INNOVATIVE APPROACHES TO RICE BREEDING

Selected Papers from the 1979 International Rice Research Conference

1980
THE INTERNATIONAL RICE RESEARCH INSTITUTE
LOS BAÑOS, LAGUNA, PHILIPPINES, PO BOX 933, MANILA PHILIPPINES

The International Rice Research Institute receives support from a number of donors including the Ford Foundation, The Rockefeller Foundation, the European Economic Community, the United Nations Development Programme, the OPEC Special Fund, the Asian Development Bank, the International Development Research Centre, the World Bank, and the international aid agencies of the following governments: United States, Canada, Japan, United Kingdom, Netherlands, Australia, Federal Republic of Germany, New Zealand, Belgium, Denmark, and Sweden.

The responsibility for this publication rests with the International Rice Research Institute.

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FOREWORD

The wide range of ecological conditions under which rice grows is evidence of the equally wide genetic variability found among cultivars of this crop. One of the primary objectives of today's plant breeders is to use this variability to develop high yielding resistant rices with the ability to resist or tolerate adverse environmental conditions. The efforts to do so require new as well as traditional breeding techniques. They also require cooperation among scientists from different countries.

The International Rice Research Conference held at IRRI 21-25 April 1979 emphasized the internationality of rice improvement research. This was reflected through the collection of papers on innovative methods of rice breeding that constitute this volume.

These technical papers report on a number of new and stimulating breeding methods and innovations that have been evaluated as breeding procedures to increase production or to improve yield stability through higher levels of resistance to insects, diseases, and other stresses.

The improved semidwarf varieties that perform so well on irrigated lands were developed through conventional breeding methods. But for plant types adapted to adverse conditions -- such as low temperature, saline soils, and drought -- innovative breeding techniques are expected to provide significant advances in a shorter time.

Dr. James Mac Key of the Swedish University of Agricultural Sciences set the tone of the session with an overview of the progress made on cereal breeding during the last 30 years. Against this background, technical discussions focused on hybrid rice breeding, distant hybridization, modified bulk population methods, mutation breeding, and finally on innovative approaches such as tissue culture.

The potential of these methods and techniques is great. The state of the science varies from country to country, from program to program, and from institution to institution. To successfully apply these methods to rice improvement, scientists involved in their development need to work together. I hope these papers will provide a base for expanded international cooperation to develop and implement innovative rice breeding methods in the quest for rice varieties adapted to diverse ecologies.

SOME ASPECTS OF CEREAL BREEDING FOR RELIABLE AND HIGH YIELDS

J. MAC KEY

The last three decades can be characterized by a more analytical approach to plant breeding goals. Since this approach was accompanied by more intensive cultivation methods, considerable yield improvements were made possible. The progress in cereal breeding was so rapid that some described it as *the green revolution.*

Generally, this term has been used to characterize the absolutely necessary, and thus more dramatic, improvements achieved in the developing countries. Being a basic approach, it was, moreover, applicable to developed countries, even those in temperate regions. Figure 1 shows a similar rate of wheat improvement through the period 1945-75 for Mexico, the classic example of *the green revolution,* and for Sweden, which has had a long and highly developed agriculture at the northern margin of cultivation (Mac Key 1979b).

Many problems in trying to raise yields are basically the same, although at each epoch of development they may have different relative weights. There is as yet no tendency for the progress curves to level off in either Mexico or Sweden. Since varietal improvement is not only a contribution in itself but also a prerequisite for more intensive agriculture, plant breeding success will continue to be decisive.

Thus, it is pertinent to evaluate the potential progress of plant breeding per se. A trend may be difficult to determine because yield performance is highly dependent on cropping intensity. The most appropriate way would be to evaluate each epoch of plant breeding at its relevant level of agronomy. This is possible where varieties have been tested in official trials over the period studied with a successive, but always overlapping, withdrawal of older varieties. The result of such a rolling comparison analysis applied to wheat in Sweden is presented in Figure 2. The genetic trends for improvement of Swedish wheat are strikingly similar to those given for grain yield per hectare in Figure 1, supporting the decisive role played by plant breeding in overall progress. The slope of the curves is very much the same and again no leveling-off tendency is observed.

This is a challenge for the future. And with today's more analytical approach there is more to be learned about how to

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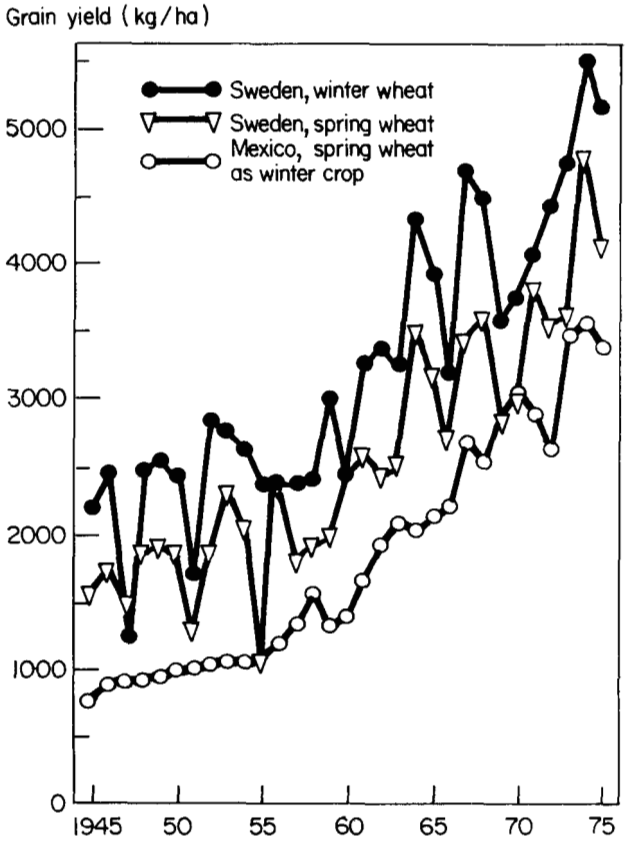
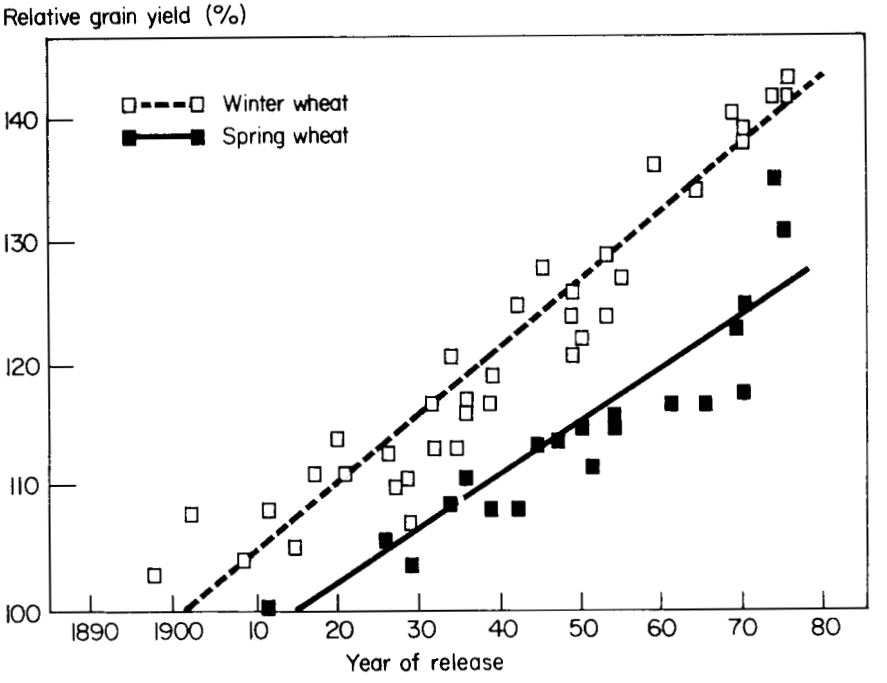


1. Improvement curves for grain yield per hectare of wheat in Mexico and Sweden during 1945–75. Average annual increase was 97 kg/ha (r = 0.97***) for Mexican winter-sown spring wheat, 102 kg/ha (r = 0.88***) for Swedish winter wheat, and 89 kg/ha (r = 0.89***) for Swedish spring wheat.

proceed. Since the conference program covers many of the problems, I will limit myself to the two where I do most of my own research. These are 1) the correlated growth pattern of the cereal plant with reference to its grain yield performance, and 2) the host-parasite interrelation.

Like the dramatic International Rice Research Institute (IRRI) and International Maize and Wheat Improvement Center (CIMMYT) programs in rice and wheat, breeding €or stiffer straw and improved harvest index was decisive in Sweden (Mac Key 1973). As under most other rainfed conditions, major dwarfing genes did not prove successful, at least in spring wheat. Progress has



2. Progress in breeding Swedish winter and spring wheat. Relative grain yield for all released varieties with yield of unbred, indigenous cultivars Sammet (winter wheat) and Halland (spring wheat) set at 100.

come from a polygenic approach. With the present type of wheat, it is obviously difficult to further diminish vegetative growth and gain in generative reproduction instead.

To illustrate, two sister varieties of Swedish spring wheat that are very much alike differ by 15 cm in plant height. When grown on upland, humus-poor mineral soils that somewhat restrict vegetative growth, the taller variety is superior. On lowland, organogenic soils that greatly stimulate vegetative abundance, the shorter cultivar is superior (Table 1).

We are faced here with a common problem in breeding cereals on rainfed land, including unbunded, upland rice. Major dwarfing genes apparently decrease vegetative growth too much for these conditions. Considering that world record yields are made with dwarf types, the genetic capacity for satisfactory leaf area development should not be restricted.

Other causes that intervene will differ in importance. In arid climates of the continental type, with sharply decreasing

Table 1. Grain yield response on different soil types for two Swedish sister cultivars of spring wheat, Kärn II and the 15 cm shorter Svenno, both obtained by reselection in Kärn I.

Soil type	Trials (no.)	Relative grain yield	
		Kärn II	Svenno
Humus content, low	40	100	99
" " , medium	471	100	101
" " , high	130	100	102
Organogenic	50	100	105

moisture gradients from the vegetative to the generative phase, the final outcome in grain yield may to a considerable extent -- 70% or more -- depend on translocation of reserves from stem and leaves (cf. Scott and Dennis—Jones 1976). In northern Europe where normally a foresummer drought is followed by rain during the grain—fillingperiod, stem reserves are less contributive, only 2—10% in many years (Austin et a1 1976).

However, in situations where water supply at any growth stage is limited, it is an oversimplification to base the ideotype construction entirely on photosynthetic considerations and assimilate transport patterns. Because shoots furnish assimilates and roots supply water and nutrients, the functional equilibrium of these two parts has important ecological significance.

Root studies offer considerable difficulties and for that reason have long been neglected by plant breeders. Available information generally comes from soil scientists (cf. Kutschera 1960, Whittington 1969, Carson 1974, Russell 1977) who are more interested in the relation between roots and soil than in correlative growth patterns.

Root and shoot develop interdependently according to a pattern based on genotype—environment interaction. During germination, root growth first dominates, followed by a compensating emphasis on shoot growth at the seedling stage (Van Dobben 1962). The distribution established at the end of this stage, under stable environmental conditions, remains almost constant during subsequent vegetative development. At the transition to the generative phase, the growth of both shoot and root stops. Except for a supply for plant maintenance, all assimilates go to grain filling (Mac Key 1973). Preferably,

Table 2. Shoot—root development of spring wheat (cultivar Prins) in relation to nitrogen supply in nutrient solution when the plant is grown in polystyrene foams.

Nitrogen conc	Plant dry weight (g)		Shoot—root ratio
	Root	Shoot	
100 mg/liter	3.67	0.36	10.1
150 "	4.56	0.35	13.0
200 "	4.75	0.30	15.7
250 "	5.93	0.33	17.8
300 "	6.81	0.32	21.1

the close interrelation between shoot and root development is studied by the Nilsson tube-culture technique (Nilsson 1969, 1973; Mac Key 1973). Each plant is grown in a transparent plexiglass tube inclined to 30° and equipped with an automatic hydroponic system. For genotypic studies, 3-7 mm spherical polystyrene foams are the preferred cultivation media. This arrangement permits continuous monitoring of shoot and root growth throughout their successive development phases. Harvest of different plant parts, including roots, is greatly facilitated.

Experiments verify that a competitive interrelation exists between shoots and root growth. In any given environment, the plant works toward optimizing contributions from shoot and root to the total metabolic synthesis. If nitrogen is the modifying factor, high nitrogen levels will increase the shoot-root ratio (Table 2) and may adversely affect drought tolerance during the generative phase.

Damage to one of these two plant parts will automatically influence the other. The situation can be illustrated by a defoliation experiment in which only the flag leaf is left (Table 3). The outcome parallels the effects of early rust infection on leaves and stems, which leads indirectly to inferior root development (Hendrix and Lloyd 1970, Doodson 1976).

Superimposed upon the genotype-environmental interactions is a genetic regulation, which limits adaptive range but allows better specialization to a particular ecological niche. This genetic steering offers possibilities for preadaptation to conditions at later parts of the vegetative period, when the relation between assimilating surface and root volume is no longer adjustable.

The genetically governed interrelation in growth and dimensions between shoot and root appears to be basically simple in

Table 3. Shoot-root development in a defoliation experiment with spring wheat (cultivar Prins) grown in tube culture in polystyrene foams.

Trait (16 replications)	Untreated control (absolute value)	Relative values (%) at complete defoliation. except flag leaf, started[a] at	
		6 WS	8 WS
Tillers (no.)	10.5	57	67
Crown roots (no.)	37.5	61	69
Plant ht (cm)	73.8	83	84
Root depth (cm)	117.4	85	91
Shoot dry wt (g)	3.88	41	48
Root dry wt (g)	0.84	44	65
Roots (%) in zone 0-20 cm	34	46	39
" " " " 20-40 "	22	25	28
" " " " 40-60 "	20	15	19
" " " " 60-	24	14	14

[a] WS = weeks after sowing.

plants such as the small grains. As to developmental pattern, shoot and root almost can be described as mirror images of each other (Mac Key 1966b, 1973). Under the balanced supply of water, nutrients, and oxygen that can be created in the Nilsson tube culture, this correlative response can be clearly demonstrated. For an international and diverse collection of spring wheats, the following correlations were found:

plant height/root depth ..... $r = 0.50 \pm 0.04^{***}$,
number of tillers/crown roots..... .. $r = 0.73 \pm 0.04^{***}$,
dry weight at heading of shoot/root.. $r = 0.85 \pm 0.01^{***}$.

Table 4 offers some concrete examples represented by tall, conventional and modern, short-strawed spring wheats from Germany and Latin America. The tall representatives have the deeper root system with a profile enabling more effective penetration to deeper zones. If such a root system is needed for reliable yields, as it is under many rainfed conditions, the dimensions of the aerial parts of the plant cannot be tailored only to photosynthetic efficiency.

The Mexican spring wheat Pitic 62 is included in Table 4 as a rare exception which combines short straw with an unexpectedly good root system. Significantly, this very variety played a decisive role in the international wheat yield breakthroughs in

Table 4. Shoot—root development of some tall and semidwarf cultivars of spring wheat grown in tube culture in polystyrene foams.

Trait (4 replications)	German cultivars		Latin American cultivars		
	Brown Schlanstedt[a]	Koli-bri[b]	Frontana[a]	Mayo 64[b]	Pitic 62[b]
Tillers (no.)	10.3	5.3	6.3	5.8	9.0
Crown roots (no.)	60.5	32.8	39.3	25.3	44.2
Plant ht (cm)	109.0	68.2	83.3	62.3	70.9
Root depth (cm)	103.3	88.3	109.1	55.3	87.0
Shoot dry wt (g)	17.0	6.0	7.9	3.5	5.2
Root dry wt (g)	1.04	0.48	0.72	0.28	0.72
Roots (%) in zone 0—20 cm	46	61	43	82	63
" " " " 20—40"	20	21	18	15	21
" " " " 40—60"	11	9	13	3	10
" " " " 60— "	23	9	26	0	6

[a] Tall cultivar. [b] Semidwarf cultivar.

the 1960s (Borlaug 1968). It is important to understand that such a desirable combination of short-root relation is not easily or automatically obtained.

To verify the applicability to field conditions of results obtained from the tube cultures, relevant data are compiled in Table 5. The spring wheat cultivar Prins is compared with its semidwarf, near-isogenic version Prins E. The deeper root system of Prins revealed by the tube experiments was verified through direct studies in the field (Haak 1978) in which natural soil was the culture medium. An isotope tracer technique was used to estimate plant uptake of calcium, phosphorus, and potassium from plow layer and subsoil. In a field of well penetrable soil, Prins demonstrated the ability to send roots deeper for nutrients resources than did Prins E.

To appreciate the correlated development of shoot and root in wheats, for example, it is important to remember their conditions in nature. They need vigorous early growth to compete for space, and a sufficient root system to guarantee drought and heat tolerance in the later part of the growing season. The growth pattern is directed toward consistency in reproduction rather than high generative productivity. There is no advantage to overproduction of seeds in an annual species that tends to grow in dense stands and has a restricted dispersion potential,

Table 5. Shoot-root development of spring wheat cultivar Prins and its near-isogenic, semidwarf version Prins E (=Norin 10 x Prins[5]).[a]

Trait	Prins	Prins E
Field performance		
Relative grain yield (X)	100	95
1000-grain wt (g)	40.8	36.6
Plant ht (cm)	95	70
Ca uptake from subsoil (% of total)	67	57
P " " " (" " ")	41	32
K " " " (" " ")	33	23
Greenhouse tube culture test in soil		
Tillers (no.)	8.3	8.0
Crown roots (no.)	11.5	13.0
Plant ht (cm)	65.5	45.4
Root depth (cm)	108.7	83.9
Shoot dry wt (g)	3.90	2.58
Root dry wt (g)	0.20	0.15
Roots (%) in zone 0-20 cm	34	50
" " " " 20-40 "	21	24
" " " " 40-60 "	19	19
" " " " 60- "	26	7

[a] Data on mineral nutrient uptake from Haak (1978) based on isotope technique and valid for a well penetrable soil profile.

owing to the need for a large and heavy dissemination unit for safe reestablishment. Almost always in the typical dense stand, only the main shoots become fertile. Because the wild types must develop rapidly they rely on translocation of reserves to a greater extent than do modern cultivars. A flexible growth pattern allows scattered, well-spaced plants to tiller profusely and thus increase their generative capacity simply by allowing more tillers to become fertile. High tillering even in dense stands implies, by correlation, an abundant crown root system for uptake of water and nutrients.

Domestication, and more obviously conscious plant breeding, implied a drastic shift in evolutionary trend. The aim became maximum generative productivity, achieved by restricting vegetative growth of the individual plant, partly by selection, partly by

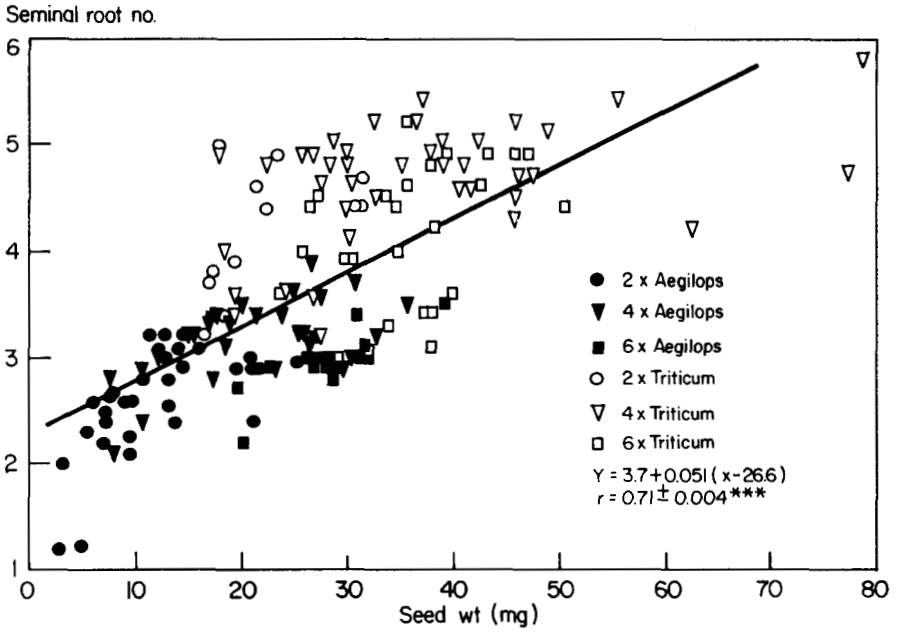
increasing population density. A shift toward monoculture to avoid interspecific and intraspecific competition was a prerequisite. Because the main culm is coarser and more vigorous, and carries a larger ear, tillering has been almost completely suppressed. Although rice is an exception a good modern stand of spring wheat has on the average only 1.2-1.5 ears/plants.

Such a shift in plant design should be accompanied by a correlative reduction in the number, volume, and depth of roots. An indication of the apparent strength of the symmetrical growth pattern in cereals is that evolution has found a way to circumvent rather than break the correlation. The solution has been a shift from a predominantly crown root- to a predominantly seminal root-dependent system. The importance of such a shift at increased stand density can amply be demonstrated by spacing and overcrowding, respectively, any variety of wheat or similar small grain (Pavlychenko 1937, Table 6).

The increasing reliance of wheat on the seminal roots has come about as a result of their increased number. This number is highly correlated with seed size, another desirable character for domestication (Fritsch 1977; Mac Key 1977b, 1979a). The evolutionary trend in the *Aegilops-Triticum* complex toward

Table 6. A field study of the root system of wheat at different spacings (after Pavlychenko 1937).

Part of root system	Plants spaced 3 m apart		Plants in 15 cm drill rows	
	sum of lengths (m)	% of total	Sum of lengths (m)	% of total
Seminal roots:				
Main axes	8	0.001	4	0.5
Laterals of 1st order	962	1.4	187	21.6
" " 2d "	7,920	11.1	504	58.1
Seminal roots, total	8,890	12.5	695	80.2
Crown roots:				
Main axes	41	0.1	3	0.3
Laterals of 1st order	4,980	7.0	58	6.7
" " 2d "	57,200	80.4	111	12.8
Crown roots, total	62,221	87.5	172	19.8
Total root system	71,110	100.0	867	100.0



3. Relation between seed weight and seminal root number tor a series of *Aegilops* and *Triticum* forms.

adaptation to denser stands and more vigorous initial growth by means of the interrelated increase of seed size and number of seminal roots is demonstrated in Figure 3. From *A. mutica,* one of the more primitive *Aegilops* species, with hardly more than 1 seminal root/seed, up to 5 or even 6 seminal roots develop in certain varieties of the *durum, turgidum,* and *vulgare* groups. Moreover, the available energy supply, i.e. the size of the endosperm rather than that of the embryo, determines how many of the primordia will develop. As a consequence, seminal root number varies within a variety according to seed position and outside influences at maturity (Mac Key 1979a). It is obvious that rice, with one seminal root (Katayama 1966), has a long way to go if a similar evolution were considered desirable.

Seminal roots are more efficient per dry matter weight than are crown roots because they are finer and more branched. They take a more vertical downward course, which allows them to go deeper and explore less water- and nutrient-exhausted zones in the soil profile. In the literature (cf. Fritsch 1977) relevant to the arid wheat-growing regions of the USSR, the

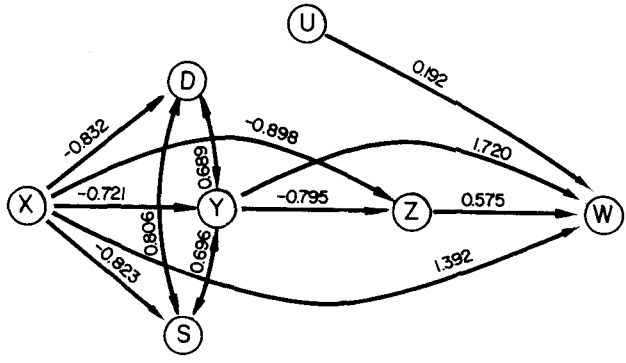
importance of a high number of seminal roots has especially been stressed for the safe establishment of the seedling.

Because of the correlation between seed size and seminal root number, attempts to select for high seminal root number may interfere with optimum balancing of the different yield components. Under arid conditions, seeds should be small but numerous, produced in many small heads per plant (Mac Key 1966b).

An example of the difficulties in surveying all correlated responses in a cereal plant is the construction of a large-glumed *vulgare* wheat. In wheat, the green surface of the ear itself contributes about one-third of all assimilates that go to fill the grains (Boonstra 1937, Stoy 1965). A large glume richer in chlorophyll could be produced by transferring the *polonicum* gene to common bread wheat (Mac Key 1966a, Fig. 4). For typical durum and triticale, also characterized by comparatively large glumes, it has been difficult to develop a thick stand of this large-glumed wheat. After a promising start, the sink capacity at ear initiation for the main culm is apparently so dominating that tillers have difficulty developing. Conversely, erect short-leaved rice with high tillering ability would have difficulty in developing large hulls and thus large seeds (cf. Murata and Matsushima 1975).

The plant breeder knows from experience that he must always compromise. But his efficiency and the reliability of his varie-

4. Pair of wheat ears viewed from two angles showing a normal *vulgare* wheat (left) and a large-glumed version carrying the characteristic at the 6x level phenotypically modified gene of the 4x *polonicum* wheat.



5. Path coefficient diagram showing interrelationship of fertile tillers per unit area (X), seeds per head (Y), kernel weight (Z), leaf area (S), culm diameter (D), and grain yield (W). The unexplained part of the variance of W is represented by U. Double-headed arrows denote simple correlation coefficients and single-headed arrows part coefficients showing cause and effect relationships (Hamid and Grafius 1978).

ties depend on his knowing how to make the right compromises. The characteristics of the wheat root system discussed above indicate that cereal roots follow the sane correlated, allometric pattern as cereal shoots.

Comprehensive studies of the aerial parts of rice, wheat, and barley indicate an almost identical basic pattern in their correla—tive responses. Hamid and Grafius (1978) and Grafius (1978) have recently made the consequences very clear: the traits set early during morphogenesis will govern almost the whole design. Basic—ally they trigger a chain reaction affecting all organs formed later. The authors have illustrated the correlated response of leaf area, culm diameter, and grain yield components in barley by a path coefficient diagram (Fig. 5).

In the cereals, the proliferation of tillers is one of the first developmental processes at the aboveground organ level. A strong, negative interrelation between the number of fertile tillers on the one hand, and culm diameter and size of leaves and heads on the other, sets the yield pattern to a large degree. Many fertile tillers mean thinner culms and smaller leaves and heads and, by the association demonstrated above, also more crown roots. In principle, this is the small grain ecotype for arid conditions. On the other hand, reduced tillering results in coarser culms, broader leaves, bigger heads, more dependence on seminal roots and thus larger seeds. In principle, this is the small grain eco-

type for maritime climates or irrigation (Mac Key 1966b). Owing to the special advantages of planting, as with most rice, varieties for such a cropping method are constructed contrary to the basic principles to compensate for low plant number per unit area.

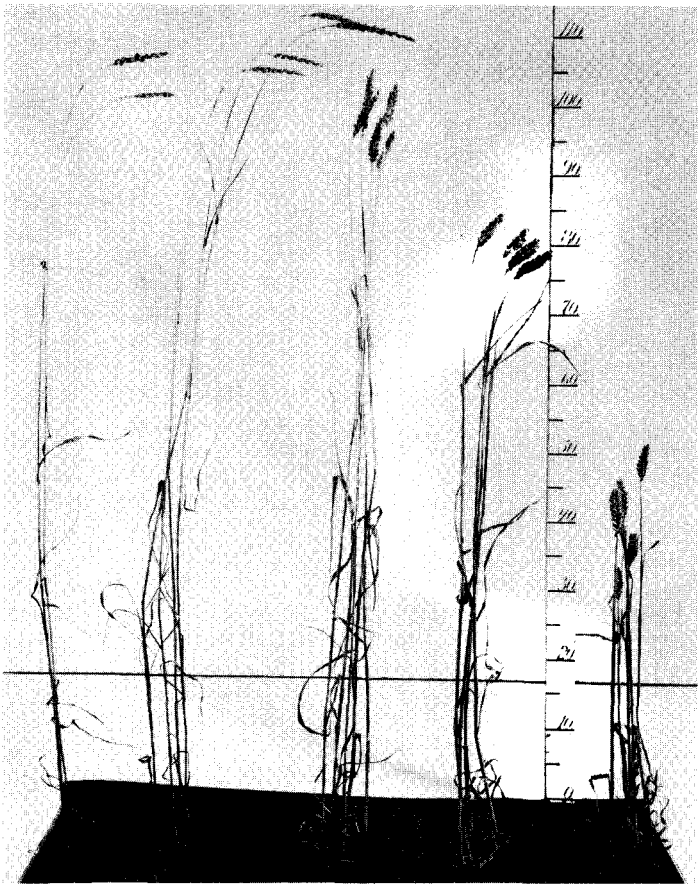
The shift from wild to domesticated plant types has proceeded through enlargement of the meristem or, ultimately, cell size. The increased importance on macroevolution in the accelerated process of domestication has favored pleiotropic repatterning (Mac Key 1979a). This is demonstrated by the evolution of factor *Q* in hexaploid wheat, which apparently arose from a triplicate (Mac Key 1954, Muramatsu 1963). It changes the general cell shape to a more stout or compact form (Li et al 1948). Consequently, the shoot meristem is similarly changed, giving rise to increased culm diameter, shorter culm and broader leaves, and larger heads, glumes, and kernels. Because of changes in organ dimensions, factor *Q* also exerts a decisive effect on the two most important characteristics of wheat domestication: the creation of non-spelting glumes and tough rachis which improve threshability and inhibit shattering (Mac Key 1954, Fig. 6).

The successful plant breeder must have respect for and knowledge of how to manipulate the developmental correlates as they are expressed under the yield component stress matrix for any given cropping practice and photoperiod regime. Grafius (1978) has collected some especially thoughtful statements in this connection, to which he even gives the rank of a natural law.

- Sinnott's (1921) law: The size of an organ is proportional to the size of meristem from which it develops.
- Grafius' (1978) corollary 1: Plasticity is inversely proportional to ontogenic proximity.
- Grafius' (1978) corollary 2: Number and size tend to have an inverse relationship.

Correlated responses are not tied to morphogenetic pleiotropism entirely. Genetic linkage may be another phenomenon which is more or less easy for the plant breeder to overcome. Physiological interrelations also have to be understood much better. The rapid progress in plant biochemistry is promising for the future.

For example, nitrogen uptake is of great concern today. Most nitrogen in cereals is taken up (often up to 80%) prior to grain filling. The protein in the leaves and stems is translocated



6. The stouting effect of factor *Q* in *vulgare* wheat illustrated by the aneuploid series of its carrier, chromosome 5A. From left: nullisomic, monosomic, disomic, trisomic, and tetrasomic plants from cultivar Skandia IIIA with 0, I, 2, 3, and 4 doses of *Q,* respectively (Mac Key 1954).

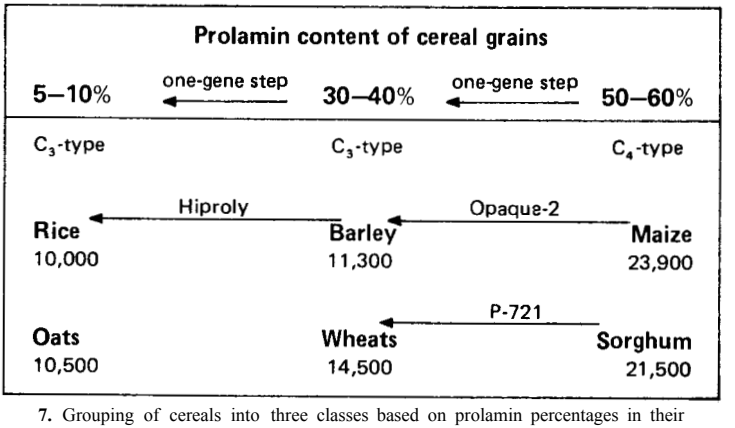
as amino acids to the developing seeds, a process mainly associated with the senescence at maturation. Such a relocation is more likely to be facilitated in ecotypes adapted for arid conditions during their generative phase, i.e. those which rely mainly on pre-anthesis photosynthesis to ensure seed production. In climates characteristic of northwestern Europe where days are long and moisture is readily available, especially during the generative phase, leaf area duration becomes decisive (Stoy 1973, Bingham 1976). Most of the assimilates to the grains are produced directly and the need for functioning leaves makes any decomposition of leaves for mere reserve storage more difficult. Thus breeding premises for high protein grains differ.

