for planting by adding 1 g ammonium sulfate, 0.5 g of solophos, and 0.5 g of muriate of potash per liter of soil. A stick with nails at 3-cm intervals is used to make guide holes in the soil surface. Standard spacing is 3 x 3 cm, but optimum spacing depends on container depth and soil fertility. Using forceps, a germinated seed is placed in each hole and covered with 1 to 2 mm soil. Only uniformly germinated seeds should be planted so even growth and competition are maintained.



RGA screenhouse at IRRI.



Direct sowing of pregerminated seeds using 3 x 3 cm spacing.

Table 1. Amount of seeds needed for a RGA program with 3,000 initial F_2 plants per cross.

Operation	Amount of seeds (g)	Remarks
Main planting	100	For RGA
Short-term reserve	100	In case population is reduced by unexpected stress.
Long-term reserve	100	For future use.
Monitoring	100	To check the potential of the cross.

If vegetative growth is vigorous and inside plants are heavily shaded, leaf pruning may be necessary 20 days after sowing. Pruning at 15 cm above the ground will prevent plants from growing too tall, but will not delay flowering (Eunus et al 1980). During pruning, plants can be inoculated for bacterial blight using the clipping technique. Premature lodging can be minimized by spraying B995 at 4,000 ppm on 35-day-old seedlings. Carts used at IRRI have metal posts at each corner so strings can be attached to support plants.

Inoculating for BLB screening during pruning at vegetative stage.

Photoperiod treatment is started 20 days after sowing for crosses using photoperiod-sensitive parents. Carts are placed inside the darkroom at 1700 h, and removed the following day at 0700 h. This process is continued until 50% of the plants have headed. Many photoperiod-sensitive plants will start flowering 60 to 70 days after seeding. Some plants with long basic vegetative phase (BVP) need 100 days to flower. The longest sowing to flowering duration recorded for a RGA population is 134 days -- a DA29/IR42 cross (Bardhan Roy et al 1982). Delayed flowering in certain crosses usually is the result of a long BVP. A plant with a 60-day BVP will take more than 100 days to mature. These crosses diminish the value of RGA because growing three generations per year is difficult. BVP should be considered when determining crosses for RGA. Crosses with long BVP and photoperiod insensitivity should be planted in the field because growth duration in RGA is not shortened and is sometimes delayed.

Table 2. Entry form used in RGA. Information for items one to six and nine must be supplied by the cooperator.

RGA No. ___

RAPID GENERATION ADVANCE

1. Name of cooperator _____
2. Address _____
3. Designation of cross _____
4. Female parent _____ Male parent _____
5. Hybridization (Yr/Season) _____
6. Institution where hybridization was made _____
7. Breeding objectives:
   Deep water rice / /
   / / submergence tolerance
   / / elongation ability
   / / photoperiod sensitivity
   / / other agronomic characters
   Cold tolerance / /
   / / at seedling stage
   / / at panicle initiation
   / / at flowering
   / / earliness of flowering
   / / other agronomic characters
   Disease and pest resistance / /

RTV	/ /	ShB	/ /		
Bl	/ /	SB	/ /	BB	/ /
GLH	/ /	RSV	/ /	BPH	/ /
GSV	/ /	CM	/ /	BLS	/ /

   Other objectives such as drought and problem soils / /
8. Generation of the seed _____
   Date submitted _____
9. RGA from F ____ to F ____

	No. of seeds	No. of plants	Sowing	Flowering	Harvest
F_2					
F_3					
F_4					
F_5					

10. Data to be collected _____
11. Special treatment made _____
12. Data collected _____
13. No. of lines returned ___ Date returned ____
14. Remarks _____

