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A Simplified Crossing Method for Rice Breeding:

A MANUAL

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Contents

Preface

The major task of plant breeders is to stay ahead of the increasing number of varietal problems that hinder rice yields, particularly that of the ever-changing biotypes of disease pathogens and insects. Plant breeders must, therefore, keep the genetic base diversified by incorporating new genetic materials through hybridization.

This manual is intended primarily for young plant breeders who wish to begin their breeding programs. It aims to provide the basic methodology for analyzing varietal constraints before establishing a crossing program. The major objective of this manual, however, is to describe new crossing processes that include collecting parents, storing cut panicles, emasculation, and pollination.

It is the author's hope that this manual will help plant breeders perform crossing activities more efficiently and more effectively.

The crossing method described by this manual grew from a technique developed by French (CIRAD/IRAT)[1] and Brazilian

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1. CIRAD = Centre de Coopération International en Recherche Agronomique pour le Développement, France; IRAT = Institut de Recherches Agronomiques Tropicales et des Cultures Vivrières, France.

(EMBRAPA/CNPAF)[2] scientists to handle seed production in a hybrid-rice project. Their generosity in sharing their information allowed us to modify the method for use in any crossing program.

Gonzalo Holguín devoted many long hours to making the numerous adjustments required to bring this method to a practical level.

The contents of this document, however, are the author's responsibility and do not necessarily reflect the views of the scientists who were instrumental in the development of the simplified crossing method.

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2. EMPBRAPA = Empresa Brasileira de Pesquisa Agropecuária, Brazil; CNPAF = Centro Nacional de Pesquisa de Arroz e Feijão, Brazil.

Introduction

A focal point of any crop breeding program is hybridization, that is, the generation of genetic variability. By having a range of different genotypes, the breeder can select material that will eventually be released as new varieties, or that can be kept as sources of favorable genetic recombination in further crossing activities. The design and implementation of a crossing program has been well covered in the text "Rice Improvement" by P. R. Jennings et al. (1979)[3].

Unfortunately, not every national rice improvement program can afford to conduct its own hybridization work because it requires substantial investments in screenhouses and equipment. Current methods are also tedious and demand large investments in labor to produce the numbers of crosses required for a program to be effective.

The objectives of this booklet are to present an improved crossing method in a step-by-step, tutorial format suitable for individual or group, informal or formal, instruction. It is hoped that, with this simplified technique, many national and state programs will be able to mount or make more

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3. Jennings, P. R.; Coffman, W. R.; and Kauffman, H. E. 1979. Rice improvement. International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. 186 p.

efficient their own crossing programs and so more effectively address the varietal problems of rice in their regions.

A Simplified Crossing Method for Rice

A simplified crossing method in rice was originally developed by IRAT and EMBRAPA for a hybrid-rice project.[4] Recently, CIAT's Rice Program improved it and is currently using it on a regular basis. This method depends on excised tillers maintained in tap water, and is intended to replace the older method that required vacuum pumps and the transfer of whole plants from field to screenhouse. It is preferred to the conventional method for several reasons, but particularly for its low costs and simplicity which allow greater efficiency in crossing. With these advantages, virtually any national rice research program should be able to conduct a hybridization program directed toward its specific needs.

The two major advantages of this new method are summarized as follows:

(1) Low capital investment and operational costs, that is, no special buildings such as

4. Taillebois, J. and Castro, E. M. 1986. A new crossing technique. Int. Rice Res. Newsl. 11(3):6.

screenhouses or greenhouses, are needed. Almost any small storeroom, or even office, is adequate for handling and maintaining plants; and, compared with the old method, less labor is needed for each pollination.

(2) Increased efficiency of pollination (Table 1), that is, a given amount of F_1 seed costs less in labor and materials; and less field space is needed for planting parents.

Table 1. Time required to perform hybridization work in two crossing methods for rice, CIAT, 1990.