In the attempts to adjust plant proteins to human nutritional needs -- for cereals mainly a matter of increasing lysine and tryptophan -- indirect complications may arise. These two essential amino acids occur in quantities that are inversely related to the prolamin fraction. Nelson (1976) has grouped cereals into three classes based on the prolamin content of the grain (Fig. 7). Maize and sorghum, which have the highest content, possess the C_4-dicarboxylic acid photosynthesis pathway, which offers higher efficiency in using not only light, water, and carbon dioxide, but nitrogen as well (Brown 1978). High-lysine mutants are characterized by moving one entire step, which suggests that two major genes exercise basic control over the relative amount of prolamin in seed proteins.

Why are the different cereals found at different levels of such a genetically simple system? The high yielding species show the lowest protein quality and vice versa. Plant breeders must be inspired to breed for compensating abilities.

Efforts to transfer the C_4-pathway to C_3-species have failed (Moss and Musgrave 1971), but distinct high lysine types have been found in maize, sorghum, and barley. They have not been found in wheat, owing to the buffering of its hexaploid constitution. Nor have they been found in oats and rice, which might already be at the lowest prolamin level (Axtell 1979).

Storage proteins are, however, not just stores of amino acids. They must fit the enzymatic makeup appropriate to different plant species (Boulter 1976). The zein proteins in maize are the most



7. Grouping of cereals into three classes based on prolamin percentages in their proteins (after Nelson 1976). Arrow with name of high-lysine mutant indicates change of class by presumably single-gene event, suggesting that differences among cereals in prolamin content could basically be controlled by two major genes. Figures below each crop are registered world record grain yields in kilograms per hectare (Anonymous 1966, Murata and Matsushima 1975, Wittmer 1975).

rapidly mobilized during seed germination, and opaque—2 kernels germinate somewhat slower than isogenic, normal inbred lines (Jones and Tsai 1977). The high prolamin content of maize and sorghum may be advantageous for rapid stand establishment under competitive conditions in the tropics. It may also be a conse— quence of their more efficient nitrogen economy, including ability to store protein as prolamin in leaves, when nitrogen is plentiful (Brown 1978). The high ability of prolamin to form multisubunit assemblies might be imperative, because it implies a reduced osmotic pressure that prevents the cell from rupturing (Wetlaufer 1973). If these were the original reasons for high prolamin level in modern maize and sorghum progenitors, they would not be controlling in intensive agriculture. Unchanged yielding ability appears to be within reach through adapting genetic background by recombination breeding (Persson 1975, Vasal et a1 1978, Axtell 1979).

Whether adjustments are of morphological or physiological nature, a precise balance with small margins is implicit. Compared to the vegetatively overemphasized wild or more primitive domesticated types, modern varieties are expected to show less tolerance for diseases and pests. Tolerance is taken here in its strict sense as the ability, either in the absence of or superimposed upon a partial resistance, to accept the parasite with less loss in (re)production. Grown in uniform stands, often with a prolonged leaf duration, modern varieties are more susceptible to attacks. Resistance in its true sense, i.e. a direct defense mechanism, increases in importance. Since more varieties require or pay off higher under a more intensive agriculture with increased investment, disease resistance in its true sense has become more important as a yield component.

Since Van der Plank in 1968 published his book *Disease resistance in plants,* there has been vigorous worldwide discussion among experts on how to use available resistance sources more efficiently and reliably. There have been proposals to completely abandon race—specific resistance in favor of nonspecific resistance alone (Robinson 1976). If race— specific resistance is used, the recommendation is that it be used in a diversified manner as in multiline varieties or gene deployment systems (Frey et al 1977). At the same time, the question of whether the two types of resistance can be kept apart has been raised (Parlevliet and Zadoks 1977). It has even been suggested that each gene carries the characteristics of both and that there will be a gradual degradation from a major to a minor effect, as related genes accumulate (Nelson 1975).

Obviously, there are difficulties in setting a sharp demar— cation line between race—specific and nonspecific host—parasite

interrelations. Nature has surely not intended such a distinction (Clifford 1975). In plant breeding as well as in epidemiology, concepts are, however, useful as long as they are concepts only. The most useful definition seems to be that race-specific resistance is an incompatibility phenomenon having a selective initial effect but none against established infections. The specificity separates a heterogeneous parasite population into an avirulent and a virulent fraction and acts, in principle, only on the former. Nonspecific resistance may also have an assortative effect but on a quantitative basis and thus be blurred by the polygenic regulation. It inhibits the spread of the pathogen by either delaying its reproduction cycle or decreasing its reproduction capacity, or both. This type of resistance is seldom complete and thus is generally expressed in degrees. The corresponding counter-adaptation pattern of the pathogen is described by degrees of aggressiveness. In principle, race-specificity acts initially on an epidemic: nonspecificity, during the course of an epidemic (Van der Plank 1968).

Nonspecificity may occur alone, but it is questionable whether race specificity does. Owing to its polygenic nature, nonspecificity to some extent occurs more or less completely hidden under race specificity (Table 7).

Table 7. Lesion area of eight rice cultivars inoculated with four isolates of *Xanthomonas oryzae* by the needle-pricking method (Ezuka et al 1975, Yamamoto et al 1977, cit. in Parlevliet 1979).[a]

Cultivar	Carries race-specific genes	Lesion area ($\sqrt{mm^2}$) caused by isolates			
		T-7174	T-7147	T-7133	Xo-7323
Nikisakae	-	10	13	13	12
Asahi 1	-	23	21	24	21
Pelita I/1	Xa-1	1	8	7	7
Norin 27	"	1	26	29	27
TKM6	Xa-1, Xa-2	1	1	16	9
Tadukan	" "	1	3	32	39
Nagomasari	Xa-3	2	2	1	13
Chugoka 45	"	2	1	2	24

[a] The steplike dividing line separates race-specific reactions (left) from nonspecific reactions (right) with difference between pairs of cultivars but not between isolates.

Every evolutionary step in host defense will put new selective pressure on the parasite and vice versa. Thus interdependent genetic systems evolve. This steady counteradaptation is reflected in the gene-for-gene relation first discovered for race specificity by Flor (1942) but valid too in connection with some types of nonspecificity. Where the gene-for-gene relation works, race specificity follows two different patterns which function as mirror images of each other. In one case, a gene for virulence in the pathogen matches (overcomes) a particular gene for resistance in the host plant (Person 1959). In the other it fits in with a particular gene for susceptibility in the host plant (Ellingboe 1976). In connection with nonspecificity, gene-for-gene relations set levels of resistance/aggressiveness, in principle, in an additive quantitative manner (Parlevliet 1979). Existence of complementary or cytoplasmic gene action does not negate the basic principle of the gene-for-gene theory. Rather, such interactions may be considered only as a rare variation on the theme. How the different models of gene-for-gene interaction work is shown in Table 8, presupposing two pairs of genes involved.

Race specificity works with major genes in an oligogenic system in which flexibility takes precedence over reliability. Nonspecificity functions in principle, although not necessarily always, as a polygenic system in which reliability is paramount. This difference appears to have given race specificity and nonspecificity different roles in an overall strategy. It appears likely that both man and nature have experimented more with race specificity and used nonspecificity as a reliable background.

It may then be more important to thoroughly understand how race-specific genes operate and function. This is not to devalue nonspecific resistance itself but rather its strategic complexity The problems here are evaluation, accumulation, and transfer.

Possible interactions in connection with race specificity have not been fully understood. Mode (1958) and Person (1959, 1966) postulated that the selective value of genes for resistance and that of their matching genes for virulence counterbalance each other towards a state of equilibrium, a balanced polymorphism. Van der Plank (1968) extended the model by suggesting that in obligate parasitism unnecessary genes for virulence are somehow disadvantageous and thus steadily selected against. This would prevent an accumulation of virulence genes into super-races. Leonard (1969c) extended the model further by *assuming,* while Harlan (1976) *stated,* that genes for resistance are also selected against in environments where they do not protect against the pathogen. The advantage of this model on the whole should be a kind of *modus vivendi,* essential for the survival not only of the host but also of its dependent parasite.

Table 8. Different systems of host plant resistance against a pathogen, presupposing two pairs of genes involved.[a]

1. *Melampsora* type race-specific resistance

Gene for virulence in the pathogen matches gene for resistance in the host plant.

Pathogen virulence genotype	Host resistance genotype --	*A-*	*-B*	*AB*
--	S	R	R	R
a-	S	S	R	R
-b	S	R	S	R
ab	S	S	S	S

2. *Helminthosporium* type race-specific resistance

Gene for virulence in the pathogen matches gene for susceptibility in the host plant.

Pathogen virulence genotype	Host resistance genotype --	*A-*	*-B*	*AB*
--	R	R	R	R
a-	R	S	R	S
-b	R	R	S	S
ab	R	S	S	S

3. Interdependent nonspecific resistance

Gene for aggressiveness in the pathogen matches gene for resistance in the host plant.

Pathogen aggressiveness genotype	Host resistance genotype --	*A-*	*-B*	*AB*
--	S	(r)	(r)	r
a-	S	S	(r)	(r)
-b	S	(r)	S	(r)
ab	S	S	S	S

4. Independent nonspecific resistance

Gene for aggressiveness in the pathogen does not match directly any gene for resistance in the host plant.

[a] *A* and *B* are two resistance genes, *a* and *b* two virulence genes or aggressiveness genes, the latter with additive effect. S = susceptibility, R = resistance, r = (moderate) resistance, r = low resistance.

During the last decade, this principle of stabilizing selection has dominated the concept of race-specific parasitism. Strategies in breeding for race-specific resistance have been changed accordingly. Methods based on diversification, such as multiline varieties and gene deployment to different geographic zones, rather than accumulation of genes for resistance, are now introduced.

The question arises whether race specificity really behaves evolutionarily strictly enough to call for a more general gene diversification strategy in breeding for race-specific resistance. One controlling approach would be to use the gene-for-gene interrelation for a genic deciphering of the racial composition of different parasite populations under different situations. Since a gene for race-specific virulence can be identified only by its matching gene for resistance, the analytical tools for such an investigation obviously must be host genotypes carrying only one race-specific resistance gene each.

The race key of oat stem rust (*Puccinia graminis* f. sp. *avenae*) happens to be appropriate from this aspect, and its limited size makes it instructive to use for demonstration. The four differentials of the old key each carry one of the dominant resistance genes *A* (*Pg2*), *B* (*Pg4*), *D* (*Pg1*), and *E* (*Pg3*) matched by the recessive and independently segregating virulence genes *a* (v_2), *b* (v_4), *d* (v_1), and *e* (v_3), respectively. Among 26 identified races, 10 show mesothetic reaction on one of the differentials. This mixed and temperature-instable syndrome is apparently due to a superimposed plasmic effect (Green and McKenzie 1967). If read as susceptibility, the system will be limited to 16 races, i.e. all possible recombinations from 4 genes. If the gene-for-gene interrelation is applied, the old international race numbers can thus be deciphered (Mac Key 1974, Table 9).

Deciphered race spectra for Sweden and the United States (1956-59), arranged not by conventional numbers but by genic constitution and degree of complexity, are given in Table 10. These two examples represent total absence and presence, respectively, of a host selection pressure. No oat varieties with race-specific resistance have ever been grown commercially in Scandinavia, but they have been consciously produced in North America since the early 1940s (Mac Key 1974, Stewart and Roberts 1970).

All races vital enough to be recorded carry the virulence gene *d* in Sweden but the gene *e* in the United States. In the sense of Van der Plank (1968), gene *d* is, as are all resistance genes, "unnecessary" in Sweden, and gene *e* is "unnecessary" in the United States, since its matching gene for resistance has not been used in breeding. Obviously, gene *d* must be important for fitness

Table 9. Race deciphering key for oat stem rust translating old conventional race numbers into combinations of virulence genes (after Mac Key 1974).[a]

Pathotype	Ratio in race complexity	Combination of genes of virulence	Conventional race no.	Host type with gene of resistance			
				A	*B*	*C*	*D*
1	1	- - - -	1	□	□	□	□
2	4	*a* - - -	11	■	□	□	□
3		- *b* - -	1A	□	■	□	□
4		- - *d* -	3	□	□	■	□
5		- - - *e*	2	□	□	□	■
6	6	*a b* - -	11A	■	■	□	□
7		*a* - *d* -	4	■	□	■	□
8		*a* - - *e*	8	■	□	□	■
9		- *b d* -	3A	□	■	■	□
10		- *b* - *e*	2A	□	■	□	■
11		- - *d e*	7	□	□	■	■
12	4	*a b d* -	4A	■	■	■	□
13		*a b* - *e*	8A	■	■	□	■
14		*a* - *d e*	6	■	□	■	■
15		- *b d e*	7A	□	■	■	■
16	1	*a b d e*	6A	■	■	■	■

□ = resistant, ■ = mesothetic or susceptible reaction.

or have a supporting function in one situation and gene *e* in another.

The Swedish race spectra show a clear tendency to form complex races despite the absence of selection pressure, while the American race spectra show no more complex races than necessary under the operating host selection pressure. Resistance genes *A* and *D* were introduced separately in the late 1940s and were combined together or with *B* in the mid-1950s (Stewart and Roberts 1970). The trend in the United States supports Leonard's (1969 a, b) earlier studies on race mixtures.

The different qualifications for reproduction in Sweden and the U.S. probably account for the seeming contradiction (Mac Key 1974). In Sweden, the complete oat stem rust cycle depends on an alternationto barberry for safe overwintering and necessitates a storage capacity for virulence genes for the safe passage from one host to the other. In the United States, the more important biotypes rely on constant asexual reproduction at the uredinial stage owing to the efficiency of the so-called *Puccinia* Path.

Table 10. Genetically deciphered spectra for oat stem rust in Sweden and the United States, 1956—59, arranged by degree of complexity (Mac Key 1977a).

Year	n	Relative prevalence (%) of oat stem rust race number/pathotype																	
		1	11 *a*	1A *b*	3 *d*	2,5 *e*	11A *ab*	4 *a*	*d*	8,10 *a*	*e*	3A *bd*	2A, 5A *b*	*e*	7,12 *de*	4A *abd*	8A, 10A *ab*	*e*	6,13 *a*	*de*	7A, 12A *bde*	6A,13A *abde*
Sweden:																						
1956	32	-	-	-	31	-	-	28	-	6	-	13	-	-	13	3	6					
1957	61	-	-	-	7	-	-	7	-	3	-	11	3	-	13	25	31					
1958	96	-	-	-	24	-	-	14	-	5	-	20	3	-	15	6	14					
1959	133	-	-	-	12	-	-	9	-	7	-	16	5	-	10	21	20					
1956—59	322	-	-	-	16	-	-	12	-	6	-	16	4	-	12	15	19					
USA																						
1956	476	-	-	-	-	16	-	-	15	-	-	66	-	-	1	2	-					
1957	522	-	-	-	-	12	-	-	21	-	0	59	-	-	2	6	0					
1958	286	-	-	-	-	14	-	-	26	-	-	54	-	-	1	5	-					
1959	230	-	-	-	-	7	-	-	11	-	-	59	1	-	10	10	2					
1956—59	1514	-	-	-	-	13	-	-	19	-	0	60	0	-	3	5	0					

Apparently, the rust has the evolutionary capability to adjust the genetic background of its virulence genes to different strategies.

In Israel (Wahl et a1 1964) and Australia (Luig and Baker 1973) cultivated oats are infected by unnecessarily complex races of oat stem rust despite the absence of barberry. Nevertheless, an alternative host is still necessary and grasses, rather than barberry, serve this function in those areas. In Israel alone,oat stem rust was found on 73 species of 37 genera of the grass family (Wahl et a1 1964).

Oat crown rust in Israel shows the same tendency to form complex races. Here alternative hosts include a number of grasses and buckthorn (Wahl 1970). Wheat leaf rust, especially in the eastern part of Europe, also seems to accumulate unnecessary virulence genes (cf. Stewart et a1 1967, Ralski 1972, Boskovic and Browder 1976, Lesovoj et a1 1976, Mac Key 1979c). Isolates with more than 4-5 unnecessary virulence genes appear frequently and often are the most numerous.

The system of describing races by their avirulence or virulence formula based on an adequate number of testers (Black et a1 1952, Watson and Luig 1963, Green 1971) also reveals that races with more than the minimum number of virulence genes necessary for survival are quite common (cf. e.g. Graham et al 1959, Luig and Watson 1970, Green 1971). Their occurrence could even be considered as normal in many parasite populations.

Although the function of race-specific genes is highly qualitative, their phenotypic effect obviously is dependent on their genetic background. If necessary, other genes are able to adjust and adapt them to a certain strategy. They may, as previously indicated, even have pleiotropic effects giving them additional functions and roles in general fitness. It is incorrect to press the autonomy as strictly as did Groth and Person (1977), Leonard (1977), or Marshall and Pryor (1978). The interaction capacity of race-specific genes goes beyond pure fitness to include interference with nonspecific ones.

Both the ability and the necessity of one and the same rust species to develop different race-specific evolutionary patterns is better understood through the important work on wheat stem rust in Australia. The parasite is totally dependent on the uredinial stage for survival throughout the year and asexual recombination is insufficient for more profound changes of once-established genotypes. The assortative effect of introducing varieties with complex race-specific resistance, therefore, implies a considerable erosion of background genes. In spite of mutation rates calculated by Parlevliet and Zadoks (1977) to be on the order of 1,000 mutants/locus per hectare per day, the Australian experience indicates an apparent restriction in evolutionary flexibility of the rust. New genes for virulence provoked by new genes for resistance will have difficulties becoming properly adjusted. For the same reason suddenly

unnecessary and now disadvantageous virulence genes cannot be properly eliminated. The more resistance genes it has to overcome, the less aggressive the rust becomes (Watson 1977).

It must be that there is a considerably greater gap between mutation rate and mutation establishment than is understood. It appears likely that newly developed genes for race specificity, both on the parasite and host side, require adjustment in their genetic background. Luig (1978) found that pathogenic mutants in rust exhibit small urediospores or delayed development, or both. Watson and Luig (1968) found that rust can change in a stepwise fashion from avirulence to virulence apart from what can be reached by passing through heterozygosity. New mutations for race-specific resistance are generally recessive and often inferior in performance. Old resistance genes occurring in nature are generally dominant, indicating an adaptation process (Mac Key 1974, cf. Jorgensen 1976, Fischer 1931). All this evidence points to evolutionary complications in developing new genes for race specificity.

Under conditions of pronounced host selection pressure eroding the gene pool and absence of asexual recombination, the adaptation process is hampered. Virulence genes are likely to remain generally unfit and preserved only when their special function is necessary. Evolution merely by mutation and selection is very much more rigid than if the annual cycle involves sexual recombination.

If mutation establishment is a problem, the rust should have the advantage of preadaptation rather than relying on prompt mutation. A considerable storage capacity could be built up in relaxed populations because of their dikaryotic constitution and the general recessiveness of virulence genes. Such an ability can be amply demonstrated by again turning to the Swedish oat stem rust (Table 11).

By using 13 genes known for race-specific resistance as traps, 11 of the matching virulence genes could be found in this rust population devoid of any race-specific host selection pressure. The number of virulence genes found is, in fact, the same as in North America where there has been conscious breeding for resistance since the 1940s. The high incidence in North America of virulence genes matching the resistance of *E (Pg3), f (pg8), h (pg9),* and *m (pg13),* but absence of the matching resistance genes in the host population (Martens et a1 1970) supports the picture of preparedness rather than mutation every time breeders introduce a new resistance gene. The European wheat leaf rust is another example of preadaptation of a high level.

The argument here has been to accept situations for both stabilizing selection and accumulation of race-specific genes within

Table 11. Known genes for race-specific resistance to oat stem rust and matching virulence genes in the Scandinavian population not subjected to any host selection pressure (Mac Key 1974).

Source	Known to hold resistance in gene		Matching virulence gene[a]
Lanark, White Russian, Minrus	*Pg1*	*(D)*	●
Exeter, Richland, Ajax	*Pg2*	*(A)*	●
Canuch, Jostrain, Foxton	*Pg3*	*(E)*	●
Podney, Torch	*Pg4*	*(B)*	●
CI 2710, RL 524.1, CL 4023	*pg8*	*(f)*	●
CI 5844	*pg9*	*(h)*	●
CI 3034	*pg11*		
Kyto	*pg12*		
A. sterilis CW490-2	*pg13*	*(m)*	●
Garry, Hajira		*(G)*	●
CI 1575		*(G)*	
Milford, Winter Turf		*(N)*	●
Posen mutant *(pg9* + ?)			●
Saia, 2x (*A. strigosa*)			●

[a]Already found in the Scandinavian population of oat stem rust.

a parasite population. Effective stabilizing selection is expected mainly in the absence of compulsory host alternation and with asexual reproduction combined with high assortative selection pressure causing overall genetic erosion. True haploidy is another important prerequisite. An accumulation of virulence genes presupposes opposite situations. Many, if not all, parasites involved in race specificity should be able to follow either trend depending on circumstances. Both situations are able to offer a *modus vivendi* because race specificity never works alone. The complex rust races in Sweden are no more dangerous than the simple ones, since host resistance is absent. The late occurrence of rust on cereals, after its first having to pass another host, offers a decisive "escape resistance." The real risk, although extremely rare, is early wind transport fron south or southeast, i.e. interference from outside the system.

Different parasite strategies mean different strategies in breeding for resistance. With existing super-races built up without host selection pressure, multiline varieties could hardly be recommended. The pathogens should rather be controlled

by a rapid and consistent introduction of multiple resistance cultivars, preferably with a reduplicated complete protection or diversification on this more complex level. Step-by-step progress would not allow a sufficient erosion of the pathogen's genetic resources for controlling its advance.

The merits of multiline varieties must also be questioned where a genetically narrow pathogen population with insufficient recombination potentials has been created. Here the inflexibility may hamper a stabilizing selection down to simpler races. When these are finally reached, a variation is created in the pathogen that might increase its evolution in undesirable directions. Diversity resources of the host imply diversification of the parasite.

With the same logic, diversification in a parasite population based on stabilizing selection places a premium on multiline cultivars as well as deployment of resistant genes. As soon as a pathogen has the evolutionary capacity to adjust its genetic balance for preserving virulence genes , diversification programs should not be expected to have extreme long-term reliability if they are extensively used within a certain region.

Resistance breeding either by diversification or gene accumulation must never occur simultaneously with the same set of resistance genes within a natural epidemiological region of the parasite.

SUMMARY

Plant breeding still has great potential for increasing yields. It is necessary, however, to better understand some basic problems involved. Two fields of research are discussed: 1) developmental allometry, and 2) host-parasite interrelation. Cereals have an integrated growth pattern, which is dynamic throughout their ontogeny. Traits set early during morphogenesis trigger a chain reaction affecting organs formed later from the same basic meristem. Even shoot and root show high correlative growth. Plant height and root depth, number of tillers and crown roots, and dry weight of shoot and root prior to grain filling show high *r*-values. Remodeling such as dwarfing may introduce the risk of undesirable effects, in this case drought intolerance. A precise balance of vegetative development for maximum grain yield will endanger disease tolerance, which places increased importance on breeding for resistance. With respect to general fitness, genes for virulence or resistance

are highly influenced by the genetic background. If such genes are unnecessary in the actual host-parasite interrelations, they will normally exhibit selective disadvantage unless an adaptation process occurs to preserve them. Mode of reproduction, host alternation, ploidy level, etc. are decisive factors. Different evolutionary trends will require different strategies in breeding for resistance.

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HYBRID RICE BREEDING IN CHINA

LIN SHIH-CHENG and YUAN LOUNG-PING

The hybrid rice research program in China was initiated in 1964. Within two years, visual inspection of the panicles during the heading stage revealed several male sterile (MS) rice plants in the fields. These MS plants could be classified into three types: pollen free, pollen abortive, and anther degenerative. These were our starting materials in hybrid rice breeding work.

Unfortunately, all three types of male sterility were controlled by the recessive nucleus genes. No satisfactory maintainers could be found by screening with wide test-crossing, nor could any be synthesized by "onion formula." Thus, in 1970 we changed our working direction from nucleic to cytoplasmic male sterility.

In the autumn of 1970, a MS wild rice plant was found in nature and was named wild abortive (WA). Its discovery was a breakthrough in hybrid rice breeding.

In 1971, the hybrid rice breeding program became the subject of the cooperative research project of Hunan province. The next year it became the cooperative project of the nation and was organized jointly by the Chinese Academy of Agricultural Sciences and the Hunan Academy of Agricultural Sciences. Through the joint efforts of more than ten research organizations within 3 years some MS lines of WA type and their maintainers were developed. Then in the autumn of 1973, some excellent restorers were selected.

Meanwhile, concentrating their efforts on the study of heterosis of hybrid rice, techniques of hybrid seed production, and improved cultivation methods, some research organizations have obtained many practical results.

In 1974, the first small demonstration field was planted to hybrid rice and promising results were achieved. Since then, the area planted to hybrid rice has been increased rapidly each year. In 1978 the total area was about 5 million ha. It has been proven practically that hybrid rice grows well, yields higher than common cultivars, and enjoys wide favor among farmers.

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BREEDING FOR THREE LINES

Three lines of WA type

WA designates a MS wild rice plant with abortive pollens. It was found by our co-worker, Li Bi-Hu, in a population of common wild rice (*Oryza sativa* L. *f. spontanea*) on Hainan island. WA is a typical photoperiod-sensitive plant with active tillering ability. It is prostrate and has narrow leaves, slim stems, easily shattered small grains with long red awns, and a purple sheath; its stigma is well developed and exserted. In short, except for its male sterility, all its characteristics are similar to those of the common wild rice on Hainan island (Fig. 1). The anthers of the WA plant are thin, yellowish, and undehiscent, and contain aberrant abortive-shaped pollens. But its sterility is unstable. At temperatures higher than 30°C, some anthers may bear normal pollens, and consequently a few selfed seeds may be produced.

1. Male sterile wild rice plant with abortive pollen found in 1970 and designated as wild abortive (WA).

In the spring of 1971, 10 cross combinations were made between WA and cultivars of Hsien (indica) and Keng (japonica) on Hainan island. In the autumn and winter of that year, fertility segregation was observed in the F_1 plants. Three kinds of F_1 plants, namely, fertile, semisterlle, and completely sterile, appeared in various proportions. The appearance of 13 complete male fertile F_1 plants, 3 of them with normal seed set, indicated that the fertility of some plants with WA cytoplasm might be perfectly normal. From these normal plants, restoring lines could probably be developed.

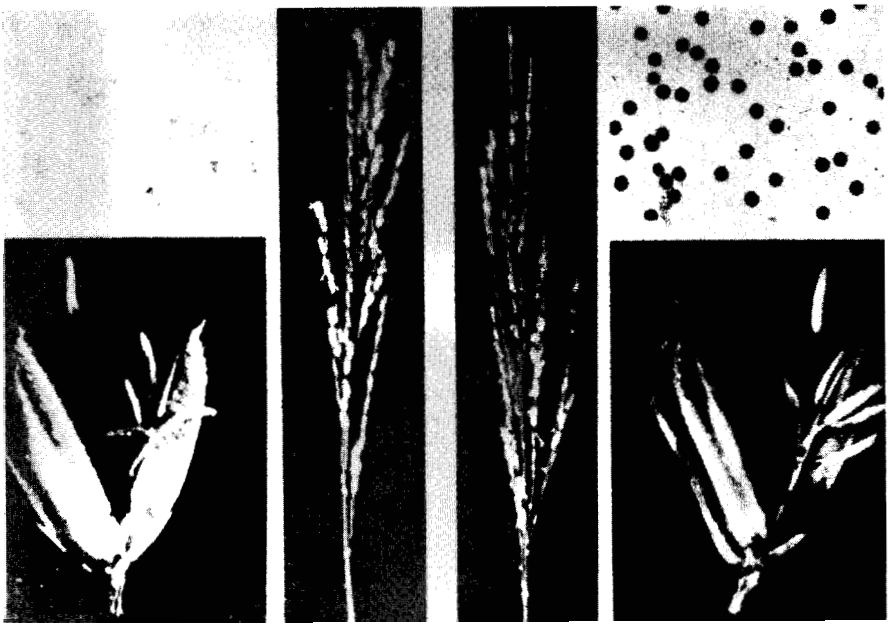
In addition to fertility segregation in F_1 plants, we found segregations in morphological characters. For example, the sheath, glume-tip, and stigma of WA are all purple, but the F_1 plants from the crosses between WA and colorless cultivars 6044 or Kwang-Ai 3784 are of two kinds: colorless and colored. A greater segregation in plant stature, panicle shape, and grain characters was observed as well. The phenomena indicated that the WA plant probably was a natural hybrid. In 1972 the meiotic division of the pollen mother cell of the WA plant was studied and some abnormal chromosome behaviors usually observed in interspecific hybrids were revealed. In addition, in 1973 plants from selfed WA segregated very widely in many characteristics and plant types -- from typical wild rice to cultivated types. It is therefore evident that the original WA plant was a natural hybrid between wild rice (♀) and a native rice cultivar (♂).

The methods of breeding WA type MS lines and their respective maintainers could be divided into two steps. First, many test crosses were made to find particular varieties that had excellent maintaining capacity. Among 731 Hsien varieties tested in 1974, 624 (85.3%) had good maintaining capacity and 18 (2.5%) had partial maintaining capacity. All the 345 Keng varieties tested exhibited maintaining capacity. Second, among the hybrid progenies, the completely MS plants, with characters inclined to the male parent, were used as the female parent and were crossed back to their respective original male parent varieties. Usually four or five backcrosses were sufficient to develop a MS line with acceptable uniformity. The breeding procedure for MS line Er-Chiu-Nan 1 is shown in the diagram on the next page.

Up to 1978, more than 100 WA type MS lines had been developed. The early MS lines, such as Zhen-Shan 97A, Er-Chiu-Nan 1A, and others, have been backcrossed 18 to 20 times. Their anthers are empty and slender, milky white or yellowish, and contain aberrant abortive pollens and a small number of rounded pollens

The breeding procedure for MS line Er-Chiu-Nan 1

Explanation			*Crossing date*
	Female parent WA	× Male parent 6044	Mar 1971
Among 18 plants, a completely MS plant with suitable characteristics was selected as the female parent	F_1	× Er-Chiu-Nan 1	Dec 1971
Among four completely MS plants, a plant characteristic of the male parent was selected ..	F_1	× Er-Chiu-Nan 1	Jun 1972
Among 12 completely MS plants, 3 plants were selected	B_1	× Er-Chiu-Nan 1	Oct 1972
Among 65 completely MS plants from 3 families, one family with suitable characteristics was selected	B_2	× Er-Chiu-Nan 1	Feb 1973
Among 6,177 plants from 20 families with 99% completely MS plants, 12 families and 3,500 plants were closely similar to the male parent in characters	B_3	× Er-Chiu-Nan 1	Jun 1973
All 3,000 plants from 10 families were completely MS and uniformly characteristic of the male parent	B_4	× Er-Chiu-Nan 1	Sep 1973



2. An example of an early male sterile line (right) and a maintaining line developed through backcrossing (left).

unstained by I-KI solution (Fig. 2). Cytological observations showed that most of the microspores become abortive in the one-nucleus stage and a few of them degenerate in the two-nuclei stage. These lines were influenced less by the environment and retain MS stability. With few exceptions, the MS lines of Hsien varieties flower normally. Most Keng MS lines flower abnormally; sometimes the florets do not open at all. Usually in the dwarf MS lines 20-25% of the panicle remains enclosed within the sheath of the flag leaf. This incomplete emergence results in lower yields of hybrid seeds.

Three methods have been used to develop restorers.

Screening. In screening for restorers many Hsien rice cultivars have been used in the experimental crosses. Results up to 1974 showed that about 12.2% of the varieties tested possessed various degrees of restoring ability. Only 24 varieties (3.3% of the varieties tested) possessed strong restoring ability, i.e. their hybrid plants were normal in seed-set. As a rule, the distribution of restorer (R) genes is somewhat related to origin and maturation of the varieties tested. The R gene frequency is high in Hsien late-maturing varieties from lower latitudes,

low in the medium-maturing varieties, and very low in early maturing varieties from higher latitudes. The R gene has not been found in any Keng variety.