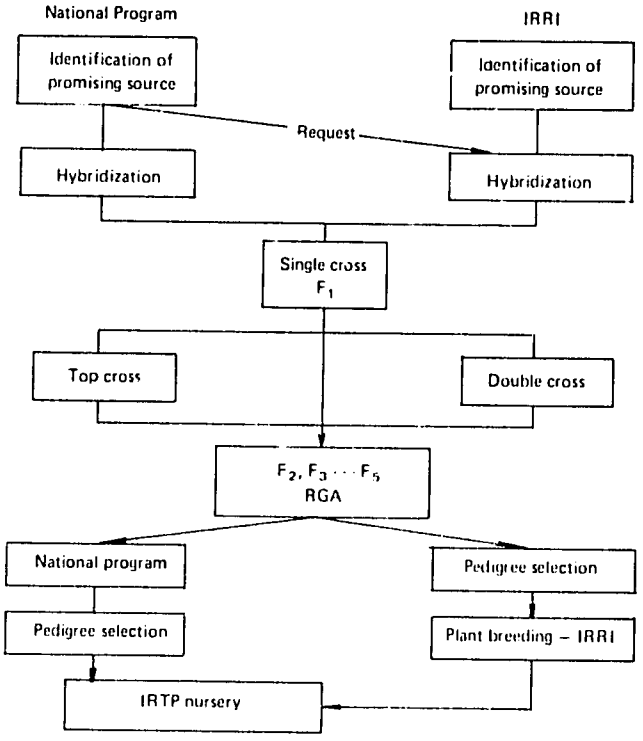


Fig. 1. General scheme for rapid generation advance (RGA).

Poor nutrition or overfertilization can retard flowering. Proper fertilization maintains healthy green plants that are not overvegetative. At 40 to 50 days after sowing, 10 g ammonium sulfate/m^2 dissolved in 10 liters of water is applied. The box is then flooded with additional water, and nitrogen is dissolved in water to facilitate even distribution. If the soil N level is low, frequent topdressing may be necessary.

Plants should be harvested panicle by panicle as they mature. Two seeds should be taken from each plant. The rest is threshed in bulk and reserved.

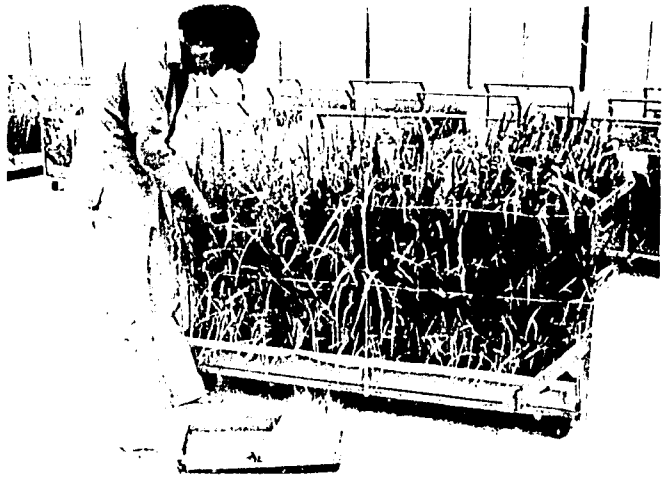
4. Planting F_4-F_5 seeds. The procedure described in steps 2 and 3 can be used through F_4 or F_5 generations although individual pots 2 1/4 inches in diameter are better for F_3 or F_4 plantings because screening is easier and bigger plants with larger panicles are produced.

At IRRI, F_4 or F_5 seeds are given to the Plant Breeding Department for field evaluation during wet season. Pedigree selection and bulk harvesting are made. Selections from the pedigree are given to the International Rice Testing Program (IRTP) for inclusion in the International Rice Deepwater Observational Nursery (IRDWON) or the International Rice Cold Tolerance Nursery (IRCTN) (Fig. 1). The cooperator who sent the F_2 seeds or requested the specific cross to be made is also supplied with bulk seeds. Seeds are planted at specific sites and subjected to rigorous area-specific selection pressure.

OPTIMAL USE OF SPACE AND PLANT CONTAINERS

Using large wooden boxes or small individual pots has advantages and disadvantages. Wooden boxes can accommodate more plants but boxes are occupied until the last plant is harvested. Small pots take more space but half that space may be available 3 months after sowing. If plants are stressed at an early stage, elimination of susceptible lines provides additional space a month after sowing.

Drought screening. A disadvantage of using wooden boxes is that dead plants occupy space until harvest.



Harvesting each panicle separately.

The number of plants grown per year is the same regardless of whether wooden boxes (127 x 127 x 10 cm) or square pots (5.5 x 5.5 x 5.0 cm) are used.

Wooden boxes may be best for early generation materials that are not screened or selected and that require only a small number of seeds per plant. Individual plant containers are better for screening than large boxes. Susceptible plants can be eliminated immediately after testing and remaining plants can grow to maturity in a smaller space. Square pots are ideal for RGA screening, which is usually done at F_4 or F_5 generation. During the F_4 and F_5 generations bigger panicles are needed because larger seed supply is required.