Method	Cross performed (no.)	Time (min.) required for:	
		Processing[a]	Prod. of 100 F_1 seeds[b]
Conventional	66	19.14	169.07
Simplified	73	11.60	54.73
LSD (5% level)		4.95	85.00
CV (%)		19.00	43.00

a. Processing included collecting plants (conventional method) or panicles (simplified) from the field, followed by emasculating and pollinating one panicle.

b. Average of 9 panicles with the conventional method, and 5 panicles with the simplified method.

Comparing the Conventional and Simplified Crossing Methods

Experiments were carried out to compare the effectiveness of hybridization by the conventional crossing method with that by the simplified method. A total of 139 crosses (Table 1) were used to estimate the time required to perform hybridization work on one panicle and so produce 100 F_1 seeds. Table 1 demonstrates that, with the simplified method, breeders need to use only one-third of their time to produce 100 F_1 seeds; for example, it took only 11.6 min. by the improved method to complete one hybridized panicle as opposed to 19.14 min. by the conventional method. Overall, the time involved in hybridization activities, such as emasculation and pollination, and related work, such as collection of plants or panicles, was reduced. Another major contribution of this improved method was the high number of F_1 seed set in any given panicle.

Establishing the Need for a Crossing Program

Because a crossing program must fit within an overall crop improvement program, some basic principles of organizing a rice-breeding program will be briefly discussed. For further details, see Jennings et al. (1979).

Identifying varietal problems

Before beginning a breeding program, the breeder must have a clear idea of the target area for which new varieties are intended. The constraints that limit rice varietal performance in that region must be carefully examined; in fact, it is indispensable that they are known and recognized.

The varietal constraints that are most readily amenable to breeding are diseases, pests, and edaphoclimatic problems. Under tropical growing conditions, rice diseases are common constraints, and are particularly troublesome because of the diversity and complexity of the pathogens involved. However, in temperate regions, major constraints are low temperatures and the need for early maturity.

How important a constraint is can be appreciated by evaluating the performance, in terms of area or tonnage, of existing commercial varieties in those areas suffering most from that constraint. By comparing the results of evaluating various constraints, an idea can be obtained of how much priority to give a certain constraint.

Ranking varietal problems in order of priority

The diagnostic study of varietal limitations allows the breeder to establish priorities, that is, to assign different weights to the various problems. These

priorities must be ranked in collaboration with scientists from many disciplines and with rice farmers from the region concerned. The priority of a constraint will depend on:

its importance in restricting current rice yields,

the effectiveness of measures to control production costs,

the constraint's effect on potential expansion of rice-growing areas, and

the likelihood of resolving the constraint through breeding.

Assessing the availability of suitable varieties within the existing germplasm

The breeder must have a thorough knowledge of the local rice varieties already available in the region to be served. These cultivars should be assessed, regardless of their previous performance, for their merits and deficiencies, agronomic characteristics, and economic acceptability. These varietal assessments will provide the basis on which to evaluate new lines. That is, the performance of new lines can be compared with the already established minima for acceptability, and so help determine whether these new lines perform adequately enough to be released.

Knowing when a crossing program is necessary

A crossing program is needed whenever the local varieties appear unsuitable, agronomically or economically, for commercial plantings, and when adapted or sufficiently superior germplasm cannot be obtained from other institutions, such as national and state research programs or the international centers of CIAT, IRRI, and WARDA.[5] As the area covered by high-yielding modern varieties expands, it becomes less and less likely that a region can satisfy its varietal needs through simple introduction and evaluation of advanced lines.

Beginning a Crossing Program

Selecting parents

Only by clearly setting the objectives for the use of a cross can the breeder hope to select adequate parents. The identification of the more serious varietal limitations will determine some of the objectives for a cross, and govern, to a large extent, the characteristics required in at least one parent.

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5. IRRI = International Rice Research Institute, Philippines; WARDA = West Africa Rice Development Association, Côte d'Ivoire.

The other parent would carry the best patterning of available characteristics, because, typically, a breeder wishes to conserve the desirable characteristics of the material already available, while adding new traits.