Transferring. Restorer genes may be transferred to any desired variety or type by cross breeding. For example, in Sinkiang Autonomous Region, Peking 300 (Keng) was crossed with IR24 to breed restorers of Keng type. In hybrid progenies, in which selection was combined with test crossing for tracing the R gene, four restorers of Keng type have been successfully developed in F_8.

Homocytoplasmic selection. Restorers could be easily developed from the progenies of MS lines x restorers. Restorers such as Tong-Fei 601, Chang 24, and others were bred in this way. All of them have the same cytoplasmic constitution as the MS lines.

Three lines of other MS types

Within 2-3 years after the three lines of WA type were developed, some other MS types were found.

Gambiaca type of Hsien x Hsien. In Szechwan province, the MS lines of Hsien type such as Chao-Yang 1A and others have been developed by using the late-maturing Hsien variety (Gambiaca) from Africa as the female parent. By test crossing, some restorers were selected from rice variety collections. Several promising hybrid combinations were released to farmers in Szechwan province. They were grown on several thousand hectares in 1978.

Tien type of Hsien x Keng. In Yunan province, the Keng MS lines such as Toride 1A and others were developed by using the Hsien variety 0-Shan-Ta-Bai as the female parent. Some restorers were selected from the progenies of Hsien x Keng crosses. Yield tests of some promising combinations are under way.

Wild rice x cultivated rice. Several types of MS lines have been developed by using various wild rices as the female parent. For example, the Hong-Lien type was derived from the cross between wild red-awned rice (spontanea) and Lien-Tong-Tsao (Hsien), the Liu-type from the Liu-Chew wild rice (spontanea) and Zhen-Shan 97 (Hsien) cross, and wild-Keng type from the cross between red-awned wild rice (spontanea) and Nan-Ken 6.

From Boro Taichung 65 type introduced from Japan, MS lines Reimei, Honishiki, and others were derived by the usual transferring procedure. The restorers C55 and C57 were derived from

a triple cross between Hsien and Keng (IR8 x Ko—Ching No. 3→ F_1 x King Ying 35). The area planted to hybrid rice of this type in Liaoning province was more than 1,000 ha in 1978.

Three basic groups of MS lines are used in China; they are classified according to the relation between restorers and maintainers and their genetic properties.

Group I - The WA is typical of group I. Gambiaca type and some types of MS lines derived from wild rice x cultivated rice are included in this group. The relation between their restorers and maintainers, their pollen abortion process, and the morphology of aborted pollens differ only slightly from those of WA type. The function of MS genes is sporophytic.

Group II - The Boro Taichung 65 is typical of group II. The Tien type in this group is gametophytic sterile. Microspores abort at approximately the two-nuclei stage. Pollens are spherical and stainable with I-KI solution but unable to germinate. The flowering habit is good. Data from test crosses show that the restoration spectrum of the BT type is wider than that of the WA type. But most restorers exist in Hsien rice, and only a few in Keng. It is easy to transfer MS from BT types to Keng varieties, but difficult with Hsien varieties. We have not yet been able to transfer Hsien rice to a satisfactory MS line of this type.

Group III - The Hong-Lien typifies group III. Microspores abort during the two-nuclei stage. Pollens are spherical and unstainable with iodine. In Hsien rice the relation between restorers and maintainers is contrary to that in the WA type. For example, the maintainers of WA type, such as Zhen-Shan 97 and Er-Chiu-Ai 4, become restorers to the MS lines of Hong-Lien type; the strong restorers of WA type, such as Tai-Yin 1, are good maintainers of the MS Lines of this type.

IDENTIFICATION OF RICE HETEROSIS

Hybrid combinations

Taking the standard variety and parents as control, 87 hybrid combinations were tested in the field by the Hunan Academy of Agricultural Sciences from 1972 to 1975. The most promising hybrid combinations selected and released to farmers were Nan-You 2 (Er-Chiu-Nan 1 A x IR24), Nan-You 6 (Er-Chiu-Nan 1 A x IR26), Shan-You 2 (Zhen-Shan 97A x IR24), Shan-You 6 (Zhen-Shan 97A x IR26), and Wei-You 6 (V 20 A x IR26). The yields of these hybrids in large scale production were 20-30% more than those of the conventional varieties. In Kiangsu province on

about 460,000 ha in 1978, the average yield was nearly 6,700 kg/ha, an average increase of about 1,500 kg/ha. The same year in Fukien province on more than 530,000 ha (nearly 1/3 of the province's total rice area), the average yield increase over conventional varieties was more than 750 kg/ha. In Hunan province, the yield of the second crop in a rice double-cropping system before 1977 was usually about 2,250 kg/ha. Then in 1977 and 1978, the second crop conventional varieties on about 1.1 million ha were replaced by hybrid rice. The average grain yield was more than 3,750 kg/ha, and yield records as high as 11,250 kg/ha were reported in many places throughout the country.

The performance and agronomic characters of the hybrid rice Nan-You 2 and its parents are listed in Table 1.

Heterosis

Vigorous vegetative growth. Hybrid rice exhibits vigorous vegetative growth. Its tillering ability, both in the seedbed and after transplanting, and growth rate are better than those of the parents. Thus, it is easy to raise luxuriant crops of hybrid rice and achieve consistent yield increases over large areas. The seeding rate is greatly reduced too. Only 15-22.5 kg hybrid seeds/ha is used, while conventional varieties need about 3-5 times that amount.

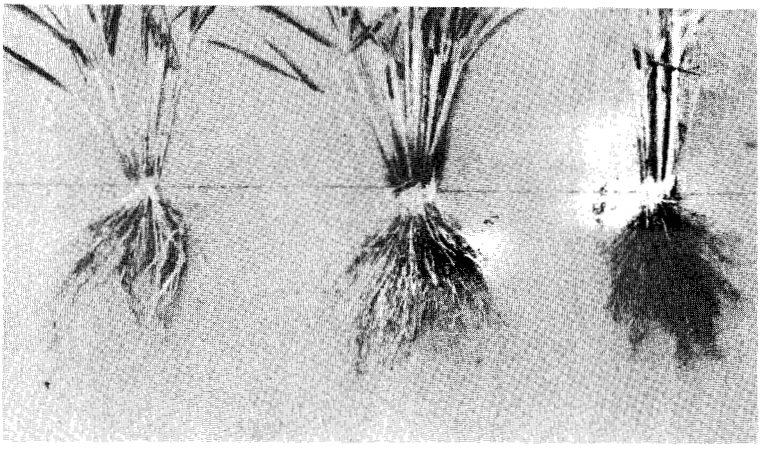
Root system. The root habits of hybrid rice were superior to those of the parent varieties in terms of penetration rate, depth and width of the root sphere, number of adventitious roots per plant (Fig. 3), and quantity of root fibrils for each growth period. To produce 7,500 kg grain/ha, the hybrid rice absorbed less nitrogen and phosphorus than the conventional varieties; however, hybrid rice absorbed more potassium. Because of its vigorous root system, hybrid rice was more lodging resistant and drought tolerant than the parents.

Panicles. In high yielding hybrid rice there are about 200 grains/panicle at a population density of 2.7-3 million panicles/ha. In ordinary varieties, the increase in panicle number per unit area is usually accompanied by a decrease in the number of grains per panicle. The ability of hybrid rice to maintain panicle size as population density increases is an important expression of heterosis.

Adaptability. Most of Hsien hybrid rices cultivated in China are crosses between the early-maturing varieties in the Yangtze Valley and medium-maturing ones introduced from

Table 1. The performance and agronomic characters of hybrid Nan-You 2 and its parents.

	Growth duration (days)	Culm ht (cm)	Panicles (1000/ ha)	Grains (no./ panicle)	Rate of empty grain (%)	1000-kernel wt (g)	Grain yield (t/ha)
Er-Chiu-Nan 1	105	67.5	307.5	91.8	31.8	27.0	4.9
IR24	136	92.2	281.3	140.5	9.1	21.6	8.7
Nan-You 2	134	101.4	277.5	176.5	15.9	26.0	10.1



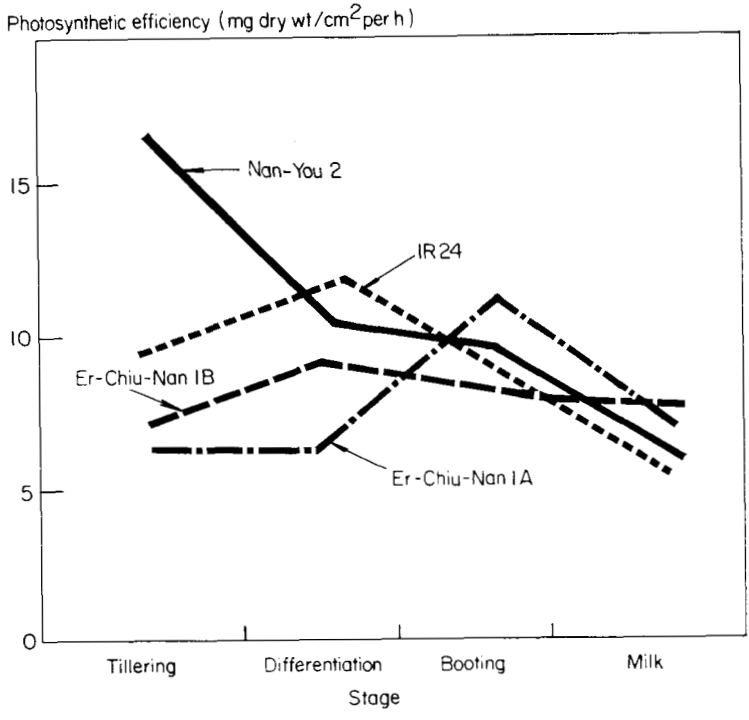
3. Comparison of the root systems of a male sterile plant (left), an F_1 progeny (center), and a restorer plant (right).

Southeast Asia. These hybrid rices are photoperiod insensitive and adapt well to day length and temperature variations. Some hybrid combinations are adaptable from Hainan island at 18°N latitude to the north of Kiangsu province at 34°N. The hybrid rice grows especially well in southern mountainous regions at elevations ranging from 500 to 1,200 m, with considerable grain yield increase. It grows well too in soil soaked with cold underground water, deep muddy soil, and saline–alkali soil.

Grain quality. The table quality and swelling ratio of hybrid rice are intermediate between the parent varieties. The protein in hybrid rice grains is about 9—11%, and Nan—You 2 contains 11.48% protein.

Heterosis in physiological and biochemical characteristics

Photosynthetic function. The photosynthetic efficiency of hybrid rice was higher than that of the parents during most of the growth period. The photosynthetic efficiency of Nan—You 2 in the tillering stage was significantly higher than that of male parent IR24, then decreased rapidly until at the milk stage it approached that of the male parent (Fig. 4). The green leaf area of hybrid rice increased quite rapidly at early growth stage, then reached its peak at the full heading stage. In general, the green leaf area of hybrid rice was larger at every growth stage than that of its parents. These facts clearly indicated that the hybrid rice has heterosis expressed in photosynthetic efficiency.

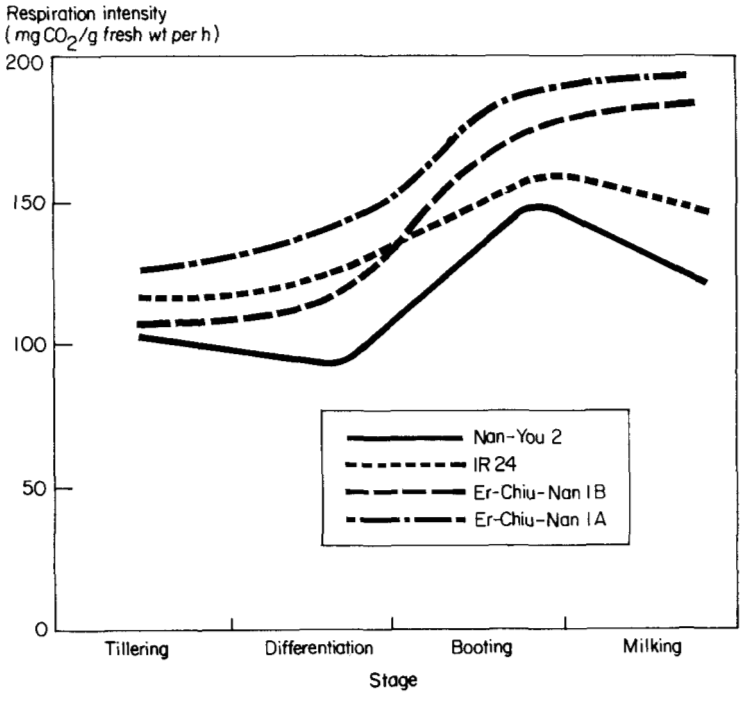


4. The photosynthetic efficiencies of a hybrid rice and its three parental lines.

Respiratory function. The respiration intensity of the hybrid rice Nan You 2 was lower than that of any of its three parental lines. Its respiration intensity curve was similar to that of the restorer, suggesting that hybrid rice is influenced by the male parent (Fig. 5). Heterosis for respiratory function was evidently negative. However, studies of mitochondrial activity revealed that hybrid rices with strong heterosis were usually accompanied with high mitochondrial activity. Male parents with strong restoring and combining abilities also had high mitochondrial activity.

Photorespiration intensity of hybrid rice was lower than that of its three parental lines at the beginning of the differentiation stage. The photorespiration intensity of the hybrid rice was even lower than that of the ordinary high-yielding varieties, which had lower photorespiration.

Root activity. The rooting ability of the rice seedling was measured by water culture. The rooting ability of hybrid rice (as expressed in the number of newly emerged roots, root length, and root dry weight) was generally higher than that of the parents or ordinary varieties. The root number of hybrid Nan-You 3 was twice that of the control Guang-Xian 3 (Table 2). The root system of the hybrid rice developed rapidly after transplanting, enhancing vegetative growth.



5. The respiration intensities of a hybrid rice and its three parental lines.

Root system activity was measured by the exudation pressure (Table 3) and the α-naphthylamine oxidation value (Table 4). The root activity of hybrid rice was generally higher than that of the three parental lines from tillering to the heading stage. It was especially higher during the booting stage. However, it was no higher than that of the best conventional varieties after heading.

Table 2. Rooting ability of hybrid rice.

	Nan-You 3 (hybrid)	Guang-Xian 3 (control)
Root length (cm)	48.4	45.8
Roots per plant (no.)	18.2	9.5
Root dry weight (mg/plant)	16.7	12.4

Table 3. Exudation pressure at different growth stages of hybrid rice Hua-Ai 158/5350 and its three parental lines.

	Exudation pressure (mg/plant per hour)				
	Tillering	Booting	Heading	Filling	Ripening
Hua-Ai 15A	81	1187	137	937	414
Hua-Ai 15B	74	1081	721	754	238
Hua-Ai 15A/5350	89	1616	1159	832	253
5350	68	1399	1048	1425	264

Isoenzyme spectra. Isospectra studies of peroxidase of hybrid rice and parental lines showed that hybrids with good heterosis might exhibit one of three characteristics in the iso-peroxidase spectra (Fig. 6):

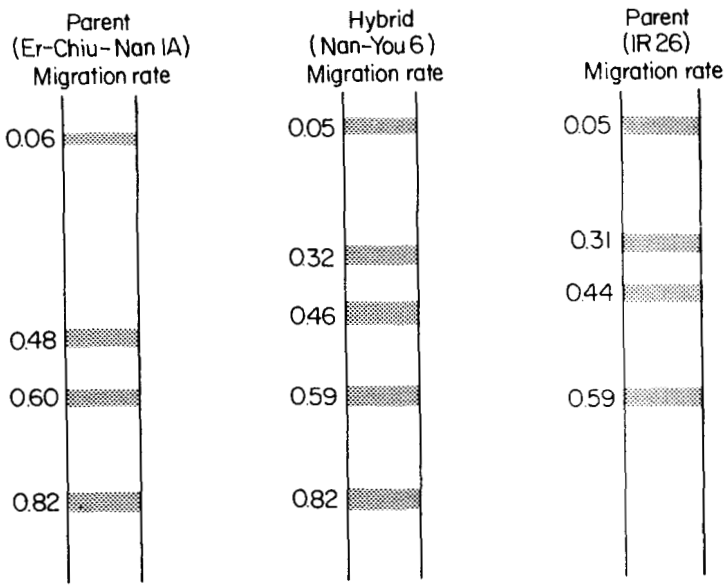
1) the bands of both parents,
2) new band or bands, or
3) band zone of high activity.

TECHNIQUE OF HYBRID SEED PRODUCTION

Through several years of experimentation and practice the following techniques have been adopted in the mass production of hybrid seeds.

Table 4. α-naphthylamine oxidation value at different growth stages of hybrid rice Hua-Ai 15A/5350 and its three parental lines.

	α-naphthylamine oxidation value (mg/fresh wt g per hour)				
	Tillering	Booting	Heading	Filling	Ripening
Hua-Ai 15A	24.4	55.8	42.5	49.5	19.4
Hua-Ai 15B	32.5	61.3	55.0	33.5	49.4
Hua-Ai 15A/5350	31.3	93.0	51.4	21.4	5.5
5350	12.6	32.5	46.3	36.9	18.2



6. The iso-peroxidase spectra of a hybrid rice and its parents.

Synchronization of heading stage

Most of the parents used in producing hybrid rice have quite different growth durations. To synchronize the heading stage, the sowing date of the early-maturing parent should be carefully adjusted to the leaf age of the late-maturing parent. For example, the early female parent of Nan-You 2 should be sown when the late male parent is at a growth stage of 10.5 leaves. To provide a few days of pollen supply, the late male parent might be sown twice; the second sowing in this case might be arranged when the leaf average of the first-sown male parent is 2.5.

Row ratio and row direction

In 1975, row ratio tests showed that within the range of 1 male and 1 female to 2 male and 8 female rows, yields of hybrid seed increased proportionately as the row ratio of the female parent increased. The yield at a row ratio of 2:8 was 3 times the yield at a row ratio of 1:1. A row ratio of 1:6 is now widely used in seed production. However, a row ratio of 1:8 has also been used, and a yield of more than 3,000 kg/ha has been recorded.

7. View of hybrid rice seed field in Hunan Province.

The design of hybrid seed fields is shown in Figure 7. An east-west row direction was adopted so that both parents received an equal amount of sunlight. Moreover, this row direction is nearly perpendicular to the direction of prevailing winds at flowering and facilitates cross pollination.

Leaf cutting

The flag leaves of the female parent are the main obstacle to cross pollination. Trials in 1975 showed that in plots with all flag leaves cut back, 42.9% more hybrid seeds were produced than in the check plots; plots with flag leaves cut back by about 1/3 from the top yielded 25.3% more seed than the check plots.

Leaf cutting usually causes the female parent to bloom earlier each day, thus enhancing seed-set.

Generally, leaf cutting is undertaken at the beginning of the heading stage. The blade of the male parent flag leaf is cut back 2/3 from the top, and that of the female parent is wholly removed. Leaf cutting affects the 1,000 kernel weight slightly.

The basal part of the panicle of the MS lines of WA type usually cannot emerge from the leaf sheath. This defect is remedied by one or two applications of 20 ppm gibberellin at the initial heading stage.

Supplementary pollination

Trials in 1975 showed that supplementary pollination increased the seed—set of the female parent by 50% more than that of the check. Supplementary pollination should be carried out at 20-30 minute intervals 3 to 5 times daily on calm days. Generally, we use the rope-pulling method for supplementary pollination.

Isolation

Pollen spread trials during the summer of 1975 on small plots isolated from pollen sources by distances of 10, 20, and 30 m received 5.2, 1.0, and 0.2% contamination, respectively. There was no contamination at distances of more than 40 m. Distances of at least 100 m are maintained between isolated hybrid seed fields and common rice fields.

PROSPECTS AND PROBLEMS

Although. the utilization of hybrid rice is just beginning in China, the future is very bright. There are plenty of paddy fields where the potential of the hybrid rice could be realized. However, plant defects have to be corrected and problems have to be studied.

Improving plant stature

First, while maintaining the present number of grains per panicle, researchers should raise the maximum number of panicles from about 3 to 3.75 million/ha. Next, the plant stature of the hybrid rice should be improved to increase the grain-straw ratio from 1:1 to 1:1.2, or higher.

Raising the seed-set rate

The seed-set rate of hybrid rice, particularly under unfavorable climatic conditions, is only about 80% -- less than that of the best conventional varieties. Recent combinations, with seed—set rate better than that of the ordinary superior varieties under the same conditions, indicate that the seed—set rate of hybrid rice can be further improved.

Insect and disease resistance

Several major insects and diseases -- bacterial blight, yellow dwarf, rice stem borer, brown planthopper, green leafhopper -- in the rice regions of south China reduce yields considerably. Most of the released hybrid rice combinations are resistant to only one or two insects and diseases. It is imperative that we select high yielding combinations that have multiple resistance against important insects and diseases.

Resistance characters controlled by dominant genes should be easy to combine in F_1 hybrids. For instance, Shan-You 6, a multiresistant hybrid extensively planted in Chekiang province, is a cross between the blast-resistant MS female parent and blight- and planthopper-resistant restorer male parent. All these three resistant traits are dominant.

Shortening growth duration

The present superior combinations of hybrid rice generally have too long growth durations to be considered as a first crop in the rice double-cropping system that prevails in the Yangtze Valley. The trait limits not only the potential planting area, but also the potential for increased yields in multicropping regions. We have exerted great efforts to solve the problem of breeding hybrid rice that is both early maturing and high yielding.

MALE STERILITY IN RICE AND ITS POTENTIAL USE IN BREEDING

J. N. RUTGER and C. SHINJO

Male sterility in rice has two principal applications: 1) to facilitate crossing in population improvement schemes, and 2) to produce hybrid rice. The first application depends mainly on genetic male sterility, and the second depends mainly on cytoplasmic—genetic systems. Both applications depend on cross—pollination, with modest amounts being acceptable for the first and massive amounts needed for the second. The hybrid rice application also requires heterosis for grain yield at levels above those of the best standard varieties.

MALE STERILITY TO FACILITATE CROSSING

Anyone who has made rice crosses can appreciate the need for ways to obtain more crosses with less effort. Genetic male steriles are potentially well suited for this use because they are relatively easy to remove from the population when no longer needed. Suneson's (1956) proposals for using genetic male sterility in composite crosses and evolutionary breeding methods are now well known (Fig. 1). Sorghum and soybean workers (Doggett 1972, Brim and Stuber 1973) have described the use of genetic male sterility in recurrent selection schemes (Fig. 2). Jensen (1978) noted that male sterile—facilitated recurrent selection procedures are useful in implementing diallel selective mating systems in wheat and barley. Fujimaki et al (1977) have discussed the use of rice male—sterile mutants for backcross breeding. Male steriles permit the breeder of self—pollinated crops to more easily utilize the repeated hybridization and selection cycles commonly employed in cross—pollinated crops.

Many genetic steriles have been reported in rice (literature to 1976 reviewed by Trees and Rutger 1978). Sterile plants are easily identified at maturity, and may be ratooned for additional tests. Male sterility can be due to pollen sterility, indehiscent anthers, anther abortion, pistilloidy of the anthers, or other causes. Trees and Rutger (1978) observed two spontaneous male steriles (Calady and Earlirose sources) that produced little or no stainable pollen, and two selections (Caloro and CS—M3sources) that produced

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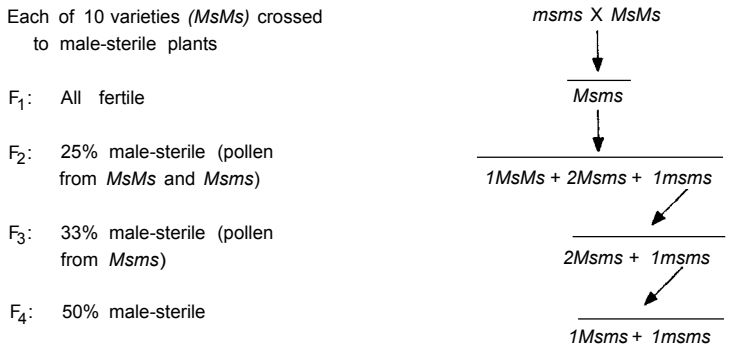
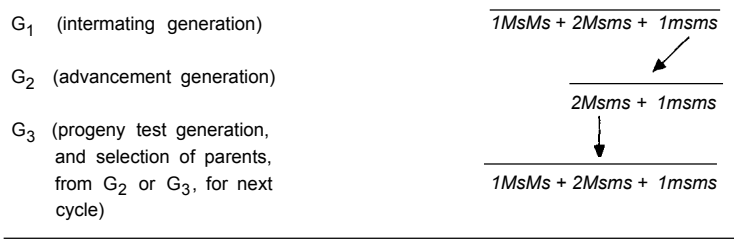


1. Utilization of genetic male sterility in forming composite crosses (Briggs and Knowles 1967).

stainable pollen but were functionally sterile because of morphological disorders. Each of the four sources was controlled by a single recessive gene (Table 1). Almost no outcrossing has been observed on the Calady source, which also seems to be partially female sterile, or on the Caloro and CS-M3 sources. When converted to short stature and planted in 25-m-wide rows alternating with tall pollinators, Earlirose male-sterile plants averaged 2.4% open-pollinated seed-set with 1 plant going to 11.3% in the semiarid California environment (Azzini and Rutger, unpubl. data). The above steriles were found by selecting completely sterile plants. In employing this rigid criterion, many partial steriles were observed but not saved. Current efforts are on selecting partially sterile plants in the hope that some are male-sterile plants exhibiting outcrossing. In this connection it may be useful to select for types in which florets are open for an



2. A possible recurrent selection cycle utilizing genetic male sterility (adapted from Brim and Stuber 1973).

Table 1. Inheritance and IKI pollen staining of four genetic male steriles in rice (adapted from Trees and Rutger 1978).

Cross	Observed F_2 segregation		P	IKI-stained pollen (%)	
	Fertile plants (no.)	Sterile plants (no.)	(3-1 ratio)	Fertile plants	Sterile plants
Calady ms/Calrose, etc.	372	114	0.25-0.50	98.6	0.0
Earlirose ms/Italica Livorno	452	161	0.25-0.50	95.5	0.8
Caloro ms/Italica Livorno	878	300	0.50-0.75	96.7	88.4
CS-M3 ms/CS-M3	547	185	0.75-0.90	99.5	95.0

extended period. Under California conditions, Tseng (1972) noted varietal differences in the time lapse between opening and closing of florets, but the open-floret period ranged only from 46 to 70 minutes.

Fujimaki et a1 (1977) reported some promising irradiation induced male steriles, under recessive gene control. Seed-set on sterile plants in segregating populations ranged from 1.7 to 8.3%.

Chemically induced sterility is also potentially useful in facilitating outcrossing, especially because any line can be used as a female. Perez et a1 (1973) found that Ethrel and RH531 caused high male sterility but also reduced female receptivity. In California RH531 caused high degrees of sterility, but outcrossing rates were negligible (Rutger, unpubl. data). Successful chemical emasculation with an agent containing primarily zinc methylarsenate has been reported in China, with seed propagation yields reaching 90-100 catties/mou (675-750 kg/ha) and seed purity as high as 91-98% (Anonymous 1978).

CYTOPLASMIC MALE STERILITY AND FERTILITY RESTORATION

In 1958, Katsuo and Mizushima obtained completely male-sterile plants in a first backcross progeny. In that cross, Chinese *Oryza sativa* f. *spontanea* was the nonrecurrent female parent and *O. sativa* cv. Fujisaka 5 was the recurrent male parent. No male sterile plants were observed in the reverse cross. They were the first researchers in the world to report male sterility conditioned by the interaction of cytoplasm and nuclear genes in rice having genome A. Subsequently, at least eight additional combinations of cytoplasmic male sterility in different varieties or species of rice have been reported (Table 2). Shinjo (1969, 1975) was the first to report the genetics of fertility restoration.

Male-sterile cytoplasm and fertility-restoring gene derived from Chinsurah Boro II

In 1959, Shinjo discovered a male-sterile cytoplasm and fertility-restoring gene in Chinsurah Boro II, a variety from India (Shinjo 1975). Both factors were introduced into a japonica variety, Taichung 65, by means of successive backcrosses. The male-sterile cytoplasm was symbolized by *cms-boro* and its fertility-restoring gene by *Rf1*.

When a plant with male sterile cytoplasm had nuclear genotype *Rf1Rf1*, it was completely male fertile (Table 3). A plant with sterile cytoplasm and nuclear genotype *Rf1rf1* was

Table 2. Male-sterile cytoplasm and nuclear donors.

Cytoplasmic donor		Nuclear donor		Effective	
Species	Strain or variety	Species	Variety	restorer	Reference
O. glaberrima		*O. sativa*	Calrose		Erickson (1969) Carnahan et al (1972)
O. perennis	Chinese strain	do	Fujisaka 5		Katsuo and Mizushima (1958)
do	Indian strain	do	Fujisaka 5		Katsuo and Mazushima (1958)
O. sativa	Chinsurah Boro II	do	Taichung 65	Rf_1	Shinjo and Omura (1966) Shinjo (1969)
do	Lead Rice	do	Fujisaka 5	Rf_1, Rf_2	Watanabe et a1 (1968) Shinjo and Watanabe (1977)
do	Bir-Co	do	Calrose		Erickson (1969) Carnahan et a1 (1972)
do	Taichung Native 1	do	Pankhari 203		Athwal and Virmani (1972)
do	Akebono	*O. glaberrima*	W0440	Rf_1	Yabuno (1977)
O. perennis ?	Wild rice	*O. sativa*	several	(yes)	Anonymous (1978)

50% pollen fertile although spikelet fertility was more than 90%. A plant with sterile cytoplasm and rf_1rf_1 was completely male sterile. These three genotypes showed no reduction in ovule fertility. Plants with normal cytoplasm, *n-boro*, were completely male fertile regardless of the nuclear genotype for fertility—restoring genes (Table 3). The F_1 plants of *(cms-boro)* rf_1rf_1 x *(n-boro)* Rf_1rf_1 segregated into partial pollen—fertile and completely pollen—sterile classes in a ratio of 1:1. The F_1 plants of *(cms-boro)* rf_1rf_1 x *(cms-boro)* Rf_1rf_1 did not segregate and all of the plants revealed only partial pollen fertility. The selfed progeny of *(cms-boro)* Rf_1rf_1 segregated into complete pollen—fertile and partial pollen—fertile classes in a ratio of 1:1. Thus in *(cms-boro)* Rf_1rf_1 plants, pollen grains with the recessive rf_1 *gene* apparently degenerate and do not participate in fertilization.

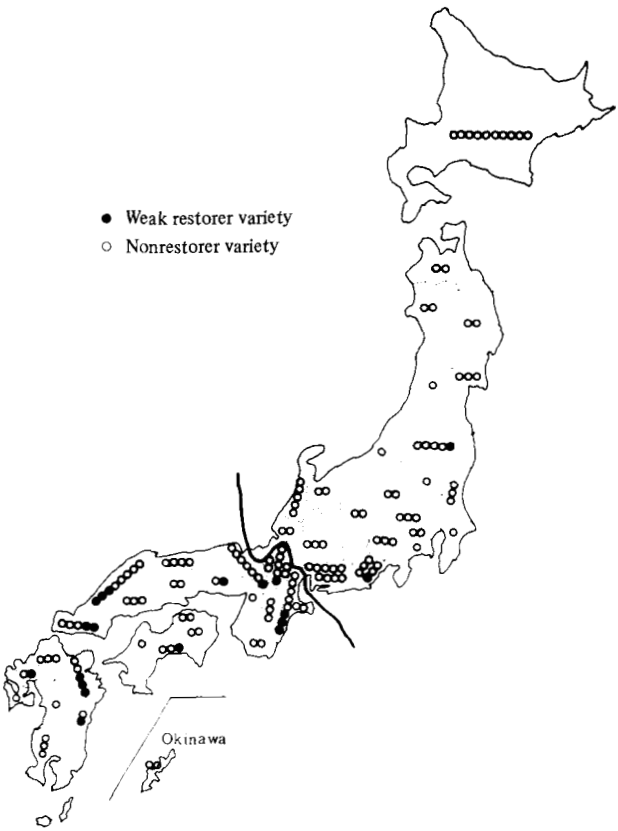
The recombination values among the three genes *fl*, *pgl*, and Rf_1 were calculated by conventional methods. The values were about 0.4% between *fl* and Rf_1, 12% between *pgl* and Rf_1, and 20% between *fl* and *pgl*. The arrangement order of the three genes is expected to be *pgl*-Rf_1-*fl*.