A plastic pot used in RGA.

Closer spacing in wooden boxes results in more dead plants at harvest. There is more competition for nutrients and light. Survival is 74% in boxes and 99% in square pots (Peralta et al 1979).

SELECTION PRESSURES DURING RGA

Selection during RGA is possible to a limited extent. Poor performers, disease-infected plants, and plants with long awns, can easily be removed. If selections are made, population is reduced for the next generation. To sow the same number of seeds for the next generation, more seeds should be taken from each surviving plant.

If environmental pressure will be applied during the growing stage and early selections made, pots should be used instead of boxes so less space is occupied after selection.

Screening for bacterial leaf blight resistance

Hybrid rice populations can be screened for bacterial leaf blight (BLB) resistance during RGA without affecting flowering responses (Eunus et al 1980c). BLB, caused by *Xanthomonas oryzae*, is one of the most serious rice diseases in Asia. The population can be inoculated with *Xanthomonas*, using a suspension of 10^9 cells/ml, 20 to 40 days after seeding by the clipping method. Lesions on leaves can be measured 2 weeks after inoculation to give a general idea of resistance. Susceptible plants will have decreased vigor and may die. Inoculation at an early growth stage, especially if one parent is highly susceptible, can reduce the population by as much as 50%.

Screening for drought tolerance

Screening for drought tolerance during RGA is possible, but has not been used with actual crosses. Tests of 10 varieties from different geographic areas have been made using plastic pots. Eighteen-day-old seedlings were water-stressed for 5 days (Eunus et al 1980a). Seedling survival percentage ranged from 22 to 89%. Actual stress measurement is not necessary, because the goal is to eliminate susceptible plants during RGA.

Five days of water stress did not delay flowering, but grain sterility was increased and plant height, panicle length, and panicle weight were reduced, as compared to the control. However, such plant characteristics during RGA is relatively unimportant because they are not part of the selection process. In cooler climates, a longer stress period may be necessary.

Screening for submergence tolerance at seedling stage

Complete submergence of the rice plant at transplanting is common in rainfed wetland rice areas where photoperiod sensitivity is required. Plants are most sensitive to submergence 10 days after soaking. Screening for submergence tolerance during RGA is done by planting in individual pots and submerging 10-day-old plants for 7 to 10 days, depending on parent resistance and environmental conditions before and during submergence (Bardhan Roy et al 1980b).

Ten days after the submergence period dead plants can be removed. In a RGA submergence test, 62% of the population died. Seedling submergence during RGA has little effect on growth duration and productivity of submergence-tolerant plants but may delay heading by 5 to 10 days and increase sterility in susceptible plants (Bardhan Roy et al 1980a).

IRRI studies show that submergence tolerance has low to moderate heritability. Because dominance and nonallelic interactions are present, selection for submergence tolerance in the F_2 generation will be ineffective because subsequent progenies of selected entries will show submergence susceptibility. Nevertheless, screening in F_2 will eliminate most of the susceptible lines and reduce population size. If screening facilities are limited, it may be best to screen in the F_3 or F_4 generations.

Screening for cold tolerance at seedling stage

To screen for cold water tolerance at F_3 seedling stage, pots should be used and 10-day-old seedlings should be subjected to 12°C water for 10 days. Water depth should be 1-2 cm from the soil surface. Susceptible lines usually die or turn brown.

In 1 RGA cross, 14% of plants died, 17% flowered early, 23% flowered normally, and 46% flowered late. Plants severely damaged by low water temperature headed later than the tolerant plants which showed little delay.

In 2 RGA crosses screened for cold tolerance at seedling stage, removing susceptible lines (score of 7 to 9) eliminated 87 and 58% of the F_3 plants, freeing greenhouse space for other uses 30 days after sowing (Pateña et al 1980a).

Selection for earliness or short growth duration

In low-temperature areas growth duration is an important factor determining varietal suitability. When using RGA for low-temperature rice varieties, eliminating plants with long duration should be standard practice (Bardhan et al 1982).

For a plant to have 130-150 day growth duration in low-temperature areas, it should flower within 70 days under RGA conditions. To select for short growth duration, plants flowering later than 70 days are removed.

Earliness selection in the F_2 generation assures that 72-84% of the F_3 population have growth duration less than 70 days (Pateña et al 1980b). Selection for earliness does not double as a selection for cold tolerance at seedling stage. If selection for earliness is to be made at F_2 generation, a minimum of 2,000 plants should be grown.