To program crosses effectively, the breeder must know the parents very well in order to use them in the hybridization program. Those sources possessing desirable new traits must be characterized for the entire range of important traits to determine which sources would be the best potential donors. Carrying along unnecessarily a host of undesirable traits does nothing but make additional work for the breeder. Brief evaluations of varietal characteristics, such as reaction to diseases, maturity period, and plant height may be carried out locally. But certain traits that require special equipment or complicated procedures (e.g., amylose content, insect tolerance, and virus resistance evaluations) may need the collaboration of international centers which work specifically in those areas.

The principal characteristics of each cultivar are usually listed in information sheets that systematically describe each trait and character (Table 2).

Programming crosses

Using the information sheets described earlier, the breeder may program crosses accordingly, usually

Table 2. Information sheet, describing major characteristics of rice cultivars.

Cultivar	Cross	Days to 50% flow.	Plant height (cm)	Grain quality[a]				Disease resistance[b]				
				White belly	Gelat. temp.	Length (mm)	Amyl. cont. (%)	BL	NB	GID	LS	BS
CICA 8	CICA 4//F1 IR 665-23-3/ Tetep	110	95	0.6	5.0	6.5	29	6	7	7	5	4
Oryzica 1	P 1223/P 1225	99	85	0.2	7.0	7.0	31	6	6	7	5	6
Colombia 1	Napal/Takao Iku 18	111	115	0.2	2.0	6.0	19	3	3	4	4	6
BR-IRGA 409	IR 930-2/IR 665-31-2-4	103	90	1.0	7.0	6.8	31	6	7	3	5	3
IAC 47	Pratão/Perola//IAC 1391	91	150	1.4	4.7	6.5	25	2	3	6	5	6
IR 36	IR 1561-228-1-2/ IR 1737//CR 94-13	98	80	0.8	5.0	5.0	31	7	9	6	5	3

a. Presence of white belly is measured on a scale of 0 to 5, where 0 = no white belly and 5 = major part of grain with white belly. Gelatinization temperature is measured on a scale of 1 to 7, where 1 = disintegrated endosperm and 7 = hard endosperm.

b. Degree of resistance is measured on a scale of 1 to 9, where 1 = resistant and 9 = susceptible; BL = leaf blast; NBL = neck blast; GID = grain discoloration; LS = leaf scald; BS = brown spot.

by choosing parents that are likely to complement each other. For example, CICA 8 is a variety which is susceptible to blast disease and "hoja blanca" virus but has good yielding ability and is tolerant of damage from the rice planthopper (*Sogatodes*). If it were crossed with Colombia 1, which possesses traits that complement those of CICA 8, some progeny of this cross would combine high-yielding potential with resistance to blast, hoja blanca virus, and insects in the segregating generations. Nevertheless, it is important to realize that when the best parents are hybridized, often only poor progeny are recovered. Thus, the breeder must maintain a good record of how cultivars combine with one another.

Mechanics of Crossing

Planting parents

Seed dormancy must be broken if fresh seeds are to be planted within 30 days of harvest. This is done by heating them in an oven for 5 days at 55 °C. Thereafter, seeds are planted in either a plastic tray or pot. About 10 to 15 plants are needed for each cultivar. Rice seedlings are transplanted to the field when they are 25 to 30 days old (Photo 1).

In general, rice varieties and/or cultivars differ markedly in their maturity period, with each having its own flowering time. For this reason, plantings

Photo 1. The healthy seedlings are transplanted to the field 25 to 30 days after sowing.

of parents are staggered on different dates to synchronize the flowering times of parents destined for hybridization. Usually each parent is planted at 14-day intervals, which, theoretically, allows crossing to occur between lines that differ in flowering date by as much as 40 days. That is, the first planting of a line that flowers at 120 days would be simultaneous with the last planting of a line flowering at 80 days (Photo 2).