One hundred and fifty wetland-rice varieties of Japan were crossed with the male—sterile line to screen for fertility—restoring genes. Out of 150 F_1 progenies, 131 were completely male sterile, and the remaining 19 had partial pollen fertility ranging from 25 to 53%. However, the spikelet fertility of the 19 progenies was far lower, ranging from 2 to 12%. The 19 male parents were found to possess weak fertility—restoring gene(s).

Table 3. Genotypes of six lines and their fertilities. (Shinjo 1975).

Line	Genotype[a]	Pollen fertility (%)	Spikelet fertility (%)
A	*(cms-boro)* Rf_1rf_1	99.8	94.1
B	*(cms-boro)* Rf_1rf_1	49.6	93.5
C	*(cms-boro)* Rf_1rf_1	0.0	0.01
X	*(cms-boro)* Rf_1rf_1	99.9	94.1
Y	*(n-boro)* Rf_1rf_1	99.9	92.5
Z	*(n-boro)* Rf_1rf_1	99.8	91.6

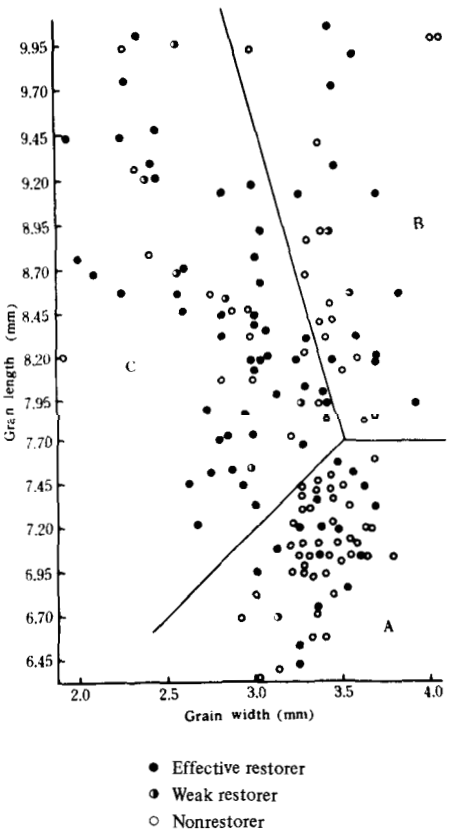
[a] *(cms-boro)* = male sterility—inducing cytoplasm derived from Chinsurah Boro II, *(n-boro)* = normal cytoplasm of Taichung 65, Rf_1 and rf_1 = dominant and recessive fertility—restoring genes.



3. Geographical distribution of weak restorer and nonrestorer varieties in Japanese wetland rice tested (Shinjo 1975).

Almost all the weak restorer varieties were concentrically distributed in the south of Japan (Fig. 3).

One hundred and fifty—three rice varieties native to different countries have been introduced into the University of the Ryukyus. The length and width of 10 unhulled grains of each variety were measured. They were then divided into three grain types -- A, B, and C -- by the method of Matsuo. Fifty—four out of 153 varieties tested were effective restorers, 28 were weak restorers, and the remaining 71 were nonrestorers (Fig. 4). The frequency of the restorer varieties in the three grain types A, B, and C was 8.5, 21.6, and 62.9%, respectively (Fig. 4).



4. Relationship between grain type and restoring gene in 153 foreign rice varieties tested by Matsuo's method (Shinjo 1975).

Distribution of mate-sterile cytoptasm in wild rice Oryza perennis

Oryza perennis was classified into four geographic plant forms by Dr. H. I. Oka and associates. About 200 strains of *O. perennis,* received from Dr. Oka, National Institute of Genetics, Japan, were crossed as the nonrecurrent female parent to 0. sativa cultivar, Taichung 65 as the recurrent male parent. Out of 130 different maternal progenies in the BC4 or more advanced generations, 62 had male-sterile cytoplasm, and the remaining 68 had normal cytoplasm (Table 4). In Asian and

Table 4. Distribution of normal and male—sterile cytoplasms in *Oryza perennis.*

Geographical plant form	Normal cytoplasm	Male—sterile cytoplasm	Strains (no.)
Asian	34	61	95
American	24	1	25
Oceanian	6	0	6
African	4	0	4
Total	68	62	130

American strains, the frequency of the male—sterile cytoplasm was about 64 and 4%. No male—sterile cytoplasm was found in the African and Oceanian strains.

HETEROSIS

The ultimate question about hybrid rice is "How much more do hybrids yield?". Several instances of significant heterosis in rice have been reported in the literature (summarized through 1975 by Davis and Rutger 1976). Virtually all published experiments to measure heterosis have been conducted with spaced transplants, with a single plant per hill. Populations usually have been small. Results are not directly applicable to the direct—seeded culture used in the USA, but may be applicable to transplanted rice. To be economically useful, the grain yield of the hybrid must significantly exceed that of the best check varieties available, which may or may not be the high yielding parent of the cross.

Experiments on heterosis conducted at Davis, California, are summarized in Table 5. Eleven of the 153 F_1 hybrids tested yielded significantly more grain than the best check variety in the experiments. Grain yield heterosis relative to the best check ranged from 16 to 63%, with an average of 41%. Contacts with hybrid corn seed producers in the USA indicate this frequency (11 of 153 combinations) and degree of heterosis (41%) would make the prospects of hybrid rice quite exciting, if sufficient hybrid seed could be produced. Another consideration that would affect use of F_1 hybrids in the USA would be grain and cooking quality. Present varieties have very specific quality factors. Unfortunately, all of the heterotic combinations so far observed have involved one or both parents with unacceptable quality.

Table 5. Summary of four experiments on heterosis in rice, conducted in 30- x 30-cm spacings at Davis, California, USA, 1971-76.

Experiment	F_1 hybrids (no.)	Number of heterotic combinations (significant at 5% probability level) relative to best check (BC) and high yield parent (HP)[a]							
		Grain yield		Tillers/plant		Seeds/tiller		100-seed wt	
		BC	HP	BC	HP	BC	HP	BC	HP
Davis and Rutger (1976)	41	2	10	0	6	0	3	0	1
7-parent diallel, 1971	21	8	9	4	4	-	-	-	-
9-parent diallel, 1974	36	1	3	0	0	6	2	12	1
11-parent diallel, 1976	55	0	16	0	5	28	8	27	5
Total	153	11[b]	38	4	15	34	13	39	7

[a]Blanks (-) indicate no data.

[b]Av best check (BC) heterosis for grain yield of these 11 hybrids was 41%.

HYBRID WHEAT AND BARLEY EXPERIENCES

It is interesting to examine progress in the development of hybrids in wheat and barley, two other highly self-pollinated cereals. In the USA, wheat and barley researchers have been pursuing hybrids 10-15 years longer than have rice workers.

Wheat

Following the discovery of cytoplasmic male sterility and fertility restorers, several hybrid wheat programs were launched in the early 1960s. Although expectations were high very little hybrid wheat is being grown. A recent article in the farm press summarized the situation: "Hybrid wheats: still looking for a home" (Curl 1978). There was a trace of hybrid wheat in the 1974 USA wheat variety survey; preliminary indications from the 1979 survey now under way are that there is still little hybrid wheat. Interviews with wheat breeders indicate two problems: 1) breeders using conventional methods continue to make good progress in raising the base yield level, and hybrid wheat breeders have had difficulty in catching up; and 2) hybrid seed production has been difficult. The production of hybrid seed involves three separate seed operations (Miller and Lucken 1976): 1) restorer increase, 2) male sterile increase, and 3) hybrid seed production. Restoration is complex, depending upon 2-4 fertility-restoring genes. Synchronization of pollination of the male with optimal floret opening of the female is a major limitation. Nevertheless, good hybrid seed yields are often obtained. Miller and Lucken (1976), for example, reported hybrid seed yields of 1,370 kg/ha in 1-1 ratio of male sterile to restorer, in drill strip widths ranging from 3.1 to 11.0 m. The fertile restorer line averaged 1,990 kg/ha, but this was apparently a low yielding line with low vigor and poor adaptability (Miller and Lucken 1976).

Barley

Ramage (1975) developed a scheme for hybrid barley production, using balanced tertiary trisomics. The system uses genetic male sterility. By appropriate genetic manipulations it is possible to develop populations that are largely male sterile, interplant these with normal male fertiles, and harvest 95-100% hybrid seed from the sterile rows. One public and several private hybrid barleys have been developed (Ramage 1975). A few thousand acres of F_1 hybrids have been grown in some years, but in general hybrid seed production has been complex.

CONCLUSIONS

Genetic male steriles are potentially useful for facilitating more crossing with less effort in population improvement schemes. Although steriles are readily found, either spontaneously or through induced mutation, outcrossing is frequently low. Attention should be given to selecting genetic steriles that exhibit considerable outcrossing. Chemically induced sterility is also potentially useful in facilitating outcrossing, particularly because any line could be used as a female.

Cytoplasmic male steriles plus restorers, the principal prerequisites for hybrid rice, are available from several sources. Restoration usually is under simple genetic control, and development of sterile, maintainer, and restorer lines is straightforward. Other prerequisites for hybrid rice are outcrossing in seed production fields, and grain yield heterosis significantly above that of the best standard varieties.

Experiences with hybrid wheat and barley indicate that hybrid seed production is a limiting step. The breeder contemplating the production of F_1 hybrid varieties in a normally self-pollinated crop must carefully weigh the advantages and disadvantages of hybrids for his particular situation.

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MUTATION BREEDING IN RICE

K. MIKAELSEN

Mutation breeding or the use of induced mutations in plant breeding has been a controversial issue for many years, but today I believe that we can discuss the topic objectively.

In recent years many successful results have been reported and a large number of mutants have been released as cultivars. Sigurbjornsson and Micke (1974) published a list of 98 crop varieties of mutant origin of which only 13 were rice. Now, more than 30 rice mutants (Appendix) have been released for practical cultivation. The question today, in my opinion is, not if mutation breeding shall be used but, how mutations can be used most appropriately in practical breeding programs. It cannot be stressed often enough that mutation techniques are only additional plant breeding tools. Crosses and hybridizations must be the basic methods for creating the variability that the breeder needs for his selections. Spontaneous and induced mutations can make valuable additions to such variability and give the breeder additional "raw material" from which to select.

A basic requirement in any breeding program is a clear definition of the breeding objectives. This is also a basic principle in the mutation breeding approach. Once plant breeders have defined their breeding objectives and assessed their variability sources, they are in a position to decide whether or not to induce mutations. This decision should be made after a full consideration of the available variation, its genetic nature (dominance or recessiveness, polygenic, gene complexes, etc.), the resources which could be devoted to the program, and the *efficiency* of selection that could be applied. Many breeders still say that there is no need for mutations as there is sufficient variability for most cultivated species, including rice, in the existing germplasm collections that has not been fully exploited. The exploration and use of this material are important in any long-term breeding program. The question I am raising for discussion is: "What is the role of mutations for changing or improving specific characters in existing well-adapted, highly productive varieties or genotypes?" I will give some examples of approaches that I think illustrate how the use of induced mutations can shorten the breeding process compared with conventional methods where certain genes have to

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he incorporated from more or less distant genetic sources. For characters where genetic variability is limited or lacking, mutation breeding methods are the only choice. The following sections discuss possible areas where the existing known variability needs to be widened.

INDUCTION OF MUTATIONS AND THEIR NATURE

The next step in the mutation breeding procedure is to produce desired variations. A large number of known mutagens, both physical and chemical, have shown high mutagenic effectiveness. In the International Atomic Energy Agency Seibersdorf Laboratory, methodological research for standardization of mutagen treatments of crop species, particularly cereals, has been carried out and clear guidelines have been established for mutagen treatments of rice (Mikaelsen et a1 1971, Osone and Mikaelsen 1971). X-rays, gamma rays, and neutrons are effective ionizing radiations for mutation induction. Ethylmethanesulfonate (EMS) is one of several chemical mutagens that could be recommended. The mutagenic effects of EMS and other alkylating agents probably are better understood than those of many other chemical mutagens and the treatment procedures are rather simple. It is important to find the dose(s) of mutagen treatments that give maximum mutation frequencies. In rice, like barley, the so-called chlorophyll mutations can be used to establish the optimal doses for seed treatment.

Because each of the recommended physical and chemical mutagens has produced most of the desirable mutant types, no single mutagen is preferred over any of the others. As a general rule, the mutagen which is most readily available is sufficient for practical application by plant breeders. The early dream of chemical mutagens that would recognize and specifically mutate particular genes has been dashed by our present understanding of the organization of genes as linear sequences of the same four DNA-bases. The chance of obtaining a chemical mutagen that will recognize the sequence of a structural gene appears remote. Induced mutations are regarded more and more as random occurrences throughout the genome, although there are reports that indicate this is not always so. Detailed analyses of a large number of mutants of the *erectoides* (Persson and Hagberg 1969) and the *eceriferum* (Lundquist et a1 1968) types of barley reveal locus specificity in response to different physical and chemical mutagens. Differences occurred in the relative frequencies of mutations at the various loci after treatment with different mutagens (gama, neutron, and chemical).

It is important to have some knowledge of the nature of the induced mutations for their proper use in plant breeding. They are basically similar to spontaneous mutations. Most induced mutations resulting from the mutagen treatments mentioned are *recessive* in nature and behave as true point mutations in crosses. Mutations occur in both qualitatively and quantitatively inherited characters. Induced mutations can generate useful variation in polygenic characters and, where appropriate selection has been applied to improve maturity time, plant height, and kernel (seed) size. Numerous other quantitative traits have been obtained from either drastic changes through mutations in major genes (macro-mutations) or small changes in minor genes (micro-mutations) .

Some other factors affecting induced variability should be recognized. Seldom is a mutation induced in a single gene without some other genetic changes occurring in the treated genotype. To obtain maximum mutation frequencies, relatively high doses of a mutagen are applied with the objective of mutating a specific gene or character. At such dose levels there is a high probability of mutating other genes or deleting groups of genes simultaneously. If selection then is applied only for a specific mutant phenotype, the selected mutant is very likely to be changed in a number of other characters at the same time and the changes may not always be in a desirable direction. It is important, therefore, that several mutants of the desired phenotype are selected in the segregative progeny populations after mutagen treatments. It is a fact that most induced mutations are deleterious to a well-adapted, economically important genotype, but when appropriate selection is applied useful mutants can be found. In barley Gustafsson (1965) tried to estimate the frequency of favorable mutants and found that 10% of the total number of mutations recorded were favorable genetic changes. From my experience, this estimate may be applicable also to rice as a modest average figure, which will depend on several factors that need not be discussed here.

The main point is that although existing effective mutagens can produce a high frequency of mutations, only a fraction of these mutations will be useful in practical plant breeding programs. The size of the plant populations from which the desirable mutants are to be selected must, therefore, be fairly high. In Tables 1 and 2 (from Manual in Mutation Breeding II) , some estimates of population sizes for various types and rates of mutations are listed. Induced dominant mutations are difficult to find: for a dominant mutation in a single gene, a very high population must be screened. This means that a large number of seeds must be irradiated or treated with chemical mutagens, and several thousand M_1 plants are needed for build-

Table 1. Number of cell progenies to be examined for various mutation rates and probabilities of occurrence.

Mutation frequency (u)	No. of cell progenies (n)		Applicable for (approx)
	P_1=0.90	P_1=0.99	
1×10^{-2}	233	465	Chromosome changes and quantitatively inherited variability
1×10^{-3}	2,326	4,652	Several recessive genes
1×10^{-4}	23,260	46,520	Single recessive gene
1×10^{-5}	232,600	465,200	Single dominant gene

ing up a progeny (M_2) population with sufficient variability for selection. Insufficient population sizes are responsible for many failures in mutation breeding.

For M_2 populations, various methods can be applied, depending on the breeding objectives. The methods are based on the pedigree method modified to account for the chimeric structure of the M_1 plants. In rice, as in other cereals, all the primary tillers can be used as they represent the maximum potential for induced genetic variability. Secondary tillers may only repeat the mutations found in the primary tillers. The M_2 material can be planted in panicle (tiller) progeny rows -

Table 2. M_2 family sizes for different segregation ratios and levels of probability of occurrence of the homozygous mutant.

Segregation ratio	M_2 family size	
	P_2 = 0.90	P_2 = 0.99
1/4	8.0	16.0
1/8	17.2	34.5
1/12	26.3	52.6
1/16	35.7	71.4
1/20	45.5	91.0

the panicle progeny method. Various bulk methods can also be applied - *single* or *multiple bulk methods.* Statistically, the bulk methods may give the highest probability for the appearance of a single mutant in the plant population sampled, but other considerations may determine the choice of method.

The induced mutations I have just discussed are the *gene* or *point* mutations. To complete the picture, I should mention that many mutagens, particularly ionizing radiations, are efficient in breaking chromosomes, and when the broken ends can restitute, many structural rearrangements of the chromosomes can occur. These so—called *chromosome mutations* are translocations, inversions, duplications, and deficiencies. Sears (1956) was the first to use induced alien translocations to transfer leaf—rust resistance from an *Aegilops umbellulata* chromosome to a wheat chromosome. Similar methods have been used since then by several geneticists to transfer disease resistance to wheat. I do not know of any example of practical significance in rice and only the future will show if such techniques will be of any use in rice breeding.

TYPES OF USEFUL MUTATIONS

Mutation studies with rice have been carried out for many years. The main results have been reviewed by Nayar (1965) and Gustafsson and Gadd (1966), but relatively few of importance for practical breeding have been reported. In recent years, successful induced mutations resulting in new varieties have been reported. Some results are presented here to demonstrate how the mutation techniques could be appropriately used in plant breeding. Many of these results come from research projects, which have been supported through technical assistance by the IAEA and the United Nations Development Programme (UNDP) under the supervision and guidance of staff from the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture. This Division also coordinated a research program on the use of induced mutations in rice breeding and production during the period 1965—77 with participants from 14 rice—growing countries. In 1977, a regional seminar on the use of nuclear techniques in rice research was convened in Jakarta, Indonesia, and recent achievements in mutation breeding were reviewed.

The kinds of useful variability that could be produced through mutations was a primary topic. Mutational changes in most important agronomic characters had been reported in literature.

Plant height

In terms of plant characters, changes in plant height have been one of the most common mutant types. The most important are those with shorter culms (dwarfs, semidwarfs). A large number of such short-statured mutants have been produced from local varieties in many countries. Most are caused by recessive major gene mutations. Some of the dwarf or semidwarf genes have been found allelic to the Dee-geo-woo-gen (TN1 and IR8) semidwarf locus, but several new loci have also been identified. It is surprising that these short-statured mutants which normally respond to high levels of nitrogen fertilizer and give satisfactory yields, have not been used to a larger extent in national programs either directly or indirectly in crosses. An excellent example is the short-statured mutant Calrose 76 selected and bred by Rutger and coworkers at Davis from the California cultivar Calrose.

Flowering and maturity time

Changes in flowering and maturing time are also plant characters that could be easily induced by mutation. The early-maturing mutants have obvious1y been of main practical interest. It is possible to shorten growth duration up to 30 days without sacrificing yield. Obviously, many major genes influence the flowering and maturity time in rice, and most of the early-maturing mutations are recessive and show monogenic inheritance. Several early-maturing mutants have been released directly as new varieties. An outstanding example is the early mutant of Basmati 70, which has been released for commercial use in Azad Kashmir under the name Kashmir-Basmati (Table 3). This mutant is being tested in other regions in

Table 3. Performance of mutant lines EF-29-1 (Kashmir Basmati) and EF-29-2 in varietal trials in Pakistan (3-yr av 1972-73 to 1974-75).

Variety or mutant	Days (no.) from seeding to flowering	Productive tillers (no./plant)	1,000-grain wt (g)	Grain yield (t/ha)
Basmati 370	125	25	18.7	3.0
EF-29-1	107	25	19.0	3.2
EF-29-2	108	26	18.7	2.9

Table 4. Results of yield trials with early-maturing mutant Nucleoryza and other cultivars in Hungary, 1973-75.

Year	Yield (t/ha)				
	Nucleoryza	Szarvas - slender control	Dubovskij 129	Av of 7 other varieties	LSD 5%
1973	5.40	6.05	6.00	5.40	6.6
1974	5.05	4.34	4.32	4.10	5.8
1975	5.39	4.07	-	4.36	7.1

Pakistan and will probably obtain wider distribution soon because it has maintained all the good attributes of the famous Basmati varieties. The early-maturing mutant Early-Cesariot (Mikaelsen et al 1971) was approved for commercial use in Hungary in 1975 under the name *Nucleoryza* (Table 4). It has excellent cold tolerance and field resistance to pests and other diseases. In the temperate climate where it is grown, it has given yields as high as those of wheat (in the same fields). When the price of rice is twice that of wheat, rice production has been attractive. Mention should also be made of the mutant varieties released in Bangladesh, IRATOM-24 and IRATOM-38, which are 20 and 30 days earlier than the original variety IR8. In Indonesia, a large number of high yielding, early-maturing mutants have been produced in Pelita I-1. Since 1975 some of these have been extensively tested by the Ministry of Agriculture in official trials throughout Indonesia, and may be released soon (Table 5) (Ismachin and Mikaelsen 1976).

Other plant characters

Changes in many other important plant characters have been reported. More tillering and increased panicle length and number are important for a productive plant type. Leaf size and shape could also be favorably altered. Grain characters are very important in rice and many improvements in grain size, shape, and shattering-resistance have been achieved. Changes in quality characters such as gluten content, protein content, and amylose content have resulted from induced mutations. Few examples exist of a new variety being released directly because of improvements in grain quality alone. Such specific improvements normally must be accompanied by some other advantages, such as increased grain yield. The main role of these particular mutations may lie in crossbreeding.

Table 5. Results of large-scale yield trials in Indonesia, 1974.

Cultivar or mutant	Yield (t/ha)		Days to
	Bogor	Sukamandi	maturity-
Pelita I-1	4.42	2.53	140-150
IR20	4.75	3.43	130-140
Mutants (47)	28 mutants >5.00	16 mutants > 4.00	110-125

Table 6. Yield performance and blast reaction of a mutant selection in multilocation yield trials in Thailand, 1971-76.

Cultivar or mutant	Yield (t/ha) Intra-station trial 1971	Yield (t/ha) Multi-location trials 1972-76	Blast reaction[a] 1971-76 8 stations
RD6 (mutant)	3.5	3.3	MR
KDML 105	2.8	2.7	S
NSPT	-	3.1	MS

[a]MR = moderately resistant, S = susceptible, MS = moderately susceptible.

Diseases and pests

Many attempts have been made to improve disease resistance in rice. Positive results, particularly for resistance to blast (*Pyricularia oryzae*) and bacterial leaf blight (*Xanthomonas oryzae*), have been reported from several countries. Results from Thailand (Khambanonda 1978) are an example. In 1977 the Department of Agriculture officially released the variety RD6, a radiation-induced glutinous mutant of the popular nonglutinous variety Khao Dawk Mali 105 (KDML 105). Selections in the M_2 populations were made for glutinous and blast-resistant mutants. One line combined these two traits and outyielded the original variety. In the advanced yield trials of 1972-76, this mutant ranked first in average yields and was superior to Niaw Sanpahtawng (NSPT), a popular recommended glutinous variety (Table 6). All the important characters of the original variety, such as photo-period sensitivity, good cooking and eating quality, aroma, and drought resistance were maintained in the mutant. The point is that the improvement in blast resistance, from susceptible to moderate, alone would probably not have been enough for recognition of the mutant as a new variety or even as genetic material for use in crosses. Breeding for disease resistance is complicated and I believe that improvements made by induced mutations, even small ones, could be better utilized.

Very little work has been reported so far on improved insect resistance by induced mutations. With the increasing importance of resistance to the brown planthopper (BPH) in Southeast Asia, work was begun a couple of years ago in Indonesia. Resistant

mutants have been selected from irradiated M_2 populations of Pelita. An early—maturing mutant, A23, one of the most promising lines in the official yield trials in recent years, has been irradiated and two BPH—resistant mutants have been selected. The material has been screened at the seedling stage in the greenhouse using the IRRI method. The results have been confirmed by the CRIA entomologists at Bogor. These mutants have been propagated for further testing by the Ministry of Agriculture. This important work has been carried out by Mr. Ismachin and Mr. Mugiono, supported by the UNDP/IAEA mutation breeding project.

THE USE OF INDUCED MUTATIONS IN CROSSBREEDING

Several mutation specialists have emphasized for many years that induced mutants could be of more practical importance if they were to be used in crossings and included as basic material in general plant breeding programs. The results with barley in Sweden provide evidence of the successful uses of mutants in crosses. A number of new varieties with mutant parentage have been released by the Swedish Seed Association, Svalov. The mutant variety Diamant from Czechoslovakia (CSSR) is another example of a mutant that has successfully been used as parent in crosses.

The use of mutants in crossbreeding is new in rice and an excellent approach has been demonstrated by Rutger and Peterson (1976) in California. The early maturing-mutant D18 from Calrose has been recombined with the short stature gene from Calrose 76 (the mutant variety) and the recessive gene for glabrous hulls from the variety CS-M3 to produce several early-maturing, short-statured, glabrous-hull lines being evaluated for possible future release. The newly released variety M7 originated in 1977 from such crosses. The induced short-statured early-maturing mutants from Calrose have the advantage of already being adapted to the California background and are much easier to use than the tropical sources of short stature. The transfer of short stature from tropical sources has been tried, but has been hampered by cold susceptibility and unsatisfactory cooking quality of the tropical donors.

CONCLUSION

I have given a short outline of mutation breeding methods and shown how they can contribute to crop improvement if they are correctly and intelligently applied. I have described how

optimal treatments with effective physical and chemical mutagens could create new variability from which desirable genotypes could be selected if specific and well-defined breeding objectives exist and proper selection methods are applied. Examples of rice mutants that have been officially approved and released directly for commercial production in several countries have been given. The potential of using mutants in crossbreeding was also discussed and at least seven new rice varieties which have mutant parents are known.

I believe that I am justified in saying that mutation breeding techniques have proven to be effective additional tools in plant breeding programs. These techniques have been most successfully used by ordinary, conventional breeders. There is no need to develop mutation breeding specialists to carry out mutation breeding work.

It has been stated often that mutation breeding techniques are sophisticated and should not be encouraged in developing countries. Because many of these countries have limited adapted genetic resources for building up good breeding materials, induced mutations may be a useful source of variability, as shown by the case in California and Hungary.

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Appendix: Mutant rice varieties.

KT 20-74	China 1957
SH 30-21	China 1957
YH 1	China 1963 (cross)
Reimei	Japan 1966
Jagannath (BBS-873)	India 1969
Delta	France 1970
BPI-121-407	Philippines 1970
Parc-1	Philippines 1970
Milyang 10	Korea 1970
IRATOM 38	Bangladesh 1971
IRATOM 24	Bangladesh 1971
IIT 48	India 1972
IIT 60	India
Nucleoryza	Hungary 1972
Mutsuhonami (Fukei No. 90)	Japan 1973 (cross)
Parc-2	Philippines 1973
Hybrid mutant 5	India 1973
Yeng-hain Taipai 45 (YHTP)	China
Fulgante	Italy 1973
Calrose 76	USA 1976
RD6	Thailand 1977
Kashmir Basmati	Pakistan 1977
Fujihikari	Japan 1977 (cross)
Hayahikari	Japan 1976 (cross)
Kagahikari	Japan 1973 (cross)
IRAT 13	Ivory Coast
Akihikari	Japan 1976 (cross 1968)
Shwe War Tun	Burma 1975
RD15	Thailand 1978
M7	USA 1977 (cross 1972)

TISSUE CULTURE IN RICE IMPROVEMENT: AN OVERVIEW

R. S. CHALEFF

Advances in the techniques of cell and tissue culture are making possible the regeneration of plants from the cultured cells of an increasing number of important crop species. We now are able to consider applying such methods to the genetic improvement of crops. Because the utility of cell culture as a breeding method depends upon the stage of development of the technique, we should first become acquainted with the present state-of-the-art for rice. Rice callus can be induced by plating surface-sterilized seed on a chemically defined nutrient medium supplemented with 2,4-D (Yamada et al 1967). The transfer of the callus to a medium lacking auxin promotes the differentiation of shoots and roots (Nishi et al 1968). However, the weakness of this procedure is that the capacity to regenerate plants (morphogenetic capacity) from rice callus diminishes rapidly with continued propagation. By the fifth passage shoot formation can no longer be induced from the callus (Nishi et al 1968). This rapid loss of morphogenetic capacity severely restricts the usefulness of callus cultures for genetic experimentation.

Callus can also be obtained from microspore cells by plating immature rice anthers on a simple medium supplemented with either 2,4-D or naphthalene acetic acid (NAA). Although many of the plants regenerated from microspore-derived callus are haploid, their ploidies will vary because of mitotic abnormalities that occur during callus proliferation (Niizeki and Oono 1971, Sunderland 1973). However, such nonhaploid plants are completely homozygous because they are produced by multiplication of a single haploid (gametic) genome. Regenerated plants that are haploid can be diploidized by treatment with colchicine (Woo and Su 1975).

INFLUENCE OF GENOTYPE ON CALLUS FORMATION

Three difficulties were encountered with the anther culture system: 1) the morphogenetic capacity of callus cultures was lost rapidly during continued culture, 2) only a small portion of cultured anthers gave rise to callus, and 3) many of the regenerated plants were albino (Ching-chu et al 1974, Chaleff

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et a1 1975, Oono 1975). But additional experiments showed that the frequency of callus formation and of albino plants depended more upon the particular rice genotype used than upon the conditions of culture.

Thus, Guha—Mukherjee (1973) reported that whereas anthers from some indica and japonica varieties did not respond to culture, callus formation occurred in 26% of the anthers of Assam 5 that were cultured. Similarly, the frequency of callus formation of Norin 20 was consistently three to four times higher than that of the variety Cigalon whether 2,4—D, NAA, or IAA was used as auxin (Fouletier 1974).

In a study of 10 japonica varieties and several F_1 hybrids, Oono (1975) found that the frequency of callus formation varied from 0.5 to 8.0%. The results obtained by Chen and Lin (1976) with 5 japonica and 7 indica varieties suggest that anthers of japonica varieties are more responsive to culture (44.7% of cultured anthers of 1 variety formed callus) than those of indica varieties (the highest frequency being 8.9%).

The dominant influence of plant genotype also is seen in my laboratory where initial studies were conducted with the variety Minehikari on the effect of the composition of the culture medium on the frequency of callus formation. The results indicate that, although the incorporation of certain compounds into the culture medium is essential, their concentrations are not critical. Thus, it is known that anthers will not form callus in the absence of sucrose, but the results in Table 1 show that the amount of sucrose in the medium is not important. Similarly, the data presented in Table 2 demonstrate that NAA is required for a high frequency of callus formation, but that frequency is not influenced significantly by the concentration of auxin.

Table 1. Effect of sucrose concentration on frequency of callus formation.

Sucrose concn (%)	Anthers plated (no.)	Anthers forming callus	
		No.	%
1	360	128	35.6
2	590	207	35.1
3	838	309	36.9
4	789	344	43.6
5	273	97	35.5
6	838	337	40.2

Table 2. Effect of NAA concentration on frequency of callus formation.

NAA concn (mg/liter)	Anthers plated (no.)	Anthers forming callus	
		No.	%
0	59	9	15.3
1	61	27	44.3
2	362	166	45.9
5	677	298	44.0
6	57	20	35.1

Different results were obtained when the amount and form of nitrogen in the medium were varied. Chih-ching et al (1975) reported that the relative amounts of nitrate and ammonium influence callus formation frequency and that a medium low in ammonium concentration is optimal. Nevertheless, it is evident that the genotype is a principal factor in determining whether rice anthers will respond to culture.

Genotype primarily determines the proportion of regenerated plants that are albino. Oono (1975) showed that callus cultures of some cultivars yielded predominantly albino plants, whereas many green photoautotrophic plants were obtained from other cultivars, including Minehikari. In my laboratory, only a small number of green plants could be obtained from calluses of the indica variety Dunghum shali and all attempts to increase the proportion were unsuccessful. Variations in light intensity and spectrum, iron and sucrose concentration, and temperature were tried, and chlorophyll precursors were added to the medium to no avail. A dramatic demonstration of genotype influence was observed in experiments with Minehikari. Under the same conditions that yielded only 5% green plants from callus of Dunghum shali, more than 90% of the plants regenerated from Minehikari callus were green (Chaleff, unpubl. data).