An F_2 cross between Silewah and Hokkai 241 from Japan was selected for earliness (Fig. 2). Only 245 plants of 2,965 seedlings grown had growth duration less than 70 days. Ten seeds, instead of two, were taken from each plant to assure the desired number of plants could be grown in the F_3 generation.

Selection for plant height

Many factors affect plant height. High temperature and close spacing may make plants tall. Low nutrient level and short growth duration may grow short plants. These factors interact during RGA, especially in the F_2 when plants are closely spaced. Plant height correlation between RGA-grown and field-grown plants is low, indicating that selection for height should not be done during RGA (Vergara et al 1980).

Screening for salinity tolerance at seedling stage

Coastal wetland areas need photoperiod-sensitive, salt water-tolerant varieties. Preliminary RGA experiments show that screening for salinity at seedling stage is feasible. Small pots are used. Ten-day-old seedlings are treated with 0.5-1% salt solution to a depth of 10 cm. Salt concentration is chosen depending on donor parent(s) tolerance or level of stress needed for specific areas.

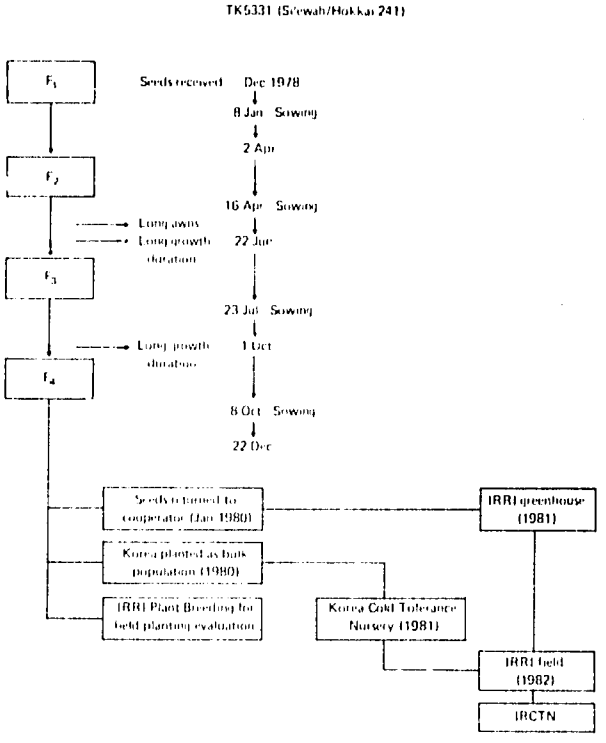
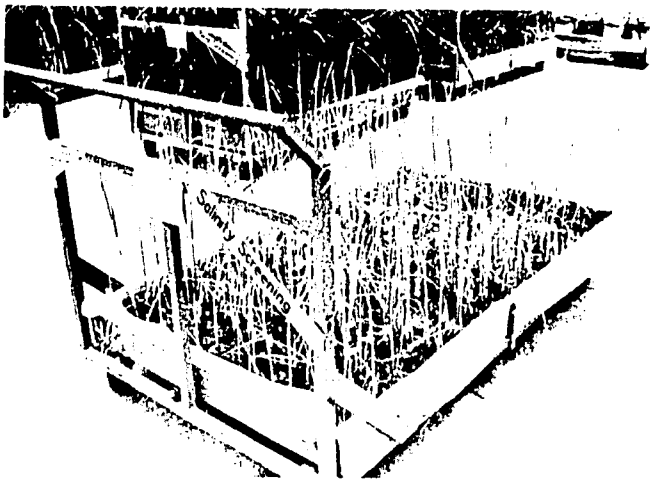


Fig. 2. Rapid generation advance of the cross TK5331 from F_1 to F_5. Four generations were planted in 1 year.



Salinity screening during RGA.

After 10 days of treatment, the salt solution is drained and susceptible plants are discarded. Pots are continuously flushed with tap water for at least 1 h and topdressed with 10 g ammonium sulfate per tray. Treatment with 0.5% salt solution has resulted in 100% survival for tolerant Pokkali and 32% survival for susceptible M1-48. Forty-seven per cent of the tolerant check survived in a 1% salt solution and all plants of the susceptible check died.