Preparing panicles for crossing

Equipment (Photo 3). The basic tools needed for crossing are:

Photo 2. Rice crossing block: parents are planted on different dates to synchronize flowering times.

fine-pointed forceps with protective cap
small, sharp, and pointed scissors
"Ebony" pencils (intense black, waterproof)
paper tags
glassine bags
metal or plastic tray
pocket knife or sickle
manila envelopes (size no. 3)
bucket, flask, or cylinder
paper clips
plastic and wooden stakes
plastic or clay pots

Excising tillers. In this simplified crossing method, the tillers which will produce the "female" panicles are removed from the parent plant in the

Photo 3. Some of the equipment needed for crossing: forceps with protective caps, scissors, manila envelopes, "Ebony" pencils, tags, paper clips and glassine bags.

field when they are in late booting stage (Photo 4) and when the pollen donor ("male" plant) has entered anthesis. Tillers should be removed from the plant the day before emasculation and maintained in the screenhouse or office. Only those tillers in which the panicle has emerged from the boot (flag leaf sheath) by about 5 to 10 cm should be selected.

Tillers are carefully cut with a small knife at soil level, as near as possible to the node from which they developed (Photo 5). It is best to excise the tillers in early morning or late afternoon when transpiration rates are low. It is important to select only healthy tillers that are free of biological or mechanical damage. **All** leaf blades **must** be

Photo 4. Panicles are ready for emasculation and pollination when they have emerged 5 to 10 cm from the leaf sheath.

Photo 5. With the use of a sickle, a "female" panicle is cut out at soil level.

removed (Photo 6) from the stem to avoid excessive transpiration. This is best done by cutting them at the base of the lamina.

The tillers are tagged with simple identification labels that give, for example, ricevariety name and collection date (Photo 7). They are maintained upright, with their stems standing in about 15 cm of clean tap water until the F_1 seed is mature (Photo 8). There are various ways by which panicles can be stored: any convenient, clean cylinder, flask, or bottle can be used, but the water should be changed every 2 to 3 days.

Emasculation

Rice panicles from plants to be used as the female parent must have their anthers removed (emasculation) before they shed pollen to avoid self-fertilization.

Before using the panicles for crossing, they should be checked to see if they are in good condition. The upper and lower parts of the panicle are normally removed (Photo 9) and only the middle part is used. Emasculation is carried out by cutting open the spikelet near its midpoint with small scissors so that the anthers can be easily seen. Care must be taken to avoid damaging the female floral parts (Figure 1). Anthers (6 per spikelet) are removed with a fine-pointed forceps (Photo 10). Again, this must be carefully done to avoid damaging the young and fragile ovary. It is

Photo 6. All leaf blades from the "female" panicle are removed.

Photo 7. Each panicle is labeled with its variety name and collection date.

Photo 8. The selected and tagged panicles are maintained in a plastic bucket that contains clean tap water.

Photo 9. The upper and lower spikelets of the panicle are removed, leaving the middle spikelets ready for emasculation.