Thus, by culturing anthers of the variety Minehikari we have realized an ideal experimental system that permits the efficient production of haploid calluses from which a high proportion of photosynthetically competent plants can be regenerated. But because the frequency of callus and green plant formation are dependent on plant genotype, cell culture techniques probably cannot be applied successfully to the more important rice cultivars grown now. It follows then that in the development of anther culture as a breeding technique, the

emphasis should be on selecting responsive varieties rather than on defining culture media that enhance response. Once this approach is adopted, germplasm collections can be screened for both responsiveness of anthers to culture and frequency of regeneration of green plants, and these capabilities can be crossed into cultivated varieties. Now let us consider specific applications of cell culture to rice improvement.

HOMOZYGOUS DIPLOIDS

One feature of anther culture that has attracted plant breeders is the ability to produce homozygous diploid plants directly from meiotic segregants. In contrast, the attainment of homozygosity in conventional pedigree and backcross breeding programs might require five to six generations. But continued selection and recombination of genetic material in these successive generations are advantages of the conventional method that are not offered by anther culture. For example, incorporation of a single desirable gene into a superior cultivar by a backcross breeding program would require that, after the initial cross, repeated crosses to the recurrent parent be made and the final generation be selfed to produce a homozygous individual.

If, on the other hand, anthers of the F_1 plant are cultured, homozygous diploids can be obtained directly from the gametes and one only needs to identify an individual that possesses 11 chromosomes from the superior cultivar and the 1 chromosome from the other parent that carries the desirable gene. If deleterious genes are linked to the desirable one, it becomes necessary to perform additional crosses and screen for progeny from which the deleterious genes have been eliminated by recombination.

At this point the advantages of anther culture as a breeding technique become less apparent. The approach of anther culture could be very useful when extensive recombination is not crucial. For example, in combining the desirable traits of two acceptable cultivars possessing complementing characteristics (such as japonica x indica) , a new genotype could be stabilized immediately by anther culture, thus precluding segregation in successive generations.

INDUCTION AND SELECTION OF MUTANTS

A potential application of cell culture that is most exciting is in the generation and selection of new alleles. The ability to culture large populations of cells on a defined medium makes available an enormous pool of genetic variability from which certain desirable mutants can be selected directly. By treat-

ment of the cells with a chemical or physical mutagen, this variability can be increased even further. Selection is accomplished by incorporating into the nutrient medium an agent that is toxic to the normal cells but not to the desired mutant. Thus, cell culture offers two distinct advantages: 1) providing genetic variability that is not available in world germplasm collections, and 2) permitting direct selection for a specific desired type. In mutant selection experiments, callus derived from anther culture is preferable to that obtained from other organs (e.g. roots) for several reasons. First, because the callus formed from the microspore cells is haploid, recessive characteristics will be expressed immediately in culture. Second, the microspores represent a population of discrete cells upon which selective growth conditions can be imposed with minimal interference from other members of a genetically heterogeneous, multicellular aggregate. Third, the calluses derived from microspores represent clones from which plants can be regenerated immediately. If multicellular aggregates are used for selection, segregation of a mutant clone would require many passages on a selective medium. By the time a clone is established the callus will have lost the capacity for differentiation.

RICE IMPROVEMENT THROUGH CELL CULTURE

A severe limitation of cell culture is that it can be applied only to select for alterations of plant functions that are expressed at the cellular level. At present, it is difficult to imagine how traits dependent upon differentiated organs, structures, and processes of the whole plant can be recognized in culture. So, for the moment, we reluctantly defer discussion of such important characteristics as yield, insect resistance, or plant height. Instead, we consider three areas in which the present techniques of cell culture might contribute to rice improvement: increasing tolerance for salt and herbicides and improving nutritional quality.

Salt tolerance. Cell lines capable of growth in the presence of normally inhibitory concentrations of NaCl have been isolated from suspension cultures of *Nicotiana tabacum* (Nabors et al 1975) and *Nicotiana sylvestris* (Dix and Street 1975). Plants regenerated from *N. tabacum* cultures tolerant of 6.4 g/liter NaCl grew when irrigated with solutions containing as much as 32.8 g/liter NaC1. This same degree of salt tolerance was exhibited by plants through at least the F_2 (Nabors et al 1979). With these experiments, it was demonstrated that NaC1—tolerance can be selected from among cultured cells and that such tolerance is due to a stable genetic transformation that is expressed in the adult plant.

Herbicide tolerance. Selection for mutants of *Nicotiana tabacum* resistant to the herbicide picloram has been accomplished in my own laboratory. In crosses of plants regenerated from four cell lines, resistance was inherited as a single dominant Mendelian allele. As seeds carrying a PmR allele were resistant to picloram, transmission of the mutant alleles through crosses could be followed simply by plating seeds on a medium supplemented with 100 µM picloram (Chaleff and Parsons 1978) . Subsequent studies have shown that 1 µM picloram killed normal month-old seedlings but did not affect mutant seedlings (Chaleff, unpubl. data).

It is appparent that increased herbicide resistance of the whole plant can be selected directly in cell culture. However, the approach may not be successful with other plant species or with other herbicides. Radin and Carlson (1978) found that tobacco cell cultures are unaffected by two herbicides, Bentazone and Phenmedipharm, to which the plant is very sensitive. Hence, the effectiveness of cell culture as a means for accomplishing a specific desired modification must be evaluated individually in each case.

Nutritional quality. The application of cell culture to improvement of plant protein quality has been explored through selection for cell lines that overproduce amino acids. Carlson (1973) screened populations of mutagenized haploid tobacco cells for the ability to grow in the presence of an inhibitory concentration of methionine sulfoximine (MSO). MSO is an analogue of methionine and of the toxin produced by the causal agent (*Pseudomonas tabaci*) of the wild fire disease. Diploid plants regenerated from three PISO-resistant calluses proved to be less susceptible than the parent plant to the pathogenic effects of bacterial infection. Two of the mutant plants contained elevated levels of free methionine in the leaves. In crosses with these plants, resistance to MSO was transmitted as a single semidominant allele.

In addition to suggesting that selection for toxin resistance in vitro may be used as a generalized procedure for obtaining disease-resistant varieties, these experiments demonstrate that genetically stable mutants of higher plants, in which regulation of amino acid biosynthesis is altered, may be recovered from cultured cells.

The ability to select specifically for plant cell lines that overproduce certain amino acids has been illustrated further by a series of experiments reported by Widholm and his colleagues over the past several years. The experiments demonstrate that in cells of higher plants, as in microbial systems, the

endogenous concentration of a specific amino acid can be increased dramatically by selecting for resistance to a structural analogue of that amino acid.

Initially Widholm (1972a, b) isolated diploid cell lines of tobacco and carrot that are capable of growth in the presence of a normally inhibitory concentration of 5-methyl tryptophan. Crude extracts of the resistant cell lines contain a species of anthranilate synthetase that is less sensitive to feedback inhibition by tryptophan and 5-methyl tryptophan than is the normal enzyme. Endogenous levels of free tryptophan in resistant cell lines of tobacco and carrot are 33 times and 27 times higher, respectively, than the normal levels. Although resistance to the analogue proved stable after protracted periods of culture in its absence, no plants were regenerated.

Palmer and Widholm (1975) recovered carrot and tobacco cell lines that overproduce phenylalanine by selecting for resistance to the analogue p-fluorophenylalanine. The increased rate of phenylalanine biosynthesis in these resistant cell lines is due to an altered chorismate mutase activity that is insensitive to inhibition by phenylalanine and the analogue. Although resistant carrot cells accumulated higher levels of free phenylalanine and tyrosine, these amino acids apparently are converted to phenols in the resistant tobacco cells. Similarly, elevated levels of free proline are present in carrot cells selected for resistance to proline analogues (Widholm 1976). Selection for resistance to ethionine was used to isolate a carrot cell line that contains 12 times the normal level of free methionine. Tobacco cell lines resistant to S-2-aminoethylcysteine (SAEC) contain 10-15 times higher endogenous concentrations of free lysine (Widholm 1976). These many examples amply demonstrate that in higher plants the regulation of the synthesis of specific amino acids can be mutationally altered, and that such mutants can be selected directly and efficiently in vitro.

Despite the encouraging results with tobacco and carrot, cell culture techniques have not been applied successfully to the improvement of food crops. An initial attempt to select in vitro for improved protein nutritional quality in rice has been reported (Chaleff and Carlson 1975). In rice and other cereal proteins, lysine is the first limiting essential amino acid (Juliano 1972). Nevertheless, screening of the world collection of rice germplasm for high lysine (by measuring dye-binding capacity with Acilane Orange G) failed to discover any existent varieties with significantly higher lysine levels (Beachell et al 1972, Khush and Coffman 1977).

By screening mutagenized populations of diploid rice cells for resistance to the lysine analogue SAEC, three SAEC-resistant rice cell lines were isolated. These cell lines contained elevated levels of free lysine, valine, methionine, isoleucine, leucine, tyrosine, and alanine. An observed 25% increase in total lysine content (following HC1 hydrolysis of callus tissue) in the variant cell lines was greater than could be explained by the increase in free lysine alone. This result suggests that the analogue-resistant cell lines incorporate more lysine into protein than does the normal cell line. Total cell protein of variant cell lines also contains higher levels of valine, isoleucine, leucine, tyrosine, arginine, and glutamate. Comparisons of growth responses to mixtures of exogenous amino acids showed that growth of one variant cell line is unaffected by a concentration of lysine plus threonine that completely inhibits growth of the normal cells. The growth inhibition presumably is caused by feedback inhibition of aspartokinase and homoserine dehydrogenase and resultant starvation for methionine. But inasmuch as the analogue-resistant cell lines had been maintained in culture for too long, efforts to regenerate plants were unsuccessful. Therefore, it is still not known whether these apparent alterations in the regulation of amino acid metabolism will be expressed in the mature plant or whether they will produce beneficial changes in the quality and quantity of the seed endosperm protein. There is reason for optimism, but the crucial questions remain unanswered. At present the more efficient selection methods, defined by growth tests with the variant cell lines, are being applied to calluses newly initiated from anther culture. As plants can be regenerated from such calluses, I hope that we shall know soon whether the in vitro approach to improved protein quality is feasible.

It may prove possible to use cell culture to introduce other agronomically beneficial mutations such as tolerance for heavy metal ions and disease resistance. Carlson's (1973) experiments with the tobacco wildfire disease have demonstrated that, in cases in which the purified toxin is available, cell culture might be employed in the production of disease-resistant varieties. More recently, Matern et al (1978) screened populations of potato plants regenerated from protoplasts for resistance to a mixture of toxins produced by *Alternaria solani,* the causal agent of early blight disease. Insensitivity of several somatically derived clones to the toxins and to the fungus itself proved stable through two generations of vegetative propagation.

The experiments cited are the more immediate applications of cell culture to rice improvement that can be envisioned. I am confident that additional manipulations and achievements will

become feasible as the technique is developed and refined further. For example, we have not even considered the possibilities of cell fusion and gene transfer. The progress made in cell culture and, hence, the benefits realized will reflect directly the amount of effort and support that are invested.

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TISSUE CULTURE IN RICE IMPROVEMENT: EXPERIENCES IN THE USSR

L. A. KUCHERENKO

In recent years there has been a rapid development of a new technique in plant biology -- the culture of organs, tissues, and cells, generally referred to as *tissue culture.* Results obtained in many countries show that the technique opens new possibilities for plant breeding and is increasingly attracting the attention of plant breeders.

The author and P.N. Kharchenko and G.G. Mamajeva cultivated the anthers of about 20-30 rice genotypes, mainly F_1 hybrids, to obtain homozygous lines. Plants of different ploidy levels, mainly haploids and diploids, were regenerated from the callus. The diploids derived from anther culture proved to be homozygous. The method makes available unsegregating material in the F_2, thus speeding up the breeding process by 2-4 years and increasing selection efficiency.

The in vitro cultivation of weak undersized seeds of breeding and germplasm material raises their germination ability. Cultivation of young embryos reduces by 20-30 days the time required for rice generation.

When cultivated on agar nutritive media, embryos of mature seeds easily form callus, from which plantlets can be regenerated. The study of 2 generations of plants restored from calluses showed that more than 30% had genetic alterations, apparently the result of spontaneous mutagenesis during tissue cultivation.

The method can also be an additional source of initial breeding material.

A comparative study of tolerance for stress factors in both cultivated tissues and whole plants was carried out. Cultured tissues and normal plants were subjected to low temperatures, the herbicide propanil, and high salt levels. Differences in the ability to tolerate the stress factors were more pronounced in normal plants than in cultured cells grown in vitro.

The addition of sodium chloride to a nutritive medium for callus cultivation resulted in plants with increased salt tolerance at the seedling stage.

ANTHER CULTURE

Anther culture is the most popular technique among breeders who are working with different crops. By using the technique in the

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first hybrid or mutant generation, unsegregating material is obtained in the second generation. This shortens the breeding process by several years. After obtaining a sufficient quantity of anther plants, theoretically one can have all the possible products of gene segregation in the F_2 or, if mutant material is used, in the M_2.

The wide adoption of tissue culture in breeding is limited by the rather small number of plants produced from anther callus. Other difficulties are the large number (about 60 to 80%) of albino plants produced, and the uncertain ploidy of the green plants.

The immature anthers of 20 to 30 rice genotypes, mainly F_1 hybrids, were studied annually. The basic media for callus induction were Murashige-Skoog and Blaydes. They were supplemented with either a-NAA or 2,4-D in concentrations of 1.5:2 and 5 mg/liter.

Fifteen to 20 anthers in a 15-mm-diameter test tube was the optimum inoculation density. About 7% of the anthers produced callus tissue.

In the callus initiation media supplemented with a-NAA, roots and shoots formed after prolonged cultivation. Only callus proliferation was observed on the media supplemented with 2,4-D.

Calluses produced on the culture media were transferred to differentiation media in an attempt to regenerate plants. Murashige-Skoog and Blaydes again were used as the basic media, but kinetin (2 mg/liter) and IAA (1:1.5 and 2 mg/liter) were substituted for 2,4-D and a-NAA. Plantlet yield from the callus tissue was 8%, and 30% of the plantlets were green.

Most of the plants were haploids and diploids, only rarely were they tetraploids or aneuploids. The absence of segregation in the progeny of the diploid plants established their homozygosity and indicates that the diploid plants originated from haploid cells of anthers (pollen grains).

The frequency of callus formation and, to a greater extent, plantlet formation was dependent more on the genotype of the donor plant than on the composition of the culture media, although that, too, is an important tissue culture component and requires further study.

EMBRYO AND SEED CULTURE

Seeds for breeding are sometimes received in small quantities. Often they are undersized, weak, immature or infected by fungus and cannot be used.

Cultivation on agar nutritive media ((White's or Murashige-Skoog) gave 50-100% germination of undersized rice seeds whose grain weight was one-third or less that of normal seeds. Under usual laboratory conditions, only 12 to 38% germination was achieved, and all of the seedlings were infected by fungus.

Since 1976 the tissue culture technique has been incorporated into the breeding process for germinating undersized F_0 seeds.

Experiments with immature seeds and excised young embryos were begun in 1975. Young embryos (5-6 days old) could be germinated in vitro, with germination rates of up to 60%. More simple yet was the cultivation of aged embryos (beginning from the 10th day after flowering) -- the germination reached 95%.

Because in rice the period from flowering to grain maturity is 30 to 35 days, the application of young embryo culture can shorten the growing period of a generation by 20 to 30 days. This is essential for year-round plant growing in greenhouses and climatic chambers and in the reproduction of late-maturing cultivars.

CALLUS INDUCTION FROM SOMATIC TISSUES

In 1976 experiments on callus induction from different rice plant organs were begun. Germinating seeds, seminal root tips, young embryos, and stem nodes were found to readily form callus on Murashige-Skoog medium supplemented with 2,4-D. Substituting 2,4-D for kinetin and IAA, plants were successfully regenerated. The regeneration from somatic tissue calluses was much more successful than that from anther callus; more than 90% of the plantlets were green.

The highest callus induction ability was shown by mature embryos and seminal root tips; the highest regeneration ability was shown by young and mature embryo-derived callus. Thus, in later experiments on callus establishment, we used mature embryos without excising them from the seeds.

The highest yields of plantlets were observed in the first three subcultures of the callus.

The experiment showed no varietal differences in callus induction ability -- 90-100% of the embryos of all tested varieties formed callus. But the regeneration ability of calluses derived from the different genotypes differed greatly.

A number of scientists have demonstrated that cultivated cells of various plants have a high level of spontaneous muta-

genesis. So one could expect to find mutant plants among regenerated ones. Until now there have been few studies concerning callus-derived rice plants.

We studied two generations of plants derived from somatic tissue callus of four rice varieties (*O. sativa* L.). Callus-derived plantlets were transferred to the soil and adult plants were obtained. We designated this generation as R_1. The plants varied in a number of traits, mainly in panicle productivity and sterility. Some plants showed profuse tillering, which had appeared even in the test tubes. Stem branching, which is rare under normal conditions, was found rather often among R_1 plants. Some plants had twisted leaves.

These observations, however, cannot be taken as firm evidence of mutant forms among such plants. Among the reasons are the possible modifying influence of some medium components and incomparable growing conditions (varying number of plants in a test tube, prolonged period of plantlet regeneration, and transplanting them to the soil).

Seeds collected from R_1 plants were sown in the field R_2 nursery to determine if mutation had been induced. The results from 98 R_2 lines investigated are presented in Table 1.

The study of the R_2 shows that 36% of the lines differed from the initial varieties; some lines have two or even three altered traits. Only the visible alterations were scored. Phenotypically unchanged lines may differ also, e.g. in grain quality and disease resistance. The differences can be established by specific analyses.

The presence of lines with altered phenotype in the R_2 indicates that these alterations are genetic in nature and probably result from spontaneousmutations that occur during tissue culture. For example, peculiarities of R_1 plants -- profuse tillering, stem branching, twisted leaves -- were not observed in R_2 plants.

Cytogenetic analysis of the material was not performed, but judging from the phenotype alone, all R_2 lines appeared diploid. None of the R_2 plant lines exhibited high sterility or other characters that could indicate a possible change of ploidy.

The data allow us to conclude that the tissue culture technique may result in recovering mutant plants that can be used as additional source material for rice breeding.

Table 1. Description of the second generation plants (R_2) obtained from callus tissue culture.

Rice variety	Subculture	R_2 lines			Main difference from the initial variety	
		Total	With altered phenotype		Altered trait	Lines (no.) with the trait
			No.	%		
VNIIR 3995	I	13	2	15.4	Late maturing	1
					Short stature	2
					Branches of abortive spikelets	1
	II	6	1	16.7	Short stature	1
	IV	7	3	42.9	Short stature	2
					Late maturing	2
					Branches of abortive spikelets	1
VNIIR 3970	I	48	18	37.5	Short stature	12
					Branches of abortive spikelets	1
					Panicle with more grains	3
					Seedlings more tolerant of water depth	3
					Awnless	1
VNIIR 3973	I	17	11	64.7	Short stature	2
					Altered panicle form	4
					Early maturing	8
VNIIR 3942	I	7	1	14.3	Decreased grain pubescence	1
Total	-	98	36	36.7	–	–

APPLICATION OF STRESS FACTORS IN VITRO

Scientists of different countries believe that mutagenesis in vitro, combined with selection pressure under the conditions of cultivated tissues or cells, followed by plant restoration may become an effective plant breeding tool.

The advantage of the method is the use of a single cell rather than a whole plant as a unit of selection. The method increases the volume of selected material and approaches the microbial selection technique. However, important problems must be resolved before the method is introduced into the breeding process. First, it is necessary to find out whether a relation exists between the response to a certain factor at the level of whole organisms and at the level of cultivated tissues or cells.

We investigated the tolerance for some stress factors at the organism and tissue levels, to determine the significance of those factors in tissue grown in vitro and in regenerated plants.

The influence of salt, herbicide, and chilling on cultivated rice tissues was studied. The plant forms with constrasting responses to a specific factor were used in the experiments, and their tolerance for that factor in vitro was compared. Mature seeds were used for callus establishment.

In vitro response to cold

The seeds of two rice varieties, one of them cold tolerant (VIR 1893) and the other susceptible (Lomello), were placed on callus-inducing media under the following temperatures: 28 (control), 19, 16, 13, and 10°C. Callus induction depended on temperature -- the lower temperature the fewer the grains that produced callus. Under colder temperatures, the callus, especially that of the cold-susceptible variety -- grew slower.

Only the cold-tolerant variety produced callus at 13°C. Neither variety produced callus at 10°C. But after 35 days at 13°C, the germinated seeds were able to form calluses when the temperature was increased. All VIR 1893 seeds produced callus on the seventh day after they were transferred to 28°C conditions, but in Lomello, callus did not appear until the tenth day after the temperature increase (Table 2, 3). The results indicated that adequate mechanisms exist for determining cold tolerance of the whole plant and of callus tissue. Thus, the selection of cell lines tolerant of chilling in vitro can be expected to result in plants with enhanced cold tolerance.

Table 2. Callus formation in germinating rice seeds at different temperatures, 15—17 and 35 days from callus initiation.[a]

Temperature (°C) during callus initiation	Rice variety[b]	Seedlings (%) with callus		Av diam (cm) of callus	
		15—17 d	35 d	15—17 d	35 d
28°	Lomello	100	100	0.68	0.99
	VIR 1893	100	100	0.68	1.00
19°	Lomello	70	-	0.17	-
	VIR 1893	100	-	0.34	-
16°	Lomello	11	100	0.20	0.53
	VIR 1893	50	100	0.22	0.60
13°	Lomello	0	0	0	0
	VIR 1893	0	40	0	0.25
10°	Lomello	0	0	0	0
	VIR 1893	0	0	0.	0

[a] - = not scored. [b] Lomello = cold—susceptible variety, VIR 1893 = cold—tolerant variety.

Table 3. Callus formation in germinating rice seeds at 28°C after 35 days of chilling.

Temperature (°C) in the first 35 days of callus initiation	Rice variety[a]	Seedlings (%) with callus		Av diam (cm) of callus	
		5—7 d	10 d	5—7 d	10 d
13°	Lomello	0	90	-	0.30
	VIR 1893	100	100	0.40	0.49
10°	Lomello	0	50	-	0.16
	VIR 1893	100	100	0.38	0.51

[a] Lomello = cold—susceptible variety, VIR 1893 = cold—tolerant variety.

Response to a herbicide in vitro

Herbicide tolerance in some cases is determined by such anatomical and morphological traits of the whole plant as pubescence, wax coating on leaves, erect leaves, etc, In such cases herbicide tolerance at the tissue level certainly is not expected.

But in other cases certain biochemical mechanisms determine herbicide tolerance. For example, propanil tolerance is determined by the activity of the arylacylamidaze-enzyme, which is present in green leaf tissues of tolerant plants and absent in susceptible ones. But there are no data on the behavior of this enzyme in cultivated tissues.

We compared the tolerance of different plant varieties and species for the herbicide propanil under callus tissue conditions.

The callus was established from the seeds of two rice varieties (the propanil-tolerant variety Krasnodarski 424 and the susceptible Kubanets 575) and from the seeds of *Echinochloa crus-galli* L. (the weed against which propanil is used).

In the process of callus induction (0-subculture) and proliferation in the second subculture, the nutritive medium was supplemented with propanil (0.01, 0.1, 0.5, and 1 mg/liter). The callus of *E. crus-galli* was more sensitive than rice callus to propanil. The difference was apparent when callus proliferated in the presence of 0.1 mg/liter propanil. When added to the medium for callus initiation, propanil showed its inhibiting after-effect on callus proliferation in the subsequent subcultures, especially on *E. crus-galli.* The callus tissues of rice varieties did not differ in propanil response (Table 4).

The analyses for arylacylamidaze showed the enzyme activity present in rice callus and absent in *E. crus-galli.* This finding conforms to the data known for whole plants of the same species.

It has been shown, then, that there is a relation between tolerance for propanil under the whole plant and callus conditions, but at the tissue level the difference in response of tolerant and susceptible plant forms is less than that under whole plant conditions. Only interspecies difference was found; intervarietal difference was not expressed.

Callus tissues' response to sodium chloride

Callus tissues of two rice varieties, one of them salt tolerant (Spalchik) and the other susceptible (VNIIR 3819), were cultivated in five subcultures: one on a salt - free medium and the others on media supplemented with sodium chloride in concentrations of 0.2, 0.4, 1.0, and 3.0%.

Table 4. Proliferation of callus tissues in the presence of propanil.

Propanil conc (mg/liter) in subcultures			Callus increase[b] in the 2d subculture			Necrosis (%)		
			E. crus-galli	Rice[a]		*E. crus-galli*	Rice	
0	I	II		S	T		S	T
0	0	0	2.8	3.3	3.3	14	5	0
		0.1	1.3	2.3	2.5	66	75	62
		0.5	1.0	1.1	1.3	100	100	100
		1.0	1.0	1.0	1.0	100	100	100
0.1	0	0	2.0	3.3	2.7	59	2	0
		0.1	1.0	2.2	2.3	100	76	72
		0.5	1.0	1.0	1.0	100	100	100

[a] S = propanil-sensitive variety, T = propanil-tolerant variety.
[b] Ratio of callus diameter to inoculum size.

When sodium chloride was added to the media in concentrations of 0.2-0.4%, callus growth inhibition in both varieties was slight. When 1.0% and especially 3.0% sodium chloride were used, callus growth greatly decreased; the tissue turned brown, indicating necrosis. After two subcultures in the medium with 3.0% sodium chloride, the calluses of both varieties perished.

Comparison of the response to sodium chloride of salt-tolerant and susceptible rice varieties under callus tissue conditions did not show any relation based on degree of salt tolerance between the whole plant and those in vitro.

Plant regeneration from callus subjected to stress factors

Plants were recovered from the callus tissues subjected to the herbicide propanil, chilling, and sodium chloride. The response to sodium chloride of whole plants derived from sodium chloride-treated callus has been evaluated.

Although sodium chloride concentrations of 1-3% greatly inhibited callus formation (about 80-90% of the tissue was necrotic), the surviving callus sectors maintained their regenerative ability. After they were transferred to an appropriate sodium chloride-free medium, the sectors proliferated well and produced plantlets from which adult plants were restored. Many albinos were found among plantlets from the callus treated with sodium chloride. All plantlets in the control (without sodium chloride) were green.

The seeds of R_1 plants were multiplied in the field nursery (R_2) and the seeds collected from the R_2 lines were sown in the laboratory for salt tolerance evaluation.

Salt tolerance was determined by comparing (at the seedling stage) plants grown on a salt-free substrate with those sown on a substrate with sodium chloride concentration of 1%. The degree of salt tolerance was expressed as the ratio of the weight of 15-day-old plants grown on the saline substrate to the weight of 15-day-old plants grown on a salt-free medium.

A number of the derived plant lines had higher levels of salt tolerance at the seedling stage than the initial plants had.

It is possible that the callus sectors that survived in the presence of sodium chloride originated from cells in which salt-tolerant mutations occurred. Under the saline media conditions, these cells possessed a selective advantage and produced more tolerant plants.

The data appear to be contradictory: no relation between salt tolerance at the organism and tissue levels on one hand, and enhanced salt tolerance in derived plants on the other. There may be a relation between salt tolerance at the levels of cultivated tissues and that of the whole plants, but at the tissue level the difference between tolerant and susceptible varieties is less evident and is not visual. Nevertheless the mechanisms of salt tolerance on both levels probably have the same basis. This provides the selection advantages for the tolerant mutant cells on saline media and the opportunity to recover plants with increased salt tolerance.

The fact that salt tolerance was expressed in the second seed generation of the derived plants could indicate the probable genetic nature of the trait. Still the possibility of prolonged adaptation cannot be entirely excluded, and the results should be considered as preliminary ones that require confirmation.

IMPLICATIONS AND PROSPECTS OF PROTOPLAST, CELL, AND TISSUE CULTURE IN RICE IMPROVEMENT PROGRAMS

Y.P.S. BAJAJ

Conventional methods of crop improvement seem inadequate to cope with the increasing demands to produce high yielding, high-protein, and disease-resistant rice. There is an urgent need to resort to new methodologies and to explore artificially induced genetic variability in rice improvement programs.

In this respect, tissue culture technology holds much promise (Table 1). Recent advances in the field of plant protoplast, cell, tissue, and organ culture (Reinert and Bajaj 1977a) have transformed this area of fundamental research into one that is dynamic and promising, not only for cell biology and genetics, but also for horticulture, forestry, and agriculture. The potentials of this technology as a powerful tool in crop improvement programs have been realized.

During the last 25 years, the culture of ovule, ovary (Bajaj 1964, 1966), and embryos (Bajaj et al 1978a) has been employed to overcome sterility, incompatibility, and dormancy; to induce polyembryony (for literature, see Bajaj and Bopp 1971); and to successfully hybridize various crops (Raghavan 1977). By meristem culture, large numbers of horticulturally important plants have been commercially propagated and freed from pathogens (Quack 1977, Mellor and Stace-Smith 1977). These established techniques have played an important role in wide hybridization and clonal propagation programs, and will no doubt continue to contribute to future demands. However, some of the recent advances in the area of plant protoplast, cell, tissue, and organ culture have attracted international attention because of their significance in and far-reaching implications for agricultural research and crop improvement programs.

It is expected that the following techniques will play a significant role, not only in the improvement of existing varieties, but also in synthesizing new plants:

- wide hybridization in crops through in vitro pollination and fertilization,
- production of haploid and homozygous plants from excised anthers, isolated pollen, and by chromosome elimination,

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Table 1. Protoplast, cell, tissue, and organ culture studies on rice.

Species	Explant	Response in culture	Reference
Oryza sativa	Seed	Callus and single cells, organ formation	Maeda (1965, 1967, 1968)
-	-	Callus, roots and shoot from embryo	Furuhashi and Yatazawa (1970)
-	-	Callus, roots and shoot from embryo	Bajaj and Bidani (unpubl. Fig. 7)
-	Segments of the seedlings	Callus	Shama Rao et al (1974)
-	-	Callus and plants	Henke et al (1978)
-	-	Callus and plants	Bajaj and Bidani (unpubl. Table 4)
-	Embryo	Plants	Maeda (1968), Tamura (1968)
-	-	Callus and organ formation	Saka and Maeda (1969)
O. sativa x *O. minuta*	Hybrid embryo	Plants	Nakajima and Morishima (1958)
O. paraguaiensis x *O. brachyantha*	Hybrid embryo	Plants	Li et al (1961)
O. sativa x *O. schweinfurthiana*	Hybrid embryo	Plants	Bouharmont (1961)
O. sativa x *O. officinalis*	Hybrid embryo	Plants	Iyer and Govila (1964)
O. sativa	Endosperm	Triploid plants	Nakano et al (1975)
-	Ovary	Plants of various ploidy	Nishi and Mitsuoka (1969)
-	Root	Callus	Yatazawa et al (1967), Lieb et al (1973)
-	Root	Callus and shoots	Nishi et al (1968)

Table 1. cont.

Species	Explant	Response in culture	Reference
-	Anther	Callus, embryos and haploid plants	Niizeki and Oono (1968), Nishi and Mitsuoka (1969), Ham (1969, 1970), Guha et a1 (1970), Guha-Mukherjee (1973), Iyer and Raina (1972), Woo et a1 (1973), Wang et a1 (1974), Sun et a1 (1974), Chen and Lin (1976), Chen (1977, 1978)
-	Frozen anthers (-196°C)	Callus and plants	Bajaj (1980)
-	Protoplasts (from callus cells)	Callus and roots	Deka and Sen (1976)

- somatic hybridization and genetic engineering through the fusion of protoplasts and the uptake of DNA,
- preservation of germplasm by freezing cultures at super-low temperature,
- production of disease-resistant plants from cell cultures, and
- induction of genetic variability in crops (mutants, triploids, polyploids, aneuploids, etc.) through protoplast and cell culture.

IN VITRO POLLINATION AND FERTILIZATION

From the plant breeder's point of view in vitro pollination and fertilization (Kanta et a1 1962) can be employed in

- wide hybridization,
- overcoming sexual self-incompatibility, and
- inducing haploids (parthenogenesis).

Basically, pollen is dusted over an aseptic culture of unpollinated ovules on a synthetic medium to effect fertilization in vitro. For wide hybridization programs, in addition to the sexual hybridization through embryo culture and somatic hybridization through the fusion of protoplasts, the techniques of in vitro pollination and fertilization, and intraovarian pollination (Kanta 1960) can be used to realize crosses in which incompatibility is due to an extra-long style and short pollen tube.