Screening for other plant traits

Screening for resistance to brown planthopper, leafhopper, blast, other diseases, and adverse soils tolerance during RGA may be possible but no trials have been made. Grain size and shape, little affected by environment, can be selected. Although awn length is determined by environment, selection for awnless grains is routinely practiced during RGA.

Screening for photoperiod sensitivity, the most important trait for rainfed lowland and deepwater rice areas, is difficult during RGA. Photoperiod sensitivity must be selected when varieties are planted in the field during monsoon season. During RGA some plants within a population are late in heading. These plants have long BVP. Selection for long BVP within a photoperiod-sensitive cross can be made by choosing plants that headed late.

TIMING OF RGA PLANTING

Although it would be easier, for comparison and record keeping, to plant all varieties in the RGA greenhouse at the same time, the method is not practical because it would sometimes leave the greenhouse empty and because the peak harvest for individual crosses would come at about the same time. Sowing RGA varieties at staggered times maximizes use of greenhouse space and optimizes labor use.

If F_2 seeds are started in early February, F_4 seeds can be returned to the cooperator or breeder by September the same year (Fig. 3). F_4 seeds can be planted in the field for evaluation although photoperiod sensitivity cannot be selected for accurately.

In crosses for low-temperature areas, receiving F_1 seeds in December and sowing in January has enabled the system to return F_5 seeds to the cooperator by January the following year (Fig. 2). For a cross made during the 1978 growing season in Japan, F_1 seeds were sent to IRRI in December. Without RGA F_1 seeds would have been planted in 1979 and F_2 seeds in 1980. With RGA, F_5 seeds were planted in Japan in 1980. Selection for awnless grains and short growth duration had also been made so chances of obtaining better lines from a bulk population were higher.

Planting F_2 seeds later in the year would have provided F_4 seeds for field planting the following year. If the F_4 seeds can be planted in May or June, selection for photoperiod sensitivity is possible.

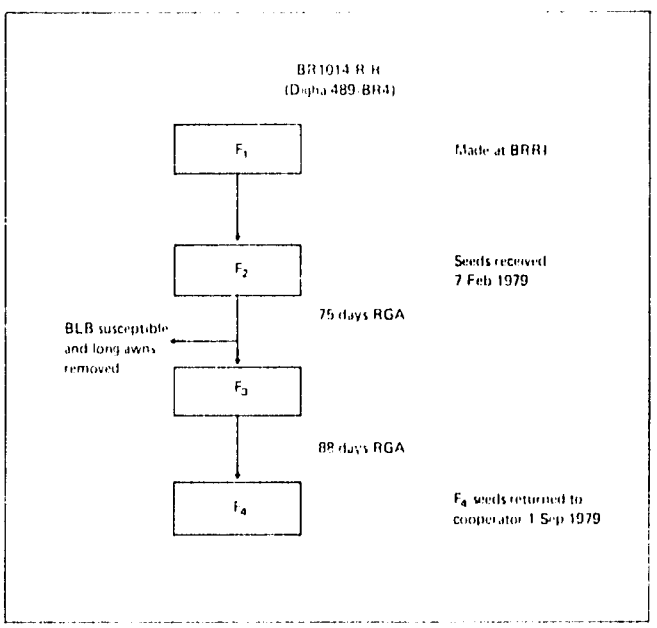


Fig. 3. Flow diagram of a cross received from a national cooperator and processed through rapid generation advance. BRRI = Bangladesh Rice Research Institute, BLB = bacterial leaf blight.

IRRI RGA FACILITIES

IRRI RGA facilities can accommodate about 200,000 plants/planting or 600,000 plants/year using wooden boxes and pots. To grow 200,000 plants in the field requires more than 1 ha at normal spacing but only 825 m^2 in the RGA greenhouse. Labor and space are minimized and insect and disease control is easier.

At IRRI, plant physiologists and plant breeders share management responsibilities for the RGA system. The Plant Physiology Department supervises facilities and the Plant Breeding Department manages field plantings and evaluation (Fig. 4). Five crosses are planted and harvested each week. Harvesting is daily. Between planting and harvesting operations harvested seeds are bulked and/or sampled for the next planting.