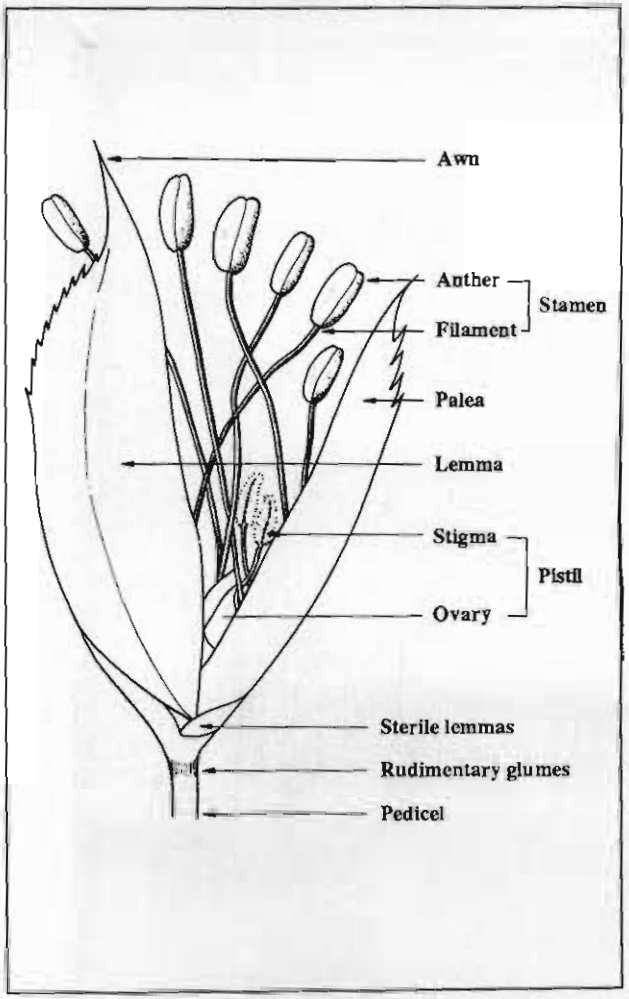

Figure 1. Rice flower, showing male (stamen) and female (pistil) reproductive organs. (Taken from Arraudeau, M. A. and Vergara, B. S. 1988. A farmer's primer on growing upland rice. IRRI and IRAT, Los Baños, Laguna, Philippines. p. 89.)

Photo 10. Removing the anthers with forceps.

Photo 11. The emasculated rice flower, showing only the pistil (female reproductive organ).

very important that **all** six anthers are removed (Photo 11, p. 20).

About 50 to 100 spikelets per panicle are suitable for adequate seed development after fertilization. An estimated 60% to 70% of pollinated florets give rise to hybridized seeds (F_1); about 10 to 15 F_1 plants are needed for a single cross and 100 to 150 F_1 plants for a double or triple cross.

The emasculated spikelets are covered by a glassine bag over each respective panicle to protect them from being naturally pollinated by unwanted foreign pollen. They are normally kept under shade until pollination, which is done the day after emasculation (Photo 12).

Photo 12. The emasculated panicle is covered with a glassine bag before pollination to protect it from unwanted foreign pollen.

Pollination

To identify easily the F_1 hybrid, the variety or line used as a pollinator or male parent should possess a dominant character, such as pubescence, earliness, or tallness. Plants developing from supposedly F_1 seed that do not possess the dominant character or characters were probably derived from self-pollination in the female plant.

Pollination is usually carried out in late morning, from 10:30 a.m. to 12:30 a.m. Nevertheless, anthesis, that is, when pollen is shed, is largely dependent on climatic conditions, such as cloudiness and temperature, so that the best time for pollination must be determined locally, according to the occurrence of anthesis.

Pollination is carried out either by bringing panicles (Photo 13) from the field to a working area before anthesis, or by transporting "female" panicles to the field (Photo 14). At CIAT, pollen sources are normally collected the day before pollination in the same way as "female" panicles are collected. Panicles used as pollen sources may be used two or three times. The variety name of the male parent and date of pollination are added to the original identification tag on the excised female tiller.

The emasculated pollinated panicles, with opened spikelets, are immediately re-covered with their glassine bags (Photo 15), which should be left

Photo 13. Pollination in the working area: the emasculated or "female" panicle is placed just below the pollen donor, which is then shaken gently to release the pollen.

Photo 14. Pollination in the field: the emasculated or "female" panicle is taken to the pollen donor growing in the field.

Photo 15. The pollinated panicle is immediately covered with a glassine bag.

on the panicle for at least 5 days. Five to 7 days after anthesis, when the stigmata are no longer receptive, the bags can be removed to enhance seed development (Photo 16). CIAT has found that panicles which remain covered for more than 5 days produce fewer F_1 seeds because of the excessive humidity and temperature which develop inside the bags.

Development of F_1 seed

The time required for the development of F_1 seed is about 25 to 30 days after fertilization. During this time, the panicles must be protected from rain

Photo 16. About seven days after pollination, when the flowers are no longer receptive to pollen, the glassine bags are removed.

and possible mechanical damage. The cylinders, flasks, jars, buckets, or other containers used to hold the tillers (Photos 17 and 18) may be stored in an office, near a window, if no greenhouse or screenhouse space is available.

Tap water can be used for the tillers, but must be kept fresh by changing every 2 or 3 days. The young caryopsis is normally visible about 7 days after fertilization. At this stage, the number of normal F_1 seed can be determined, and crosses may be repeated if F_1 seed set is insufficient. About 50 to 80 seeds from a simple cross are required to generate a sufficiently large F_2 population, that is, 3000 to 5000 plants.