As a result of in vitro fertilization, hybrid embryos have been obtained in many plant species (see Rangaswamy 1977, Zenkteler and Melchers 1978) in which normal in vivo hybridization fails.

An improvement of ovule culture isplacental culture in which ovules are not cultured siagly. Instead the whole of the placenta, which bears the ovules, is cultured (Rangaswamy and Shivanna 1967) . This method helps to minimize the damage incidental to excision that adversely affects the entry of the pollen tube into the ovule. Also the necessary nutrients are partially provided by the mother tissue. This technique has been used successfully in maize (Gegenbach 1977, Bajaj 1979a) where an isolated pistil occasionally grows, but when cultured in groups such pistils develop into fertile caryopses.

In vitro fertilization can also be employed in overcoming sexual self-incompatibility. This has been successfully applied in *Petunia axillaris* and *P. hybrida* (Rangaswamy and Shivanna 1967, Niimi 1970), and fertile seeds have been obtained.

The use of in vitro pollination may be extended also to the production of haploids through parthenogenesis. This has been achieved by pollinating the ovules of *Mimulus luteus* with pollen from *Torenia* (Hess and Wagner 1974).

Intraovarian pollination is the direct injection of a pollen suspension into the ovary (Kanta 1960). The pollen germinates directly in the ovarian cavity on the surface of the ovules, fertilization is effected, and embryo and seeds are formed.

It is apparent that, at least in rice, the use of in vitro fertilization may be useful in distant hybridization involving other cereals.

HAPLOIDS AND HOMOZYGOUS PLANTS

Haploids are of great significance, especially for detecting mutants and producing homozygous plants. They are required in large numbers for successful breeding programs. However, conventional methods employed by the plant breeders are time-consuming and the frequency with which haploids are produced is very low. In this respect in vitro techniques are quick and efficient, and some ensure large and repeatable production. Anther culture technique (Guha and Maheshwari 1964) is rather simple and has proved useful in obtaining haploids from about 70 species of plants from diverse families (Reinert and Bajaj 1977b), notable ones being wheat, barley, rye, potato, coffee, tomato, and rice. Although the frequency of androgenesis is generally low (Table 2), it can be considerably improved (Bajaj et al 1977) by 1) taking the anther at a suitable stage of pollen development (late uninucleate stage just about to enter first mitosis has given encouraging results, 2) subjecting anther cultures to 3-5°C to prevent pollen abortion and thus increasing the number of potential pollen embryos, 3) adding charcoal to the medium, 4) growing the plants under optimal conditions and periodically supplying nutrient solutions, and 5) culturing isolated pollen and pollen embryos.

The culture of isolated pollen (Nitsch 1974, Bajaj and Reinert 1975, Reinert et al 1975, Bajaj 1978a) offers the additional advantage of the plants originating only from the

Table 2. Effect of various media on inducing androgenesis in anther culture of rice cultivar Basmati 370.[a]

Medium	Anthers cultured (no.)	Pollen (no.) studied in the growing anthers	Multinucleate/ cellular pollen (no.)	Multinucleate/ cellular pollen (%) in the growing anther
MS + 2,4—D (2 mg/liter) + kinetin (0.2 mg/liter) + CM (7%)	90	1010	20	2
MS + 2,4—D (2 mg/liter) + kinetin (0.5 mg/liter) + potato extract (200 g/liter) + 4% extra sucrose	119	624	0	0
B5 + 2,4,—D (2 mg/liter)	105	468	12	2.5
Blaydes + IAA (4 mg/liter) + kinetin (2 mg/liter)	112	759	22	2.9

[a] Source: Bajaj and Bidani, unpubl. data.

pollen and resulting in a homogeneous population of haploids. In anther culture, the plants originate not only from the pollen but also from various other parts of the anther. As a result, a mixed population of plants of various ploidies is produced. The culture of isolated pollen and their protoplasts (Bajaj 1974a) would also yield an ideal material for mutation, transformation, and biochemical studies. There is the possibility of obtaining virus-free plants from infected stocks.

The production of barley monoploids by the selective elimination of chromosomes, often called the *bulbosum technique* (Kasha and Kao 1970), involves the crossing of *Hordeum vulgare* with *H. bulbosum.* The young embryo, which invariably aborts in nature, is dissected out and cultured. The chromosomes of *H. bulbosum* are eliminated during culture to produce a monoploid (x = 7). This work extended to wheat (Barclay 1975) has given positive results with the production of monoploids (n = 3x = 21). The method ensures production of monoploids in large numbers (Jensen 1977) and should be extended to other crops.

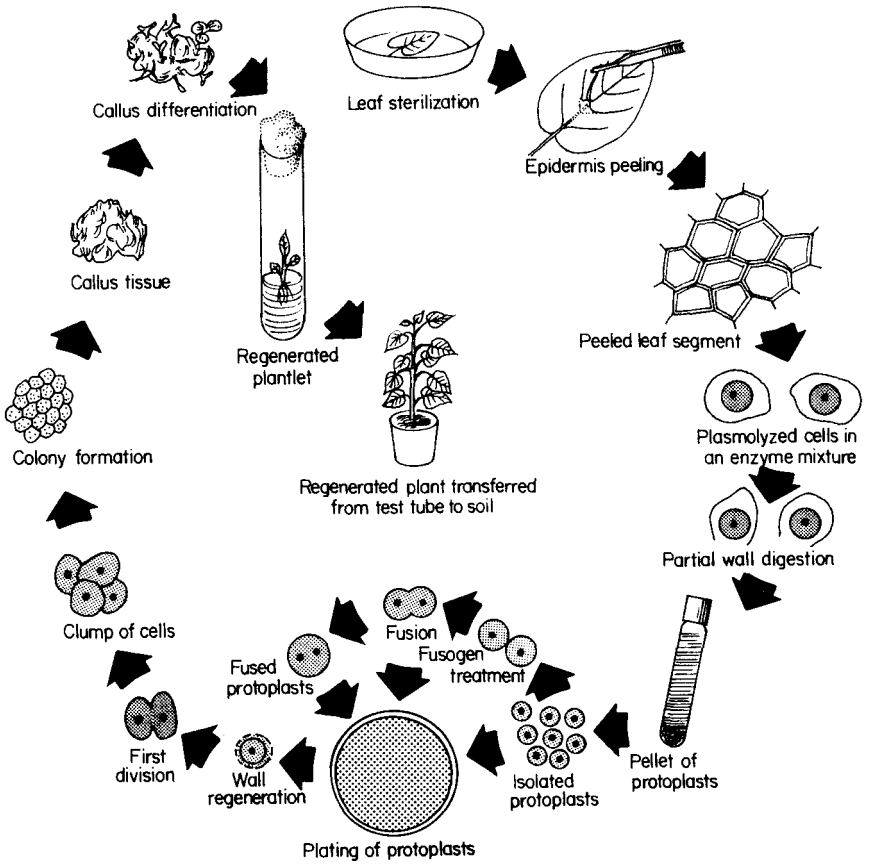
Another approach worth pursuing, which often occurs in nature, is parthenogenesis, i.e. inducing egg or sperm to develop further. This can be attempted through in vitro and intraovarian pollination with foreign pollen.

In rice, excised anthers (Niizeki and Oono 1968) cultured at the uninucleate pollen stage have given the best response on Blaydes' medium (1966) supplemented with 6% sucrose. Increasing the concentration of sucrose to 9% increases the frequency of albino plants (Chen 1978). However, the production of albino plants from the anthers of cereals is a common trait (Clapham 1977). Various cultivars show a strong genotypic growth response (Guha-Mukherjee 1973).

The in vitro production of haploids and homozygous plants has greatly facilitated breeding programs, especially those of rice, tobacco, and barley. Through this technology two new rice cultivars (Hua yu I and Hua yu II) have been released in China (Anon. 1976).

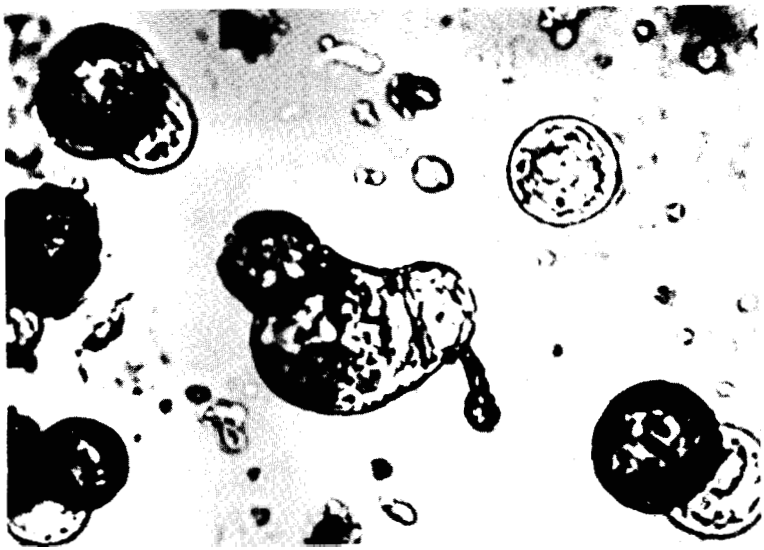
SOMATIC HYBRIDIZATION AND GENETIC ENGINEERING

One of the most significant developments in the field of plant tissue culture during recent years has been the isolation (Cocking 1960), culture (Nagata and Takebe 1971), and fusion of protoplasts (Power et al 1970); and the subsequent regeneration of parasexual hybrids (Carlson et al 1972) . This



1. Isolation, culture, and fusion of leaf protoplasts to regenerate an entire plant (Bajaj 1974b).

is especilally important because of its far—reaching implications for crop improvement (Bajaj 1974b, 1977a). Although still in the formative stage, this technology has already played an important role in opening new vistas and has awakened the interest of plant physiologists, molecular geneticists, and



2. Adhesion and fusion of mesophyll protoplasts (dark) with callus-cell protoplasts (light). Note the formation of a "somatic hybrid cell" in the center (Bajaj et al 1975).

plant breeders. In the future, protoplast manipulation will be one of the most frequently used tools in agricultural research with unlimited potential for genetic engineering and plant improvement.

Large quantities of protoplasts now can be enzymatically isolated and, when cultured, regenerate a cell wall and divide to form callus which in turn can be induced to regenerate an entire plant (Fig. 1). To date, entire plants have been regenerated in about 20 plant species of the genera *Asparagus, Brassica, Citrus, Datura, Nicotiana, Petunia,* and *Ranunculus.*

In rice, so far only roots have been reported to differentiate from protoplast-derived callus (Deka and Sen 1976). In this connection, it is pertinent that not only rice but cereals generally pose a number of problems, and cause disappointments. But this is a challenge and an optimistic view should be taken. More basic and fundamental work regarding the cultural requirements of cereals will lead to breakthroughs in this field.

Protoplasts can also be induced to fuse with one another, and the fused product (Fig. 2) eventually forms a "somatic hybrid." The discovery of polyethylene glycol (Kao and

Michayluk 1974) as a fusogen has given a great impetus to somatic hybridization studies as this chemical is easy to manipulate, causes a high frequency of fusion, and seems to have no deleterious effects on the fusion product.

There are a limited number of reports on the successful regeneration of somatic hybrids, i.e. *Nicotiana langsdorfii* + *N. glauca* (Carlson et al 1972, Smith et al 1976), between two chlorophyll-deficient mutants of tobacco (Melchers and Labib 1974. Gleba et al 1975), *Petunia hybrida* + *P. parodii* (Power et a1 1976), *Daucus carota* + *D. capillifolius* (Dudits et a1 1977). *Datura innoxia* + *D. stramonium* (Schieder 1978). *Nicotiana sylvestris* + *N. knightiana* (Maliga et a1 1977), and *Solanum tuberosum* + *Lycopersicon lycopersicum* (Melchers et ai 1978).

Extending this work to other plants is a matter of refining the technique, and needs an imaginative mind and a skilled hand. Undoubtedly there will be problems; for example, incompatibility at certain levels needs to be explored. We expect chromosomal mosaics, deletions, and eliminations, just as in animal cells. However, some of these phenomena could be useful in introducing and increasing genetic diversity in crops.

The pinocytotic property of the protoplasts makes them an ideal tool for use in genetic engineering studies. This opens up new possibilities and a host of scientifically interesting aspects to be exploited in plant improvement. The isolated protoplasts take up DNA, viruses, bacteria (Davey 1977), nuclei (Potrykus and Hoffmann 1973). and chloroplasts (Giles 1977), and therefore are useful in plant-modification studies (for literature, see Bajaj 1977a). So far, there is no evidence that the DNA or the nuclei are biologically functional within the protoplast. Efforts to culture them have been unsuccessful.

The uptake of *Azotobacter vinelandii* into the protoplasts of the fungus *Rhizopogon* has been demonstrated. This appears to be the first report on nitrogen fixation by a eukaryote cell (Giles and Whitehead 1976). The modified fungus grows on a medium devoid of combined nitrogen. The symbiotic nitrogen-fixing bacterium (*Rhizobium*) can be introduced into legume protoplasts during the enzymatic digestion of the cell wall (Davey and Cocking 1972). This uptake occurs through the invaginations of the plasmalemma during plasmolysis. Legume root-nodule protoplasts containing packets of bacteria have also been successfully isolated (Davey et al 1973). These nodule protoplasts can be fused with nonlegume protoplasts, and the investigations can result in new endosymbiotic relations. The introduction of free nitrogen-fixing bacteria, e.g.

Azo tobacter, and the blue-green algae into nonlegumes are possibilities for the future. The ability to fix nitrogen can also be transferred to the protoplasts by plasmids. which are much smaller than bacteria. The incorporation of nitrogen-fixing *(nif)* genes into nonlegumes (Child 1975, Scowcroft and Gibson 1975), especially cereals, which are already photo-synthetically efficient, will render these plants self-sufficient for increased protein synthesis. This is another area where geneticists and agronomists can combine their efforts.

The introduction of *nif* genes and plasmids from legumes into cereals, and the transfer of disease resistance to susceptible plants via the incorporation of DNA are being attempted in various laboratories. The implications for rice improvement are evident.

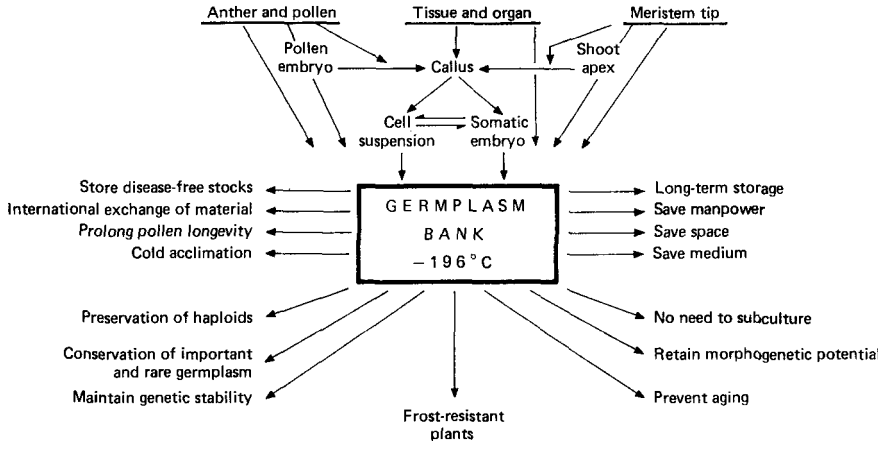
FREEZE PRESERVATION OF GERMPLASM

Isolated cells, tissues, and organs are the ideal systems for studying the fundamental aspects of the biology of freezing. They offer possibilities as well, for a number of applied studies. The regeneration of entire plants from haploid (Bajaj 1976a) and diploid cells (Nag and Street 1975, Bajaj 1976a), meristems (Seibert 1976, Bajaj 1977b,c), pollen-embryos and anthers (Bajaj 1976b, 1978b) frozen at -196°C in liquid nitrogen demonstrates the utility and potential of freeze storage technology for the preservation of germplasm (Fig. 3).

The excised anthers and the haploid callus of rice cultivar Basmati 370, frozen in liquid nitrogen (Bajaj 1980), occasionally (5 out of 927 anthers) revived, produced haploid callus and differentiated. However, the haploid calluses are highly unstable in culture, undergo endomitosis, and revert to their diploid state in a relatively short time. For this reason, their preservation by cryogenic methods is worth considering. This work is relevant to the preservation of haploid cultures, which are important for the induction of mutations, and to studies on biochemical genetics.

Phenomena such as chromosomal aberration, mutation, and change of ploidy, although undesirable for maintaining the uniformity of clones, can be used, nevertheless, as variables to be incorporated into breeding programs. New cell culture lines and mutants can be banked and used to meet research needs.

Freeze preservation could be used to induce hardiness through acclimation (Tumanov et al 1968. Steponkus and Bannier



3. Diagram of how freeze preservation technology may be employed to establish a germplasm bank (Bajaj 1979c).

1971, Bannier and Steponkus 1976), and possibly to select cold-tolerant mutants that could then be induced to regenerate "frost-resistant plants."

One of the most profitable areas is pollen storage (Collins et al 1973, Nath and Anderson 1975). This procedure would solve some of the problems often connected with incompatibility and pollen longevity. It also would 1) facilitate the crossing of plants that flower at different times, and are separated by space, or are grown at different places, and 2) reduce the dissemination of diseases and insects often associated with the transport of whole plants (Collins et al 1973). Freeze preservation will also aid the international exchange of pathogen-free stocks. Thus the technology would be ideal for the establishment of germplasm banks of rare stocks (Bajaj 1976b, 1979b, 1979c, Bajaj and Reinert 1977).

With the rapid increase in the number of varieties it is becoming difficult, if not at times impossible, to maintain or preserve some of the stocks which are not needed in current breeding programs. Thus, some of the germplasm which may not seem to be so important today, but might be needed later, is ignored or completely lost. The establishment of germplasm banks (Bajaj 1979b, 1979c) is especially needed for preserving materials that are threatened with extinction.

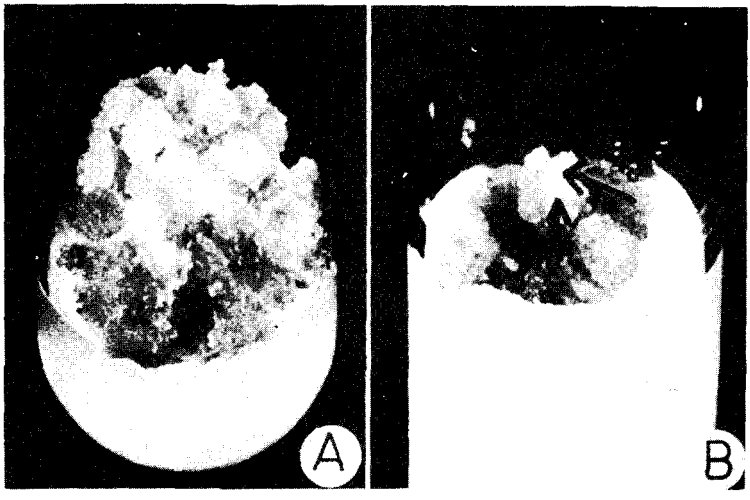
DISEASE—RESISTAST PLANTS

The production of pathogen—free stocks by the culture of excised meristems of shoots (Morel and Martin 1951) and roots (Bajaj and Dionne 1966) is an established technique, and a large number of plants, especially those of horticultural importance, have been freed from viruses by using this method (Quack 1977). Meristem culture is being commercially exploited by the flower industry.

Recently, inducing disease resistance through cell culture was attempted. There are many significant advantages in studying the mode of action of pathogens and pathotoxins at the cellular level. Isolated protoplasts offer the further advantage of being devoid of the cell wall, thus the mechanism of infection and the action of pathotoxins on the membrane can be elucidated. Not only can the toxin—resistant cell lines be isolated; toxin—resistant plants can also be regenerated from them. This procedure opens an entirely new field of study for plant improvement programs.

The work on the effect of toxins on the growth and development of plant tissue cultures was started in 1968 (Bajaj and Saettler) with a view to understanding the mechanism at a cellular level, and later to select toxin—tolerant cells. Toxin filtrates from the host—specific pathogen *Pseudomonas phaseolicola* and the non—specific pathogens *P. syringae* and *P. morsprunorum* were used, Only the toxin filtrate from *P. phaseolicola* induced halo on the leaves and inhibited tissue culture growth by 77% (Bajaj and Saettler 1970). But after an initial setback, some cells or islands of callus resumed some growth (Fig. 4). These cells presumably were tolerant of the toxin, and might eventually be used in the plant regeneration studies.

During the last few years, significant progress has been made in regenerating tobacco plants resistant to methionine sulfoximine (a compound similar to the extract of *Pseudomonas tabaci*, which causes wildfire disease) (Carlson 1973). Pelcher et al (1975) demonstrated the differential response of protoplasts, isolated from resistant and susceptible plants of maize, to the host—specific race "T" toxin produced by *Helminthosporium maydis*. Recently, complete maize plants (male—fertile) resistant to *Helminthosporium* were obtained from callus cultures of a male—sterile, toxin—susceptible variety (Gengenbach et al 1977). Bajaj et al have observed the differential growth inhibition response of callus tissue cultures of pearl millet *(Pennisetum americanum)* grown on a



4. Effect of toxin filtrate of *Pseudomonas phaseolicola* on bean callus cultures: A) control, B) grown on toxin filtrate for 35 days. Note growth inhibition and survival of a few tolerant cells (Bajaj and Sacttler 1070).

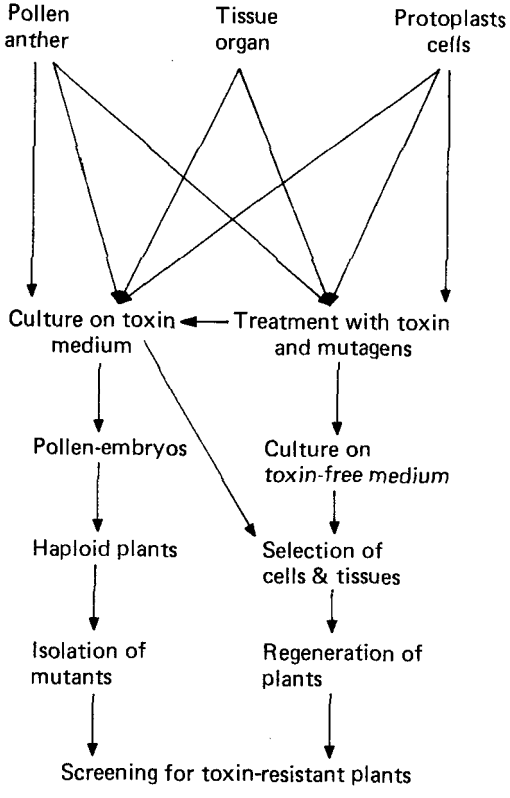
medium containing ergot extract (*Claviceps fusiformis*). Higher concentrations of ergot extract (Table 3) stopped the growth initially, but a few cells survived (possibly mutants) and resumed growing. In one instance, an albino shoot developed from a patch of these cells. The procedure for the isolation of toxin-resistant cells is outlined in Figure 5.

Other possibilities worth exploring are the fusion of protoplasts from a disease-resistant and a susceptible plant, and the eventual regeneration of a resistant somatic hybrid. This could also be achieved by the uptake of isolated DNA from a disease-resistant plant and incorporating it into the susceptible protoplasts. At present, these approaches are only of academic interest, but with some refinement in technology they will undoubtedly help in achieving the desired goals. Thus, there is a possibility of extending this work to the tissue culture of rice. Considerable loss in the rice crop is brought about by numerous maladies caused by various bacteria, fungi, viruses, nematodes, and insects (Khush 1977). Some of them, especially blast (*Pyricularia oryzae*), sheath blight (*Corticium sasakii*), brown spot (*Helminthosporium oryzae*), bacterial blight (*Xanthomonas oryzae*) and bacterial streak (*Xanthomonas translucens*), reach epidemic proportions. Therefore, it is worthwhile to explore the selection of cell culture lines that are potentially tolerant of or resistant to the crude extracts and toxins of some of the pathogens, and the regeneration of plants from such cultures.

Table 3. Growth response of embryos or seeds of pearl millet *(Pennisetum americanum)* on MS medium containing extract from ergot *(Claviceps fusiformis)* -infected earheads.

Medium	Germination (%)	Roots	Shoot	Callus [a]	General remarks
MS control	66	Normal	Norma1	Profuse callusing	
MS + seed extract	60	Slightly inhibited	Not effected	Slight inhibition	Germination is not effected. Extract at higher concentrations stops the growth of roots and
MS + ergot extract (24 g/liter)	65	Inhibited	Not effected	Inhibition	callus. Occasionally a few cells survive and continue to divide.
MS + ergot extract (48 g/liter)	66	Completely stopped	Not effected	Completely stopped	

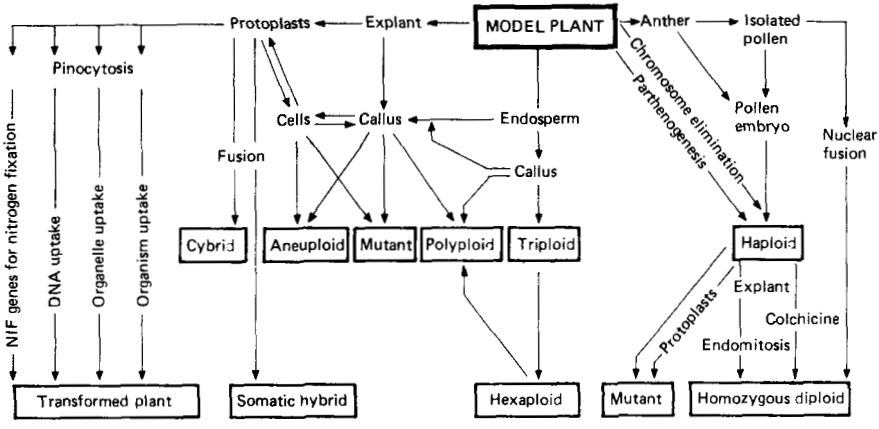
[a] For callus experiments MS medium was supplemented with 5 mg 2,4-D/liter (Bajaj et al unpubl.) .



5. The procedure for the in vitro induction and selection of toxin-resistant plants (Bajaj et a1 1980).

INDUCTION OF GENETIC VARIABILITY

Because of the depletion of germplasm pools in agricultural crops, plant breeders must resort to unconventional methods for synthesizing new combinations of germplasm. Cell and tissue cultures are a valuable means for inducing genetic diversity and creating new gene combinations that do not exist in nature (Fig. 6). It has been repeatedly observed (D'Amato 1977) that plant cells in cultures show a wide range of genetic changes such as polyploidy, mutations, chromosomal breakage, unequal mitosis, etc. These genetic changes somewhat limit the use of tissue culture; nevertheless, tissue cultures provide plant breeders the means to select desirable genomes, which normally may not be available in nature. For instance, numerous lines of sugarcane (Heinz et a1 1977) have been obtained from cell cultures and the plants have been studied for agronomic traits. Moreover, virus-resistant plants have been regenerated from those cultures.



6. Introduction of genetic variability into crops through protoplast and cell culture (Bajaj 1979c).

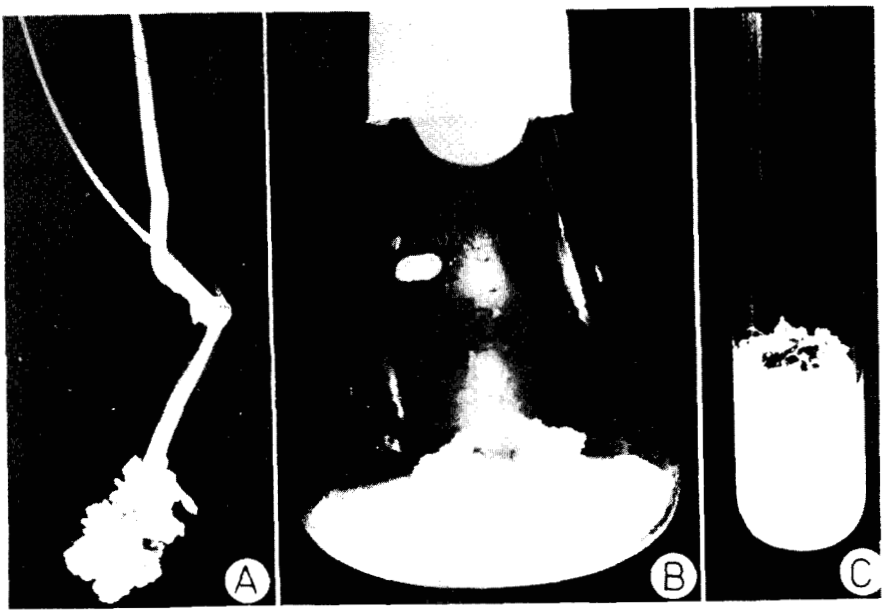
Polyploid plants can also be produced from colchicine-treated protoplasts and cell cultures. Cell and tissue culture would be ideal for inducing changes in ploidy, especially in cases where polyploids are desired and normally are not available. From tissue cultures plants of various ploidy levels, aneuploids, etc. have been obtained in tobacco (Murashige and Nakano 1966), sugarcane (Heinz and Mee 1970), *Asparagus* (Malnassy and Ellison 19701, *Brassica* (Horak et al 1971), and *Atropa* (Bajaj et al 1978b). Likewise, rice plants of various chromosome levels have reportedly been regenerated from cultures of ovaries (Nishi and Mitsuoka 1969) and triploid plants from endosperm (Nakano et al 1975).

Perhaps the induction of mutation at a cellular level is one of the most important approaches. With the availability of plating techniques (Nagata and Takebe 1971), it has become easier to utilize cells, protoplasts, and pollen for inducing mutations. The great advantage of this technique over working with whole plant populations, is that millions of cells can be treated at one time with various mutagens, both radiations (Bajaj et al 1970) and chemical (Sung 1976), and plated in one petri dish. That makes the selection of mutants much more efficient. There are reports on the isolation of mutants resistant to various amino acid analogs and drugs, i.e. N-methyl N-nitrosoguanidine (Lescure 1969, 1973), 5-bromodeoxy-uridine (Carlson 1970), streptomycin (Binding et al 1970, Maliga et al 1973), methionine sulfoximine (Carlson 1973), methyltryptophan (Widholm 1974), p-fluorophenylalanine (Palmer and Widholm 1975), and valine (Bourgin 1978).

Table 4. Differential genotypic growth response of excised segments from seedlings of various cultivars of rice cultured on BM + 2,4—D (2 mg/liter).[a]

Cultivar	Root	Mesocotyl	Shoot
Basmati (B370)	+++	+++	++
Palman	++	+	+
HM95 (HM)	-	+++	+++
PRl06	+	++	+
Chowsung	+	+++	-
HM95	-	++	++
Jaya	-	+	+
IR8	+	+	-
Crythroceros	-	++++	+

[a] - no callusing, + poor, ++ good, +++ very good, ++++ excellent callusing (Bajaj and Bidani, unpubl. data).



7. Induction of callusing and organogenesis in rice cultivar Basmati 370. A) Two-week-old culture of an embryo on MS + 2,4-D (5 mg/liter), showing proliferation of the roots. B) Root-derived callus mass 3 weeks after subculture on MS + 2,4-D (1 mg/liter). C) Organogenesis in callus 2 weeks after transfer to a low level 2,4-D (0.5 mglliter) medium (Bajaj and Bidani, unpubl. data).

Attempts are being made to select mutant cell lines that are resistant to various toxins (discussed earlier), salts (Nabors et al 1975, Meredith 19781, herbicides (Boulware and Camper 1972, Oswald et al 1977, Chaleff and Parsons 1978), drought and cold (Dix and Street 1976), low photorespiration (Day 1977), and protein-rich and nutritionally limiting amino acids (Green and Phillips 1974; Widholm 1974, 1976).

To induce genetic variability in rice, much work on callus induction, cell suspensions, cell culture, and their nutritive requirements has been conducted (Maeda 1965, 1967, 1968; Yamada et al 1967; Yatazawa et al 1967; Furuhashi and Yatazawa 1970; Wu and Li 1971; Watanabe et al 1971; Lee et al 1972; Lieb et al 1973; Ohira et al 1973, 1975; Henke et al 1978) (Table 4; Fig. 7). Plants of various ploidies have been regenerated (Nishi and Mitsuoka 1969, Nakano et al 1975). However, the differentiation of plants (Reinert et al 1977) from callus cannot be induced at will and that is one of the problems that delay further progress. More basic work needs to be carried out on this aspect before the technique of cell plating can be successfully applied to rice.