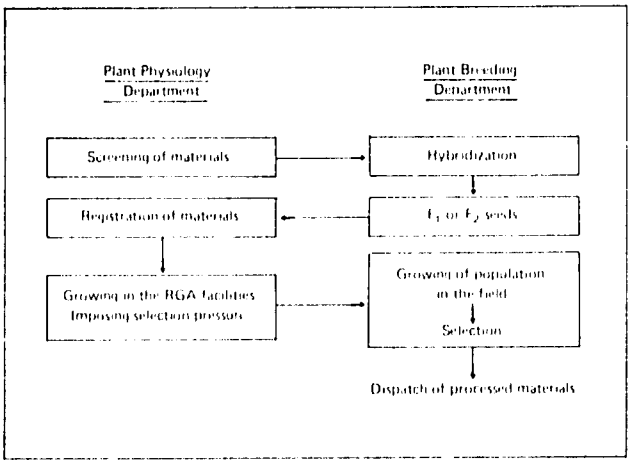


Fig. 4. Management of the IRRI rapid generation advance system.

SPECIAL USE OF RGA FOR RICE COMPOSITES

Genetic male sterility in indica rice makes it possible to combine more than 4 parents to develop a population. Chaudhary et al (1982) outlined a method for using RGA facilities to produce a composite cross of outcrossing rice plants if genetic male sterility is maintained in 50% of the individuals. RGA is important in male-sterile-facilitated composites because flowering can be synchronized by manipulating photoperiod and planting seeds closely to allow cross pollination. Free flow of genes can take place as it does in cross-pollinated crops.

By allowing the plants to complete BVP before inducing flowering, a very high percentage of the population flowers within a short time and outcross. In a trial involving 5 male-sterile F_2 populations, 75% or more of the plants in each population flowered within 4-9 days when plants were grown for 40 days under long day length before subjecting to flower inductive short days (Fig. 5). Shorter flowering range permits a plant that flowers at 0 day to be pollinated by other plants heading 2 days before and 2 days after 0 day.

Close spacing allows maximum outcrossing. In the field, 50 m^2 accommodates 1,000 F_2 plants, lessening the chances that 1 plant at 1 end of the field intermates with another at the opposite end. In RGA the farthest distance between 2 plants in a population is 1.5 m. The flowering panicles can be shaken, using a stick to stir pollen and promote outcrossing.

Composites for photoperiod-sensitive deepwater rice using IR36(ms) have been made (Chaudhary et al 1982) to increase the elongation ability of progenies and create genotypes with wide adaptability. The method is illustrated in Figure 6.

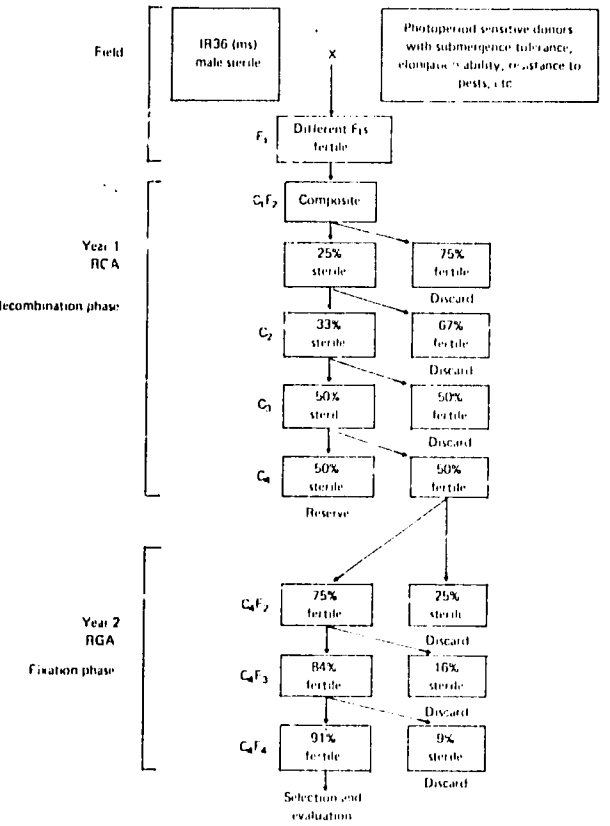


Fig. 6. Scheme to develop and handle male-sterile-facilitated composite.