Photo 17. The pollinated panicles are stored in cylinders containing clean tap water.

Photo 18. Plastic buckets, containing clean tap water, provide an alternative for storing pollinated panicles.

Harvesting and handling F_1 seed

Mature F_1 seed is easily recognized. The naked seeds without glumes become yellowish brown and their endosperm becomes hard and shiny. Half of F_1 seed is covered with glumes from the female parent (Photo 19). These glumes should be removed when seeds are collected.

Each cross is kept separately in a small manila envelope on which is written the cross's identification and date of harvest. F_1 seeds can be stored in any standard household refrigerator.

Producing F_2 seed

Before planting, F_1 seeds are heat treated in an oven at 50 °C for 7 to 10 days to break dormancy and ensure uniform germination. Note that this is a less severe treatment than that applied to the more hardy normal seeds which have their protective structures intact. The seeds are then germinated in a petri dish containing moist filter paper or sown directly in commercially available "Jiffy" pots (Photo 20). After about a week, the young seedlings in the petri dishes are then transferred and maintained in pots for 2 weeks before being transplanted to the field. Those sprouted in the "Jiffy" pots can be transplanted directly to the field after 3 weeks. Each F_1 plant must be grown, separated from the others, at distances of about 50 x 50 cm.

Photo 19. Mature F_1 seed, half covered with glumes.

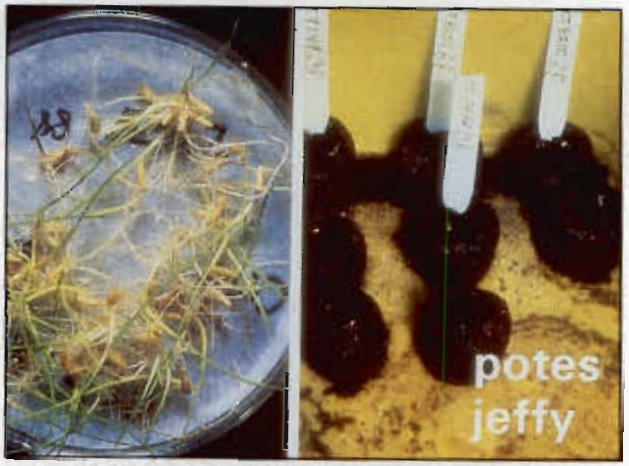

Photo 20. F_1 seed germinating in petri dish (left) and in "Jiffy" pots (right).

If a breeder is not extremely well acquainted with the parents of a cross, then the "female" parent should be planted near the F_1 plants so to identify accidental selfs among the F_1 population (Photo 21). The progeny of self-pollination should be rogued immediately.

The F_2 seed of a single cross may be harvested in bulk. However, seed of individual F_1 plants from double or triple crosses should be kept separate.

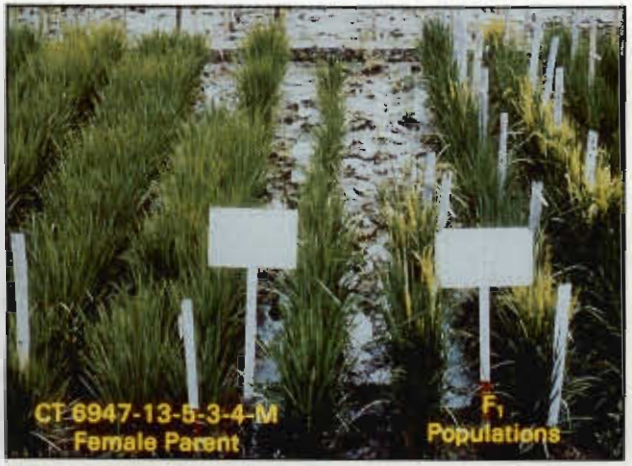

Photo 21. F_1 seedlings are transplanted to the field, beside their "female" parent.

In Conclusion

The new crossing method, because of its simplicity and low capital requirements, will help national program scientists work more effectively. Those national programs who wish to establish their own crossing program can readily do so and, therefore, develop crosses that are specific to particular ecosystems.

For further information, interested readers may contact

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