CONCLUSIONS AND PROSPECTS

Progress in plant tissue culture during the last decade has established it as a powerful tool in agricultural research and plant improvement programs. Problems regarding the cultural requirements of the cells and other factors controlling the differentiation of entire plants eventually will be resolved by concentrated effort and an interdisciplinary approach.

Studies on protoplast culture and fusion have established three main points: 1) an isolated protoplast is totipotent and capable of regenerating a complete plant , 2) protoplasts can be induced to undergo intra- and inter-specific fusion to form a somatic-hybrid, and 3) the pinocytotic property of protoplasts makes them an excellent material for genetic engineering and cell modification studies.

Protoplast, cell, and tissue culture could play a significant role in rice research programs to improve existing cultivars and to achieve wide hybridization with other cereals and legumes.

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STATUS OF JAPONICA-INDICA HYBRIDIZATION IN KOREA

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ENVIRONMENTAL CONDITIONS FOR RICE CULTURE IN KOREA

The Korean peninsula is located in a typical temperate zone: between 33 and 43°N latitude and between 124 and 131°E longitude. Because Korea is bordered on the north by Asia and by sea on the three other sides, continental and oceanic climates are often intermixed. Day lengths vary with the season: 9.5 hours in late December, 14.5 hours in late June, and around 12 hours in late March and late September. The period when daily temperatures are above the 13°C required for rice culture is comparatively short, lasting about 150 days from late April to late September. Annual precipitation varies from 1,100 to 1,200 nun, with 70—80% concentrated between late June and early September. The rice season normally begins in mid—April and ends in mid—October, varying with sites and cropping systems.

Irrigation facilities in 1978 were sufficient for about 85% of the total paddy area, but water shortage at optimum transplanting time often occurred in poorly irrigated fields.

The major rice diseases are blast, sheath blight, bacterial leaf blight, and stripe and dwarf virus. The important insect pests are stem borers, planthoppers, and leafhoppers. Heavy nitrogen application, early planting, and high planting density increase damage from diseases and insect pests.

The major environmental conditions dictate the various breeding targets. Breeding programs were focused primarily on rice, which is the staple food in Korea.

RICE BREEDING IN KOREA

Systematic rice breeding in Korea started in 1906 when the Agricultural Experiment Station was founded in Suweon. The 3,000 or so native rice varieties grown throughout the country were gradually replaced by Japanese varieties after 1906. Pure line selection was first practiced for rice breeding in 1912, with the first hybridization in 1915. The first home—bred rice variety was released in 1938. All the rice varieties

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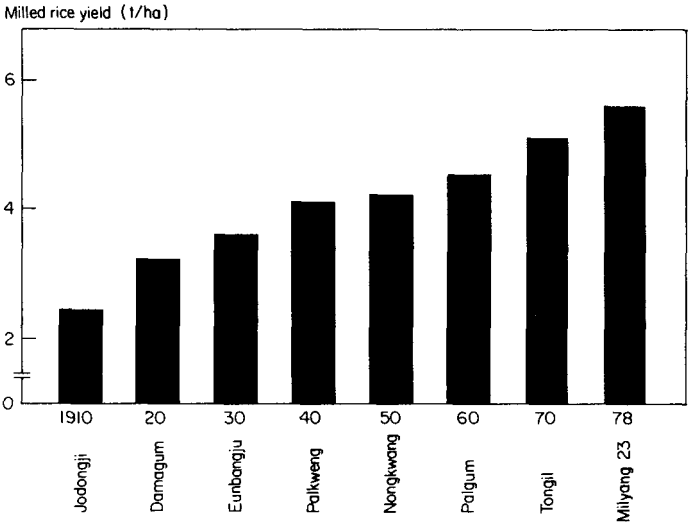
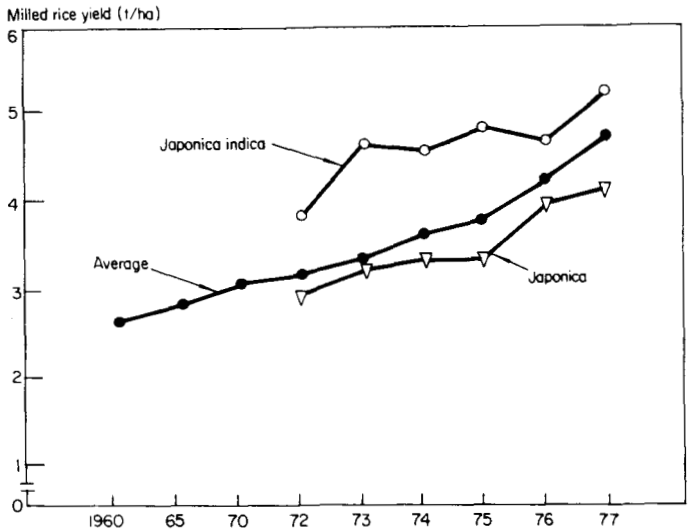


1. Periodical changes in productivity of cultivated rice varieties.

cultivated in Korea were japonica until 1971 when Tongil, the first japonica-indica variety, was released in the country. Historically, rice varieties cultivated or released in Korea can be grouped as follows: land varieties cultivated before 1910, introduced varieties from Japan cultivated mainly from 1920-1945, home-bred japonica varieties cultivated primarily during 1955-72, and locally bred japonica-indica varieties cultivated from 1975 to date.

Continuous breeding efforts have increased the productivity of rice varieties cultivated or released in Korea. Figure 1 indicates that the productivity of the new rice varieties is almost 2.5 times that of Korean native varieties, and that yield increases as much as 1 t milled rice/ha were achieved every 20 years by varietal improvement. Tongil, which was selected from a japonica-indica hybrid population, showed a 30% yield increase compared with japonica varieties. Improved semidwarf varieties from japonica-indica crosses after the release of Tongil also had high productivity.

The national average rice yield per hectare was also increased (Fig. 2). The yield increases were derived from the combined effect of improved rice varieties; cultural techniques; and meteorological, irrigation water, and paddy soil factors. The japonica-indica varieties always yielded 20-40% more than traditional japonica varieties in farmers' fields from 1972 to



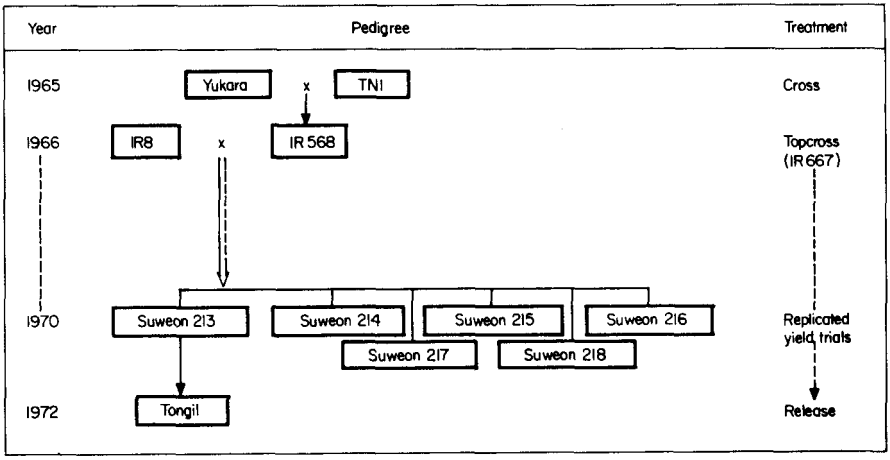
2. Changing trend in national average rice yields in Korea.

1977. Their high productivity, came from the well-known semidwarf indica variety IR8, a short plant with stiff and strong stems, and erect leaves.

TONGIL, THE FIRST SEMIDWARF VARIETY FROM JAPONICA-INDICA HYBRIDIZATION

During the 1960s, the major factors limiting rice yields in Korea were lodging, blast and stripe virus diseases, and low productivity of the rice plant itself. The major rice breeding targets at that time were for resistance to lodging and blast. However, many Korean rice breeders felt it was difficult to achieve those breeding targets with hybrid populations of japonica varieties. And semidwarf indica varieties that showed blast resistance were crossed to japonica varieties in an effort to maintain the ecological adaptability and eating quality of the japonica parent. To overcome grain sterility in japonica-indica hybrid populations and incorporate high productivity, the F_1 of all japonica-indica crosses were crossed back to semidwarf indicas.

IR667 (IR8//Yukara/TN1) was one of those top crosses well-adapted to Korean conditions. As shown in Fig. 3, six Suweon numbered lines were derived from this IR667 cross in 1969. The Suweon 213 line was named *Tongil* in 1971, and seeds of this first japonica-indica variety were initially released to Korean farmers on a large scale (180,000 ha) in 1972. Tongil was characterized morphologically by medium-long and erect leaves, thick leaf sheaths and culms, short plant height but relatively long panicles, open plant shape, lodging resistance, and easily



3. Development of Tongil improved rice variety.

shattered grain. It was highly productive throughout the country, except in the cool mountainous regions (Table 1). Tongil also showed high resistance to blast and stripe virus diseases. Apparently it received from the japonica parent Yukara its adaptability to Korean growing conditions (temperate region), early maturity, and a grain shape and eating quality acceptable (although not ideal) to the Korean people. Short plant height; long panicles; stiff and strong stems; erect leaves; resistance to lodging, blast, and stripe virus diseases; and high productivity were attributed to the semidwarf indica parents TN1 and IR8.

Although Tongil significantly outyielded the traditional japonica rice varieties and had a number of desirable characteristics, it also had several weaknesses. Its grain and eating qualities were less acceptable than those of japonica varieties. It lacked low temperature tolerance; did not have a broad spectrum of resistance to blast, bacterial leaf blight, and brown planthopper; and was not adapted to late-season culture.

BRIDGE PARENT IMPROVEMENT

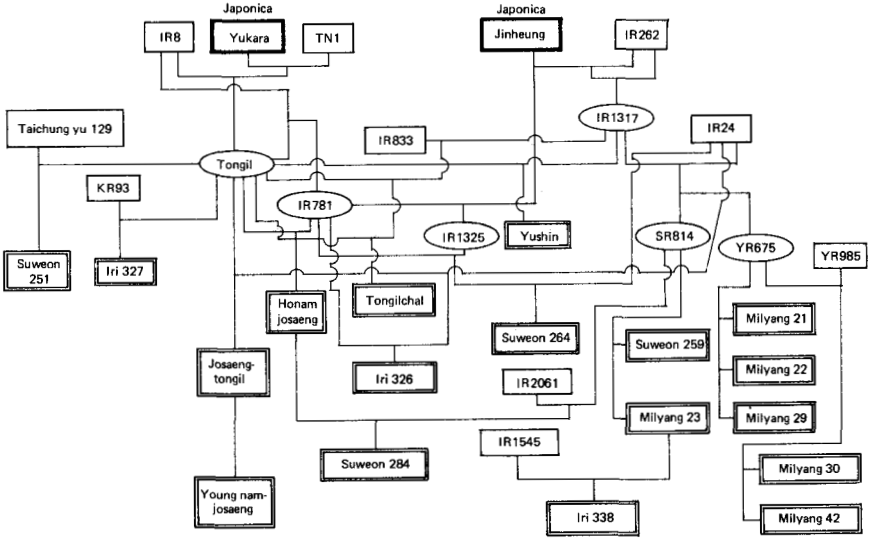
The number of crosses between japonica and indica varieties was increased in Korea beginning in 1968, and Korean rice breeders working at the International Rice Research Institute (IRRI) also made other japonica-indica crosses. The F_1 of all japonica—indica crosses were backcrossed to their semidwarf

Table 1. Milled-rice yield of Tongil in joint production and individual pilot farms in 1971 (Office of Rural Development, Korea).

Classification	Variety	Item	Milled-rice yield of <4 t/ha	4.0 t/ha	4.5 t/ha	5.0 t/ha	5.5 t/ha	>5.5 t/ha	Total	Av yield (t/ha)
Joint production	Tongil	Locations (no.)	38	59	170	166	84	33	550	5.00
		Percentage	6.9	10.7	30.9	30.2	15.3	6.0	100.0	
Individual pilot farm	Tongil	Locations (no.)	9	6	22	26	8	7	78	5.00
		Percentage	11.5	7.7	28.2	33.3	10.3	9.0	100.0	
	Japonica Check	Location (no.)	36	28	11	2	1	-	78	3.98
		Percentage	46.2	35.9	14.1	2.6	1.3	-	100.0	

indica parents or to another semidwarf variety. Some of these lines [IR781 (IR8*2//Yukara/TN1), IR1317 (Jinheung/IR262*2), and IR1325 (IR781//Jinheung/IR262)] performed better under Korean conditions, but they did not have acceptable grain qualities. Korean rice breeders in 1970 began to use these semidwarf japonica-indica pedigree lines, including Tongil itself, as the bridge parents for further development of other breeding targets: grain and eating quality, cold tolerance, adaptability to late season cultivation, and resistance to diseases and insects (Fig. 4). As a result, 18 Tongil-type rice varieties were released to farmers during the period 1974-79 (Table 2). Among the bridge parents during this period, IR1317 showed the best combining ability and was responsible for the production of 11 released rice varieties. The newly-developed varieties were almost the same as or had better productivity and agronomic characteristics than Tongil.

The development of Tongil-type rice varieties from japonica-indica hybridization has contributed significantly to Korean rice breeding and cultivation. The most significant characteristic is the more productive plant type compared with that of the japonica varieties in the country for thousands of years. Tongil is more productive and permits more light transmission



4. Bridge improvement from japonica-indica crosses.

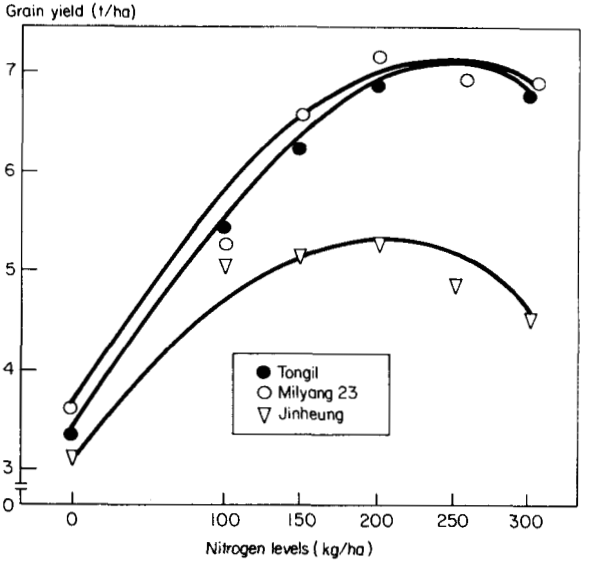
Table 2. Japonica-indica high yielding rice varieties released in Korea.

High yielding rice varieties					
1971	1974	1975	1976	1977	1978
Tongil	Josaengtongil	Yushin	Milyang 21	Suweon 264	Suweon 284
	Youngnamjosaeng	Suweon 251	Milyang 23	Iri 327	Iri 338
	Tongilchal	Milyang 22	Suweon 258	Iri 326	Milyang 42
				Milyang 29	
				Milyang 30	
				Honamjosaeng	

into the lower portion of the plants. Korean rice breeders intensively use the Tongil plant type because it is now known that Tongil's productive plant structure, which originated from the Chinese indica semidwarf variety Dee-geo-woo-gen, is simply inherited. Because of their semi-dwarf plant type, the Tongil-type rice varieties show high lodging resistance.

The nitrogen response of Tongil-type japonica-indica varieties significantly surpasses that of japonica rice varieties (Fig, 5). Consequently Korean farmers apply high levels of nitrogen fertilizer, more than 200 kg/ha, on their rice fields, although government agencies recommend maximum application of 150 kg/ha on Tongil-type rices.

An additional significant advantage of Tongil-type rice varieties is their high disease-resistance. These varieties resisted blast disease in Korea up to 1977, and their resistance to stripe virus disease continues. The leading japonica varieties show moderate susceptibility to both diseases. Before the release of Tongil, lodging, and blast and stripe virus diseases had been the major constraints to rice yield increases in early-season culture of japonica varieties with heavy nitrogen application.



5. Nitrogen responses of japonica-indica crosses (Tongil and Milyang 23) and a japonica (Jinheung) in 1976.

Korean farmers readily welcomed Tongil-type varieties because no rice blast and stripe virus symptoms appeared in their fields from 1971 to 1977. But in 1976, blast fungus races capable of attacking Tongil-type rice varieties began to appear in the southern mountainous part of Korea and prevailed throughout the country in 1978. Among 18 Tongil-type rice varieties, 4 -- Milyang 30, Suweon 284, Milyang 42, and Iri 338 -- still show moderate resistance to newly appearing blast fungus races. Blast resistance has broken down in the remaining varieties. This phenomenon had been anticipated. Most Korean rice breeders had tried to incorporate broad-spectrum resistance to blast disease into Tongil-type varieties. Most Tongil-type varieties were resistant to bacterial leaf blight pathogen group II, which was widely distributed throughout the country. Only Milyang 30 showed resistance to brown planthopper, which severely damaged rice in 1975 and 1978.

Wide regional adaptability or good locational yield stability was another merit of Tongil-type japonica-indica varieties. Tongil-type varieties adapted well to all locations in which they were tested and showed high grain yield compared with japonica varieties. The reasons are not certain, but Tongil-type varieties are less photoperiod-sensitive than japonicas.

Tongil itself lacked grain shape and eating quality readily acceptable to Korean consumers. After its release, the first rice breeding target in Korea was improvement of grain shape and eating quality. As a result, most of the Tongil-type varieties released later showed considerable improvement in physiochemical eating qualities (Table 3). Among the varieties well accepted by farmers, Suweon 264, Nopoong, and Milyang 23 did not significantly differ in eating quality characteristics from the japonica rice varieties Akibare and Jinheung. The protein content of Tongil-type varieties is substantially higher than that of the japonicas. That indicated that through well-programmed breeding pressure, grain shape and eating quality could be improved in the hybridization of japonica and indica varieties.

BACKGROUND FOR JAPONICA-INDICA HYBRIDIZATION BREEDING

Grain sterility and genetic complexity were major problems in japonica-indica hybridization projects. However, both Japanese and Korean rice breeders had developed blast-resistant rice varieties from japonica-indica crosses by the backcross breeding

Table 3. Improvement of rice quality (IRRI 1978).

Variety	Protein (% at 12% H_2O)	Amylose (% dry basis)	Gel consistency		Alkali spread (1.15% KOH)	Instron cooked rice	
			100 mg	120 mg		Hardness (kg)	Stickiness (g.cm)
Suweon 251	7.1	18.2	99	89	5.0	3.8	168
Suweon 258	6.9	19.2	95	83	4.9	4.2	162
Suweon 264	8.2	18.6	92	86	5.0	4.4	123
Nopoong	8.3	18.2	95	86	5.2	4.6	134
Milyang 21	8.9	15.0	93	80	4.7	4.9	111
Milyang 30	7.9	18.0	88	74	4.8	4.9	112
Raegyeng	7.2	14.0	86	80	4.9	5.0	126
Milyang 23	7.1	19.5	92	72	5.0	4.7	122
Akibare*	6.7	17.5	90	82	6.0	4.6	139
Jinheung*	6.6	18.2	98	74	5.2	4.8	122

*Japonica check.

method. The major difference was that the Japanese made the backcross to japonicas while the Koreans backcrossed to semidwarf indica varieties.

Because of the nonallelic relations for plant type between japonica and indica varieties, plant heights of F_2 populations derived from single japonica-indica crosses showed normal distribution, which indicated genetic complexity. However, backcrosses or topcrosses to semidwarf indica varieties separated the F_2 populations into two genetic groups, simple and complex (Table 4). The distribution of the F_2 plant height in family I of both topcrossed and backcrossed japonica-indica hybridization was simpler than that in family II. Consequently, rice breeders can easily select semidwarf F_2 plants in family I, which may include plants with good productive structure. Plant heights of BC_2F_3 lines selected from backcrossed populations by this method were distributed almost the same as those of the recurrent semidwarf parent (Table 5). The F_3 lines were selected only visually in the greenhouse during the generations of BC_1F_1, BC_2F_1 and BC_2F_2, It is apparent that semidwarf plant types can be completely recovered by the backcross or topcross method in any japonica-indica cross.

The grain yield of BC_2F_3 lines selected from backcrossed populations was slightly lower than that of their recurrent parent IR667-98-1-2-2, but some lines yielded as high as their recurrent parent (Table 6). Grain yield is not such a simple genetic trait for advancing selections as the yield variations among crosses and among lines in a cross seem to imply.

The amylose content of the rice grain is a selection indicator for the assessment of eating quality. Most japonica rices have low amylose content, less than 20%; indica varieties have medium or high amylose content. Large genetic variation in amylose content might be expected in hybrid populations or pedigree lines derived from japonica-indica crosses. Environmental variations also affect amylose content even within the same variety. Thus, it is difficult to select low-amylose pedigree lines. The only way has been to screen large hybrid populations through chemical analyses. Many of the pedigree lines recently tested in Suweon have low amylose contents.

Another way of selecting lines low in amylose is by the use of waxy lines, the carriers of highly productive plant types. It was found that low, medium, or high amylose contents can be easily restored in the hybrids with waxy lines. If a proper waxy carrier line and amylose restoring lines -- both of semidwarf plant type -- are available, we can formulate a

Table 4. Frequency distribution of plant height of parents and F_2 plants in (japonica and indica) topcross and backcross combinations.

Variety,cross	Generation	Frequency distribution of plants of given ht												Plants observed (total no.)
		36 cm	42 cm	48 cm	54 cm	60 cm	66 cm	72 cm	78 cm	84 cm	90 cm	96 cm	102 cm	
TN1	P_1		1	14	9	1								25
Yukara	P_2			9	13	3								25
IR8	P_3	1	13	8										22
$P_3//P_2/P_1$														
Family I	F_2	14	57	20	14	3	1	1						110
Family II	F_2	2	4	1	4	5	8	9	3	8	5	3		52
IR262	P_4		5	11	7	1	1							25
Jinheung	P_5								1	10	14			25
$P_4//P_5/P_4$														
Family I	F_2		3	18	35	20	8							84
Family II	F_2				2		2		4	5	3	3	2	21

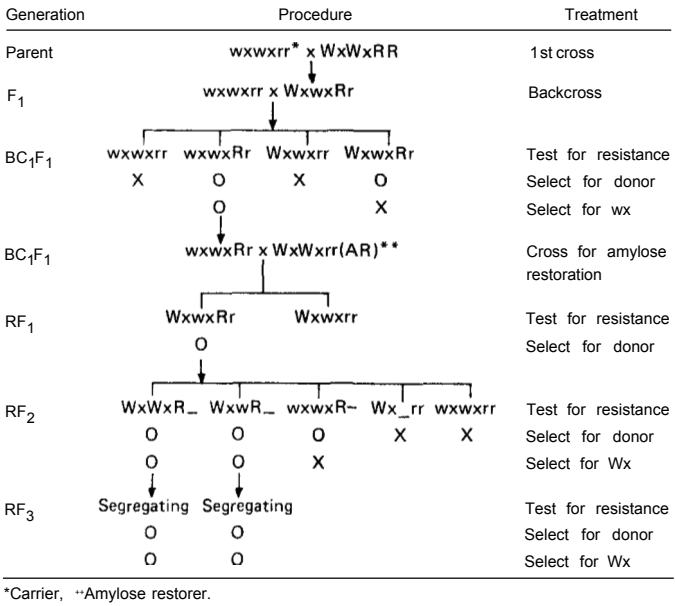
Table 5. Plant height of F_3 lines selected from backcrossed populations of japonica-indica and javanica-indica crosses.

Cross	Lines (no.) of given plant ht							Lines observed (total)
	56-59 cm	59-62 cm	62-65 cm	65-68 cm	68-71 cm	71-74 cm	74-77 cm	
IR667///IR667/0woo M.//IR667	1	3	4	10	5	2		25
IR667///IR667/Hiko M.//IR667	4	4	7	1	5	1		25
IR667///IR667/M. Sinaguing//IR667	2	3	4	7	5	3	1	25
IR667-98-12-2		4	1	3	1			9

Table 6. Grain yield of F_3 lines selected from backcrossed populations in japonica—indica and javanica—indica crosses.

Cross	Lines (no.) of given grain yield								Lines observed (total)
	<5.0 t/ha	3.0—5.5 t/ha	5.5—6.0 t/ha	6.0—6.5 t/ha	6.5—7.0 t/ha	7.0-7.5 t/ha	7.5—8.0 t/ha	8.0—8.5 t/ha	
IR667///IR667/0woo M.//IR667	3	3	3	6	6	3	1		23
IR667///IR667/Hiko M.//IR667			1	7	8	1		1	18
IR667///IR667/M. Sinaguing		2	3	5	6	6	3		25
IR667—98—1—2—2				1		3	4	1	9

very efficient breeding scheme, as shown in Fig. 6. Suppose, for example, that a blast-resistant indica variety is used as a donor parent and crossed to a carrier semidwarf waxy line selected from japonica-indica hybridization. The F_1 will be backcrossed to the waxy carrier as soon as the F_1 flowers. BC_1F_1 seedlings will be grown and blast screening conducted, and the survivors will be grown to the flowering stage. Through the pollen test, homozygous waxy plants can be selected. If the selected plants are not as good as the breeder desires, they will be backcrossed to the waxy carrier line as many times as he wishes until the selected plants show a reasonably favorable appearance. The progeny will be crossed with an amylose restorer (AR). This AR line must have an amylose content acceptable to the breeder in addition to other desirable agronomic traits. The hybrids (RF) will be tested for blast disease resistance, and the survivors will be grown through harvest. With the RF_3, seed selections could be made first for nonwaxy endosperm, and then for blast resistance through nursery tests. By the RF_4, homozygosity for both blast resistance and nonwaxiness will be attained. The final selections would have the same amylose content as the AR, besides possessing blast resistance. Throughout the procedure, chemical analysis for amylose content can be omitted.



*Carrier, **Amylose restorer.

6. Selection scheme for resistance, assuming that a dominant gene is responsible for resistance.

The carrier technique can be used successfully whenever the breeding objective is recovery of the semidwarf plant type, as when japonica-indica hybridization is practiced. This program will be most successful when the waxy carrier lines and AR possess desirable agronomic traits and reasonable combining ability for plant type and yield so that the final hybrid population ultimately segregates as acceptable preferred lines.

PROBLEMS OF JAPONICA-INDICA HYBRIDIZATION

The blast disease situation in Korean rice culture at present is similar to what it was before the release of Tongil. More than half of the pedigree lines tested were susceptible to blast in 1978, although in 1977 most breeding lines showed resistance. Plant pathologists feel that in Korea the dominant blast fungus races have changed from races that

Table 7. Delay of heading due to 1°C mean temperature decrease during the vegetative stage. Korea, 1973, Crop Experiment Station.

Variety	Days delayed (no.)	Origin
	Japonica	
Suweon #82	3	Korea
Kanto 79	2	Japan
Nongbaek	7	Korea
Jinheung	6	Korea
	Japonica-indica	
Tongil	12	Korea
IRl325-27-2	11	Korea
IRl317-316	7	Korea
	Indica	
YR6-100-9	8	Australia
IN.421-73-2	19	IRRI
Milfor 6(2)	5	Philippines
IR24	10	IRRI

Table 8. Varietal response to cold water at Chuncheon in 1978 (Crop Experiment Station).

Varietal group	Variety	Response to cold water			Fertility (%)
		Heading	Tillering	Height	
Japonica (native)	Daegoldo	Good	Good	Good	77
Japonica (improved)	Nongbaek	Moderate	Good	Good	57
Japonica-indica	Suweon 285	Poor	Poor	Moderate	24
	Josaengtongil	Poor	Good	Moderate	0
Indica	Nilo 48	Poor	Good	Poor	3
	KN-lb-361	Moderate	Poor	Poor	69
	ARC 6000	Good	Good	Poor	81

originally attacked japonica varieties to races now attacking indica varieties. It is felt that indica and japonica varieties with a broad spectrum of blast resistance should be used as donor parents to incorporate variable blast-resistant genes into a variety.

Cold susceptibility is another weakness of Tongil-type japonica-indica rice varieties. Air and water temperatures in spring (March to May) and autumn (September to November) are not optimum for rice plant growth because Korea is located in a typical temperate zone. The rice plants are subject to low temperature during both the nursery bed period in the spring and the ripening period in autumn. In the central-northern and mountainous areas of Korea, rice seedlings always suffer cold damage. The result is leaf discoloration and stunting. During the vegetative period, the decrease in daily mean temperature delays heading and low autumn temperatures result in a decreased percentage of grain maturing.

Tongil-type japonica-indica varieties are generally much more sensitive than japonica varieties to decreased daily mean temperatures during vegetative stages (Table 7). Delayed heading affects yields less in japonica varieties than in Tongil-type varieties. Responses to cold-water treatment from vegetative stage to maturity show similar trends (Table 8). Many of the japonica-indica crosses were made to introduce the cold tolerance of japonica varieties into Korea. Single crosses between japonica and indica varieties yielded more cold-water-tolerant plants, but most of them had undesirable plant types. In the

backcrosses to semidwarf rice varieties, many desirable plant types could be selected, but few of them showed improved cold—water tolerance. At a recently established cold-water testing field in Chuncheon, an expanded program is under way to screen cold-tolerant hybrid populations and improved breeding lines. Breeding materials at any growth stage can be evaluated. Several indica varieties that exhibit cold tolerance will be used as gene sources in future crosses.

Improvement of rice grain properties and eating quality is still an important target in japonica-indica breeding programs. Short growth duration, and resistance to bacterial leaf blight disease and to brown planthopper are other japonica-indica breeding targets in Korea.

The trend in crossing programs was such that during the first 4-5 years, mostly backcrosses and topcrosses were made to restore the semidwarf, highly productive plant type in japonica-indica hybrid populations. Double- and multiple-crosses were made to incorporate into the hybrid various desirable genes for disease and insect resistance, cold tolerance, good grain and eating quality, etc.

The majority of Korean rice breeders once thought it necessary to introduce climatic adaptability, grain and eating quality, and cold tolerance into hybrid populations from japonica varieties which had been cultivated in Korea for several thousand years. However, the current breeding philosophy of our japonica-indica hybridization programs is that bridge parent materials should be developed first by the backcross or topcross method, and then combined by double or multiple crossing.

ADVANCES IN REMOTE HYBRIDIZATION OF PLANTS IN CHINA

SHAO QI-QUAN (SHAO CHI-CHUAN) and
JIANG XING-CUN (CHIANG HSING-TSUN)

Remote hybridization is, in essence, interspecific and intergeneric hybridization. It is an important way of producing new varieties of crops, even new species. However, it often gives rise to difficulties, e.g., interspecific incompatibility including the absence of seed-set on crossing and sterility of F_1. Progress has been made in this field in China.

Plant hormone treatments, culturing of hybrid embryos in vitro, chromosome doubling of F_1, and test-tube fertilization of corn ovaries were tested as means to overcome interspecific incompatibility. Good results were obtained in wheat, cotton, sorghum, sugarcane, rice, etc. Although much work has been done in this field, only a few examples will be discussed here.

WHEAT

First, I would like to talk about wheat and the results obtained in octoploid Triticale by a research group under the guidance of Prof. Pao Wen-Kwei (Bao Wen-kui) in the Research Institute of Crop Breeding and Cultivation, Academy of Agricultural Sciences of China. Octoploid Triticale with chromosome configuration AABBDDRR was successfully established through screening of about 20 "bridge varieties," such as Chiang-tung-min, and Red Star spring wheat, which were very easy to cross with rye. A large number of diverse hybrid varieties were thus produced for further selection. New high yielding, stiff-strawed, and disease-resistant varieties were released and planted in about 53,000 ha in Yunnan-Guizhou plateau. Octoploid Triticum-Agropyron with genotype AABBDDEE was obtained by intergeneric hybridization between *Triticum aestivum* (♀) and *Agropyron elongatum* (♂) and chromosome doubling of the F_1. This work was carried out by a research group of the Institute of Botany of North-Western China under the guidance of Prof. Li Zen-shen. Backcrossing these octoploids with wheat produced alien addition lines, a rather stable aneuploid, with genotypes AABBDD + 2 (IE up to AABBDD + 2(7E). A new variety Xiao yen No. 4 was bred from the cross between wheat

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Fengshou No. 1 and Triticum-Agropyron octoploid Xiao yen No. 759 (an alien addition line). Xiao yen No. 4 is a medium-maturing variety that is high yielding, semiwinter hardy, semidwarf, and rust and drought resistant. It is now grown on more than 266,000 ha in China.