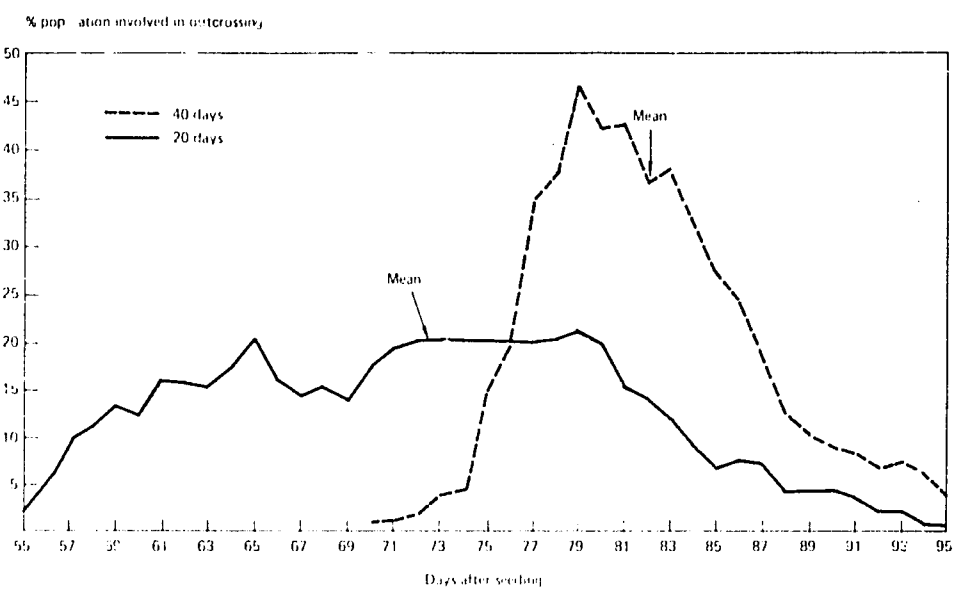


Fig. 5. Period of long day treatment before floral inductive short day treatment and percentage of composite population involved in outcrossing.

A salt tolerance composite has also been developed through RGA. The composite cross seeks to increase the level of salinity tolerance beyond that of the best parents and to obtain salinity tolerance in a genotypically diverse background to allow wide adaptability.

STATUS OF RGA AT IRRI -- 1979-82

By June 1982, about 600 RGA crosses involving 745 generations and a million plants had been grown in the IRRI RGA facilities. Most crosses were for low-temperature, deepwater, and rainfed wetland areas.

In 1980, five RGA lines were included in the IRCTN. In 1982, 9 entries, 5.4% of all IRRI entries, came from RGA. In the Cold Tolerance Nursery at Chuncheon, Korea, about 50% of the IRRI entries were from RGA. In the IRDWON, 11 entries, 20.7% of all IRRI entries, were generated through RGA.

Bulk seeds from RGA crosses have been sent to Bangladesh, Burma, Australia, Columbia, China, India, Indonesia, Japan, Korea, Liberia, Malaysia, Nepal, Thailand, Turkey, the West Africa Rice Development Association, and the International Institute of Tropical Agriculture.

It is too early to expect a variety from the RGA facilities at IRRI although the effectiveness of RGA in Japan has been clearly demonstrated. However, the increasing number of entries in the IRDWON and IRCTN indicate that RGA facilities have indeed shortened the breeding cycle and have made available fixed bulk populations within a short time.

INTERNATIONAL COOPERATION

Participants in the 1978 International Deepwater Rice Workshop in Calcutta, India, recommended that breeders working with photoperiod-sensitive rices should utilize IRRI RGA facilities (Ikehashi and HilleRisLambers 1979). Participants agreed that breeders could send five crosses per year to IRRI for RGA, or suggest crosses for IRRI to make and run through the RGA. Researchers at the 1978 International Cold Tolerance Workshop at Suweon, Korea, have also decided to use RGA to accelerate cold tolerance breeding for high-elevation and high-latitude areas where only one rice crop per year is possible (Vergara and Coffman 1979).

If the best crosses from each national program are sent to IRRI for RGA and sent back to the respective breeding centers for further in situ selection, turnaround time for national breeding programs can be reduced 2 to 3 years. The same seeds can also be sent to other national breeders.

Different national programs have different facilities to test for environmental and biological stresses. A RGA cross may be planted in the deep-water tanks in Thailand and selected for internode elongation ability, or tested in West Bengal for submergence tolerance and in Bangladesh for ufra disease. A portion of the seed from the selected lines can be sent to IRTP for future inclusion in the international nurseries to benefit other areas with similar varietal requirements. In future, a coordinated testing system may have to be developed to utilize existing capabilities of national facilities and accelerate breeding programs.

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