COTTON

Procedures to overcome the low seed set of interspecific hybrids and the sterility of F_1 plants were developed by Prof. Liang Zheng-lan and his group at the Institute of Genetics, Academia Sinica. Reciprocal backcrosses were made of 21 combinations between *Gossypium hirsutum* and *G. barbandense* (26 genomes, n = 26), and *G. arboreum* and *G. herbaceum* (13 genomes, n = 13). Once on the first day of crossing and once a day on 4 succeeding days, the hybrid bolls received a treatment of aqueous, boll-set, spray solution which was alternated daily between 50 ppm GA and 40-320 ppm NAA. Ninety percent boll set was achieved, with an average of 2.3-3.6 well-differentiated embryos per boll. More than 80% of the embryos were cultured successfully in vitro. The resulting plantlets grown in test tubes had a survival rate of 80%; 40% of these survived transplanting to pots and grew into whole plants.

In 1975, 10 ppm colchicine was added to the medium to increase the effect of colchicine treatment. Fertility restoration of the F_1 plants was 100%. The F_1 plants were grown in pots over the 3 winter months. The bolls averaged 4/plant, and the large seeds, 4.8/boll. More than 95% of the seed germinated. This technique resulted in F_2 seeds or seeds of backcrosses being obtained in a single year so field trials could begin the year following.

SUGARCANE AND SOGHUM

A significant difference usually occurs in the ease of crossing depending on the choice of male and female parents in remote hybridization. The hybridization of sorghum with sugarcane is a good example. If sugarcane is used as the female parent, and sorghum as the male, the cross is easily accomplished, but the reciprocal is very difficult. From the standpoint of agricultural production and broadening the genetic resources of the sugar crop, it is necessary to use sorghum as the female parent and sugarcane as the male because land suitable for sorghum production is much more widespread than land suitable for sugarcane. A new variety that yields sorghum grain from

the ears as well as sugar from the stem has been bred by crossing sorghum with sugarcane in Hainan Sugarcane Breeding Station of the Research Institute of the Sugarcane Industry and Food Science, Guangdong province. The species has been released for production. The new hybrids are now experimentally grown in nine provinces -- Guangdong, Guangsi, Shansi, Honan, Shensi, Hubei, Sichuan, Liaoning, and Gansu. Crystallized sugar can be produced from the stem. In general, the output of the stem is about 22.5 to 52.5 t/ha. It contains 12—13% saccharose, about 1.79% reduced sugar, and about 61.0% juice. Cytological observation of pollen mother cells showed a significant variance in chromosome numbers. For example, a variety, designated 73/18 exhibited variance in 9 of 10 plants examined. Most of the cells had n = 28 chromosomes. The pollen mother cells were 33—66% larger than those of the sorghum parent, although the chromosomes were smaller.

RICE AND SORGHUM

A research group under the guidance of Prof. Zu De—ming, Institute of Crop Breeding and Cultivation, Academy of Agri—cultural Sciences of China, investigated intergeneric hybrid—ization of rice with sorghum. Hybrids were first obtained in 1960 with rice variety *Rinfang* as the female parent and sorghum variety *Henjali* as the male. The cross was made by emasculating the rice flowers with hot water and pollinating them with sorghum pollen. Hybrid seeds from this cross were sown the next spring. The F_1 plants were tall and strong with wide leaves. But heading did not occur until early October, a month and a half later than in the female parent. Because temperatures were decreasing, the rice plants were transferred to the greenhouse where, after heading, very large spikes with many flowers appeared, but no seed was set. The F_1 plants were highly sterile. In the spring of 1962 stocks were transplanted into a net chamber, where they grew vigorousla and produced many tillers. This time a few seeds were set but they were poorly filled. More seeds were sown in the spring of 1963. Some failed to germinate, some gradually died in the seedling stage, and only 26 plants ultimately survived. The F_2 plants exhibited different performance characteristics and segregated widely, a phenomenon never before observed in crosses between japonica and indica subspecies of rice. Strong segregation also occurred in the F_3 and F_4. Similar variation was observed in progenies from a combination of the rice Jingyin No. 1 with sorghum variety Yuan Za No. 10. Many more violet and black spots were found on the spikes. It was not until F_8 plants were obtained in 1973 that cytological

observations were initially made. Variation in chromosome number of the root-tip and pollen mother cells were observed in the lines 923 and 5216 along with significant morphological variations. These lines became more stable in F_{13} and the genotype was similar to that of the rice plant. The superior lines of rice-sorghum hybrid have large spikes and grains, are resistant to leaf blight and drought, and have high photosynthetic efficiency. They are now undergoing production testing.

In the Laboratory of Cytology, Institute of Botany, Academia Sinica, the process of fertilization of the rice-sorghum hybrid was cytoembryologically observed. Studies under the guidance of Prof. S. H. Wu showed that sorghum pollen germinated well on rice stigma, and some pollen tubes elongated normally and penetrated the embryo sacs. Double fertilization and embryos with 8 to 10 cells and endosperm nuclei were detected in 10.8% of the materials that were fixed 3 days after pollination. The embryos with a maximum 80 cells were in the material fixed 5 days after pollination. The maximum endosperm was in materials fixed 7 days after pollination. But no differentiated embryos or mature seeds were obtained. It is suggested that, although rice may be fertilized with sorghum, hybrid embryos are very difficult to develop normally. In vitro culture of hybrid embryos may be valuable.

Isozymes in rice-sorghum hybrids were investigated by Prof. Zhon Guang-yu who guides a group at Shanghai Institute of Biochemistry, Academia Sinica, studying the molecular basis of remote hybridization. In the offspring of Rinfang/Henjali hybrid, a new band of esterase isozyme absent in the parent Rinfang was observed. The same band was also found in wax ripening and fully matured sorghum seeds and seeds 3 days after germination. An intermediate band between those of the rice and sorghum occurred in the electrophoretogram of the alcodehydrogenase isozymes.

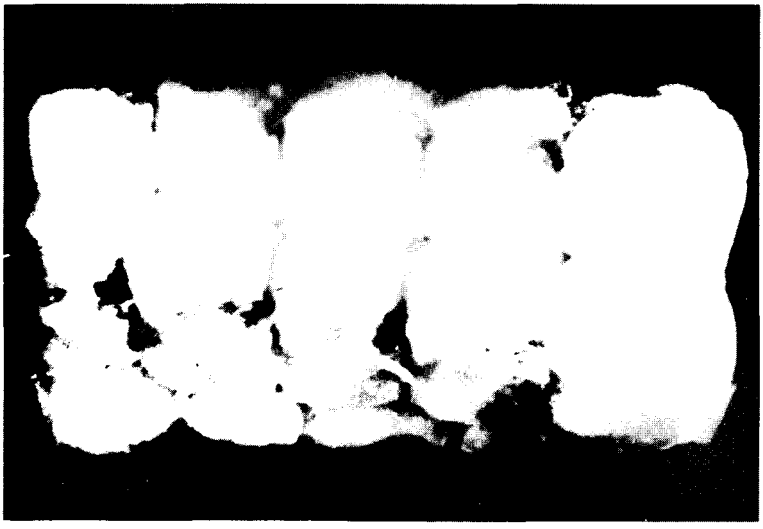
TEST TUBE FERTILIZATION

I would like to discuss briefly the use of the test-tube fertilization technique in remote hybridization. Since the pollination and fertilization of *Papaver somniferum* L. ovules in vitro were first reported (Kanta et a1 1962), similar experiments have been carried out by others with other plants of seven represented genera in Solanaceae, Crufiferae, and Dianthus of dicotyledons. The test-tube fertilization technique was applied to overcome the incompatibility of

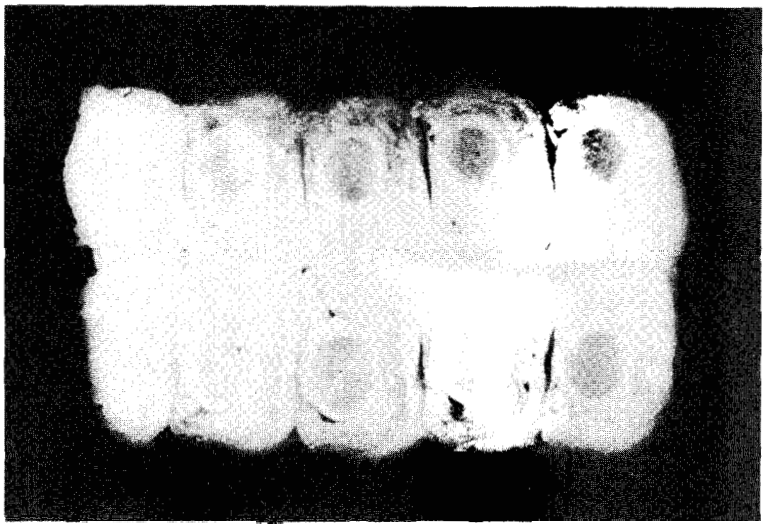
interspecific hybridization, and several interspecific hybrids of Dianthus (Zenkteler 1965) and Compositae (Watanabe 1977) were obtained. The potential of this technique in genetic and breeding investigation of crops was immediately recognized. Experiments with corn ovaries pollinated in vitro were first carried out in 1975, but not until 1977 was successful fertilization reported (Gengenbach 1977). Fertilization took place on a simplified medium prepared mainly from natural potato extracts (Shao et a1 1977).

Then experiments on the in vitro fertilization of ovules were carried out. From unpollinated corn a block with 10 ovaries was cut under sterile conditions. The style and ovary wall were removed from the upper third part of the ovary. A large number of naked ovules were obtained which then were inoculated on White—agar medium in test tubes. Sterilized corn pollens were directly placed on the cut of the naked ovules.

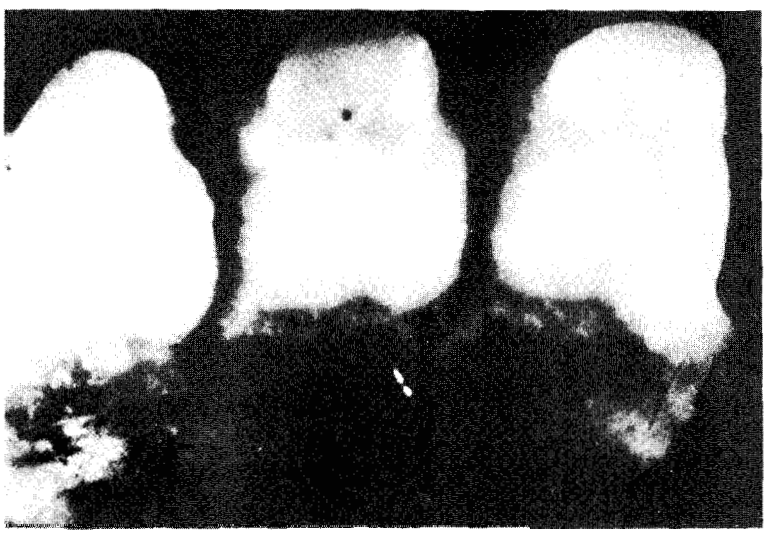
Figures 1 through 6 demonstrate the successful in vitro fertilization of corn ovules.



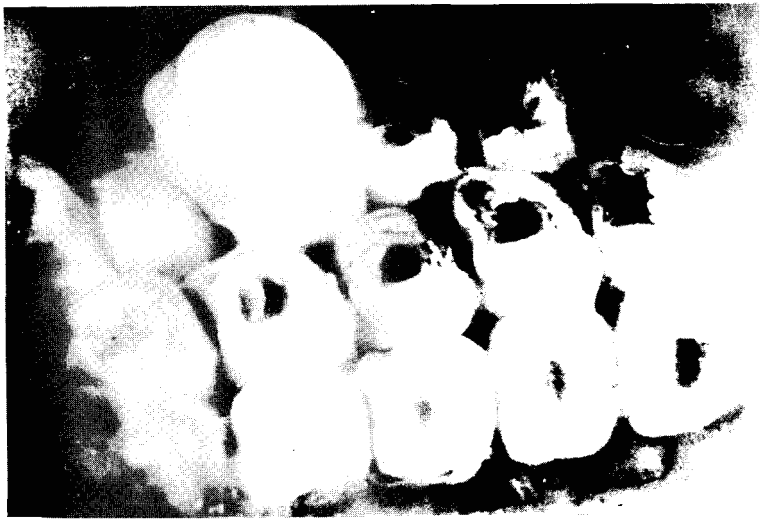
1. A block of intact corn ovaries before cutting.



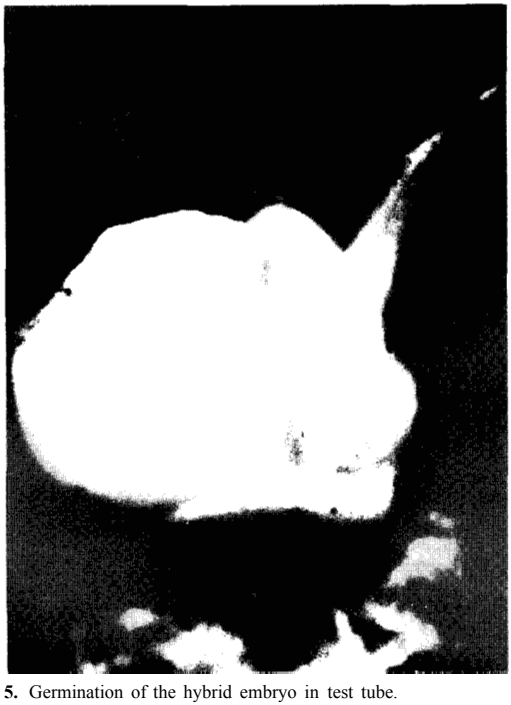
2. Naked ovules after cutting off the upper 1/3 of the ovaries.



3. The elongation of the ovules 24 h after cutting.



4. Seed forming after pollination of naked ovules.



5. Germination of the hybrid embryo in test tube.

Table 1. Experimental results of test-tube fertilization of corn ovules.

Test no.	Combination	Ovaries treated (no.)	Ovules received		Seed formation	
			No.	%	No.	%
1	Patangpai x Progeny Chase	216	151	69.8	2	1.32
2	Patangpai x Progeny Chase	532	314	59.8	2	0.64
3	Patangpai x Progeny Chase	535	263	49.1	7[a]	2.66
4	Patangpai x Progeny Chase	41	12	29.2	1	8.33
5	Patangpai x Progeny Chase	71	30	42.2	0	0.00
6	Huobai X x Progeny Chase	803	406	50.5	0	0.00
7	Shuanshubai x Progeny Chase	1129	556	49.2	1	0.18
8	197-1 X x Progeny Chase	1201	886	73.7	1	0.11
9	197-1 X x Progeny Chase	946	707	74.7	0	0.00
	Total	5574	3325		14	
	Average			59.6		0.42

[a]Five seeds have endosperm only.

6. Seeds set after in vitro fertilization of ovaries.

Nine sets of experiments were carried out during the summer of 1978. Fourteen seeds were obtained from test—tube fertilization of 3,325 ovules and seed set was 0.42% (Table 1). The hybrid embryos matured about 20-22 days after pollination, i.e. germinated in the test tubes. The chromosome number of the root-tip somatic cells was 20, thus verifying that the plants obtained from test-tube fertilization were intervarietal hybrids, but not parthenogenetic haploids. Studies on the intergeneric hybridization of corn and sorghum are in progress.

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MODIFIED BULK POPULATION METHOD FOR RICE BREEDING

H. IKEHASHI and H. FUJIMAKI

The bulk breeding method has long been used by many breeders working with self—pollinated crops. Its relative advantage over the pedigree system has been discussed by Allard (1960) and Frey (1975). Yet innovating this method through some new ideas such as rapid generation advance and male sterile—facilitated composite may increase its importance. Although new breeding methods such as tissue culture and distant cross are still far from large—scale implementation, it is already technically feasible for breeders to apply the bulk method.

So far not many rice breeders have utilized the bulk method in the tropics probably because the pedigree selection of biparental progeny provides them with adequate progress. But in some areas of rice breeding, it is obvious that the bulk method is most efficient. During the 30 years since the bulk method was first employed in the rapid generation advance (RGA) for rice breeding in Japan, this technique has steadily gained popularity among rice breeders (Kikuchi 1978). In 1977, 21 out of 33 rice cultivars released by the government were developed by RGA. Furthermore, 24 leading varieties, which now cover 44% of the total rice area in Japan,were bred through RGA (Nakane 1979).

This paper lists the various modifications of the bulk—population method and their scope, particularly from the perspective of male—sterile facilitated composite populations.

International collaboration is an entirely new challenge for breeders. Some collaborative breeding programs will be discusssed as it seems that the potential of the innovated bulk method can be exploited most efficiently in international programs.

BULK POPULATION METHOD AND ITS INNOVATIONS

Historically the bulk method of breeding has been adopted to handle extremely large volumes of breeding materials in an inexpensive way and to take advantage of natural selection. To improve populations before pedigree selection, segregating plants should be exposed to environmental or biological stress. Nilson Ehle of Sweden was one of the first breeders to adopt

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this method in an attempt to combine the winter hardiness of the Square-head variety of wheat with the high yields of the Stand-up variety of winter wheat to obtain high yielding and winter hardy types (Allard 1960).

The most serious question in the bulk method is whether survivors tolerant of a given stress are also superior in productivity. Early works on the use of variety-mixture have been reviewed by Allard (1960). Suneson (1956) advocated a method of evolutionary breeding based on the improved productivity of survivors in segregating populations after several generations of exposure to natural selection. Generally, experience gained with bulk populations suggests that natural selection will cause change in a desired direction or no change at all. In early breeding work at the International Rice Research Institute (IRRI), the bulk method proved to be impractical for breeding high yielding dwarf types, which are poor competitors. Because the common source of dwarfism is genetically recessive, selection of the short-statured plants in early generations has been the most effective method to obtain fixed lines (IRRI 1966, 1967).

Now, the most common use of the bulk population method is to obtain homozygous lines with minimum effort and expense.

Single seed descent

A modification of the original bulk method that has attracted many plant breeders, is a breeding procedure in which fixation with minimum bias from potential variability is the major aim. In soybean, oats, and wheat, several workers have recently reported the advantages of the procedure under various yet unfixed terminologies, i.e. modified pedigree method, random method, or single seed descent (SSD) method (Brim 1966, Kaufmann 1971, Empig and Fehr 1971, Boerma and Kooper 1973, Knott and Kumar 1975).

In Japan most rice breeders have adopted this modified bulk method procedure under the name bulk method following extensive collaborative studies in the late 1950s (Okabe 1967, Kikuchi 1978). To prevent any drift in segregating populations, the SSD method proposes that a single seed should be harvested from each F_2 plant to grow F_3 populations and that the same procedure be repeated until F_5-F_6. Ikehashi and HilleRisLambers (1977) discussed the details of this method in relation to rice breeding.

Rapid generation advance

Rapid generation advance (RGA) is not a breeding method, but a way of growing bulk populations. Since the major aim of SSD

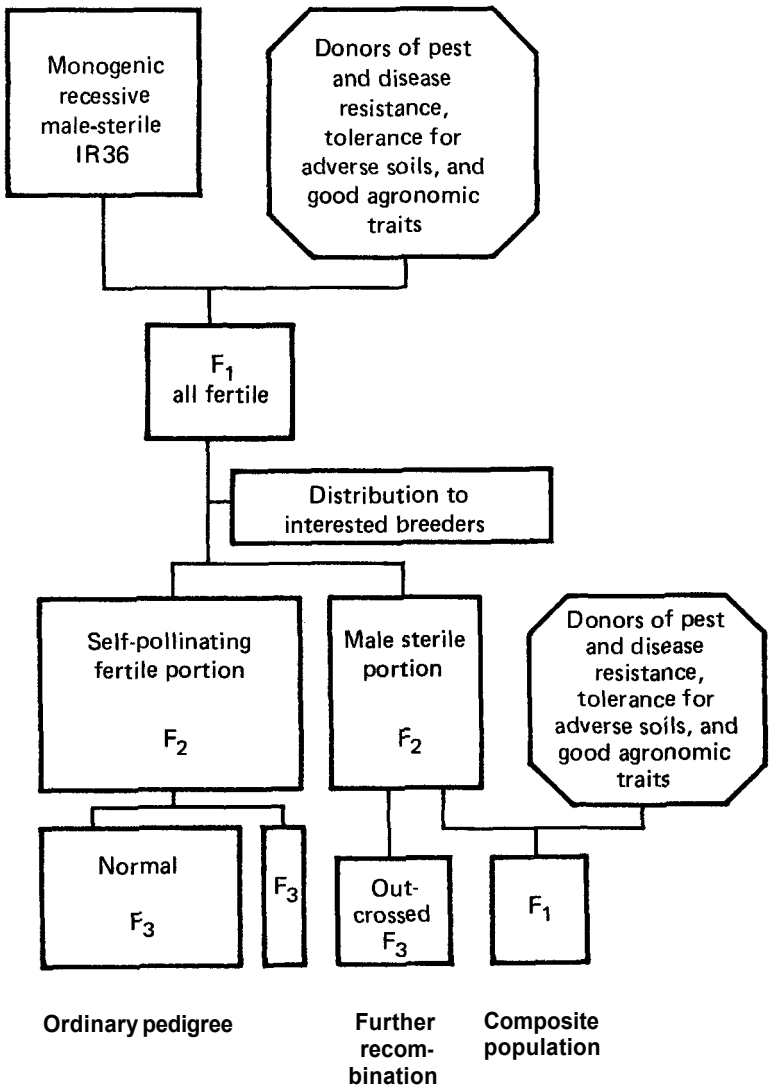
is to attain homozygosity prior to pedigree selection, it is natural to come up with the idea of rapid cycling of breeding materials that shortens the growth duration of a given population. A large number of plants can be accommodated in limited space and dense spacing because only one or two seeds from each plant are enough to compose following generations. Thus, the SSD method is the most appropriate for RGA. Success in RGA largely depends on the minimum growth duration that can be induced in a specific growth condition. In wheat breeding, it is possible for breeders to grow five to six generations in a single year (Mukade 1974). For breeding of photoperiod-sensitive rice in the tropics a test at IRRI demonstrated the feasibility of growing three generations a year (Ikehashi and Nieto 1977).

Male sterile-facilitated composite

In traditional methods used for improving self-pollinating crops, genetic recombination occurs to a limited extent in a hybrid population because selfing populations rapidly approach homozygosity. One method for overcoming the limitation imposed by biparental crosses is multiple crossing. Although multiple crossing increases the opportunity for genetic recombinat ions among alleles from various source strains, it is difficult to get enough F_1 seed to retain all of the possible parental alleles.

Another method for increasing genetic variation is to make a composite, a method extensively applied to barley breeding by Suneson (1956). The F_2 of several crosses are bulked into a composite population. This procedure ensures the maximum variability for selection in diverse environments, but each of the component plants can have germplasm from no more than two parental strains because there is no outcrossing among hybrid plants in the population.

Male sterility was incorporated into a composite hybrid population to enhance recombination. From the very early stage of the use of the bulk method, the male sterile-facilitated composite was tested by Suneson (1945). Source material in the Suneson experiment was a series of composite populations derived from a number of parents by a succession of pairings and crossings of F_1 plants. One of the composites included male sterility to realize continuing gene recombinations through natural crossing concurrent with natural selection (Suneson 1956). Several barley composites developed by means of the male-sterile genes were investigated by Jain and Suneson (1966) for quantitative genetic changes in variability and productivity. Great genetic variability was observed in the composite with a high degree of outcrossing, but the increase in genetic variability was not accompanied by a corresponding increase in productivity.



1. Use of monogenic male sterility (Singh and Ikehashi, unpubl. data).

So far male-sterile-facilitated hybridization has not been employed systematically in rice breeding. However, male sterility was used in Japan very recently for some initial trials of recurrent selections and backcrossing (Fujimaki et a1 1977, Fujimaki 1978, 1979). It is available, too, for indica rice as some strains of monogenic, recessive male-sterile mutants have been developed from IR36 at IRRI (Singh and Ikehashi, unpubl. data) (Fig. 1).

Integrated population breeding

The original concept of the bulk method has been remarkably diversified by SSD, RGA, and male sterile—facilitated composites. Breeders are now in a position where they can integrate one or more of the bulk method-related means to build the most efficient system for any breeding objectives. It is particularly significant that all the modifications have been developed to handle maximum genetic diversity with the least cost and in the shortest period.

RELATIVE ADVANTAGES OF THE BULK METHOD

To construct the most efficient system based on the various means that have been developed through the bulk method, their general advantages or disadvantages should be taken into account.

A long list of literature citations would be needed to cover all the arguments on the advantages and disadvantages of the bulk method. Because it would be almost impossible to accommodate the great variation of breeders' opinions on the subject, only some basic considerations in the evaluation of the breeding method will be discussed here. Major genetic processes after hybridization should be recovery of recessive phenotypes, fixation, and enhanced genetic recombinations.

Recovery of recessive types

When an economically important trait is controlled by several recessive genes, the ratio of desired plants to those bearing dominant gene-governed characters will be small in the F_2 population. Recessive types should be recovered in later generations until half the population manifests the recessive character. Therefore, breeders should find more of the plants with the recessive character in later generations. Brim (1966) considers this an SSD advantage.

Advanced fixation

Attaining homozygosity before pedigree selection is apparently the major reason many breeders are adopting the bulk method.

Sakai (1951) examined the delay of fixation as a function of close linkage and increased chromosome numbers, then concluded that individual selection of autogamous plants in hybrid progeny should be commenced in later generations. Therefore the bulk method would be preferable to the pedigree method, so far as characters that are difficult to identify are concerned. In the same paper, Sakai introduced a concept of the efficiency

of selection -- which means the recovery of true—bred types from selections that are phenotypically similar -- and argued that a very large number of individuals would be required to get a certain rate of recovery. This number would become very small in later generations as fixation advanced.

Enhanced genetic recombinations in F_2–F_3

To visualize quantitative changes in correlated characters may not be so easy in the course of generations. But theoretical investigations of the expected length of parental linkage blocks revealed that a considerable amount of genetic recombination is expected in the F_2 and F_3 population under self—pollination (Hanson 1959a).

Computer simulation was used to estimate the frequency of genotypes of linked loci through succeeding generations at varying levels of linkage and chromosome numbers. It was shown that the proportion of good recombinants from undesirable linkages can be increased up to the F_4—F_5 through additional recombination (Ikehashi 1977). Postponement of selection to later generations, therefore, would be an efficient way of getting more desirable recombinants (Table 1).

Little experimental work has been done to verify the theoretical estimations. In rice hybrid populations, Okada and Nara (1958) reported the increase in variance of some agronomic characters from F_2 to F_5 and concluded that the segregating genotype in each generation had been accumulated in the bulk—harvested population in the course of its fixation. Kaufmann (1971) reported the transgressive variations of the progeny lines from SSD, which contributed to substantial progress in his wheat breeding.

Simplified handling procedure

The bulk method has been developed to handle the large genotypic variation involved in multiple crosses or composite populations. It is commonly used to obtain homozygous lines with minimum of effort and expense. Such operational merits as reduced field space and economy in early generation record keeping have attracted breeders.

Evaluation of nearly fixed lines is a distinct advantage in the bulk method because the tremendous segregation within each line makes it very difficult to evaluate pedigree selections at F_3. This is particularly so when the components of the cross have widely different characters. The feasibility of RGA is a remarkable advantage of SSD (Grafius 1965, Brim 1966).

Table 1. Population size and well-recombined fractions in SSD (Ikehashi 1977).

Population size	Recombination value	Individuals (no.) in the well-recombined fraction[a]						
		F_2	F_3	F_4	F_5	F_6	F_7	F_8
200	0.3	0.3	1.5	2.4	2.5	2.0	1.7	1.7
	0.5	2.4	5.2	6.0	6.4	5.5	5.1	5.0
400	0.3	0.8	2.9	5.4	4.6	4.5	4.3	3.9
	0.5	5.4	9.3	12.5	12.2	10.5	10.3	9.8
800	0.3	1.8	10.1	10.1	9.5	8.5	8.0	7.9
	0.5	10.8	20.1	24.3	23.6	20.0	17.8	17.1
1600	0.3	3.4	16.0	20.8	21.0	20.3	19.8	19.3
	0.5	20.7	40.7	48.3	45.1	40.4	37.0	36.3

[a] Means of 10 trials.

NEW APPROACHES IN THE BULK METHOD

Under selfing, fixation of each locus advances very rapidly. In a monogenic segregation between two inbred parents, the F_2 progeny is 50% heterozygous and 50% homozygous. Heterozygosity is reduced to 50% in every selfing generation. In a digenic linkage, only double heterozygotes generate new recombinants when a crossover occurs between the two loci concerned. Therefore, the rapid approach to homozygosity under selfing, where the parental genotype is permitted to constitute a major portion of the hybrid population, can be a barrier to the production of new recombinants.

Intermating to promote genetic recombinations

For promoting frequent recombinations, intermating can be remarkably effective as a large number of heterozygotes are generated in every generation. Theoretic estimates of the expected lengths of parental linkage blocks under various mating systems were presented by Hanson (1959a,b). According to his prediction, effective recombinations are increased to a great extent by repeating intermating between progenies originating from a polyparental cross.

Jensen (1970) enumerated three disadvantages of the conventional breeding systems for a predominantly self-pollinated crop: 1) the limited size of the gene pool utilization (usually two parental crosses), 2) the restrictions of genetic variability and recombination potential through intensive inbreeding, and 3) the absence of intercrossing among hybrid progenies. To overcome these disadvantages he proposed a new breeding technique called diallel selective mating (DSM).

Genetic variations of cotton hybrid populations were largely expanded (Miller and Rawlings 1967), and undesirable genetic associations between fiber strength and high yield were effectively reduced by intermating among hybrid progenies (Meredith and Bridge 1971). These theoretical and experimental studies show the effectiveness of intermating among hybrid progenies to advance effective recombinations in predominantly self-pollinated crops.

Intermating in self-pollinating crops requires additional time and money for one generation. If we think of the increased recombinations in the course of selfing from F_2 to F_4, the merits of intermating should be very distinct to compensate the hybridization cost. By computer simulation, Baker (1966) evaluated the amount of intermating required for efficiency against possible drift of the useful recombinants. He concluded

that 20-30 intercrossings among randomly selected F_2 plants would be advantageous over selfing in the F_2 population. However, his estimate was based only on digenic linkage, so the amount of intermating should be larger in actual breeding practice.

Stam (1977) compared random mating and selfing by computer simulation of continued individual selection. He concluded that random mating gives no substantial advantage over selfing during early generations (up to F_4), irrespective of the number of loci and the intensity of linkage. But in later generations, random mating always is superior to selfing, especially when many loci are involved, because there is an optimum moment for a single round of intermating to be inserted. Likewise, Bos (1977) evaluated by mathematical means the relative advantages of intermating and selfing. He concluded that if there is no selection in the F_2 population, then random mating of F_2 plants has little effect as far as the following generations are concerned, and plants with the desired recombination are fewer in the F_3 from intermating than in the F_3 from selfing. This conclusion follows from the fact that random mating yields all kinds of homozygous and heterozygous genotypes and not an increased fraction of desirable homozygotes. Earlier, Pederson (1974) found also by computer simulation that truncation selection is usually preferable to intermating as a procedure for increasing the proportion of desirable homozygotes in a population.

Although the benefit of intermating, as advocated by Hanson (1959a), could be considered valid in breaking up parental linkage blocks, it would not be easy to demonstrate the distinct advantage of intermating over continued selfing. Experimental verification would be subject to sampling error and bias introduced by experimental materials and conditions.

At least from the practical point of view, it should be noted that unless there was prior selection of the desirable plants, intermating among the progeny would not be worthwhile. Therefore, particular attention should be paid to intermating in a system of recurrent selections.

Male-sterile-facizitated recurrent selection

Recurrent selection is a breeding technique that increases the frequency of desirable genotypes in a hybrid population by cyclic applications of intermating and selection. The technique has been used exclusively in heterosis breeding for cross-pollinated crops. Breeders of self-pollinated crops, however, must not be discouraged from employing recurrent selection when random mating is achieved without great difficulty.

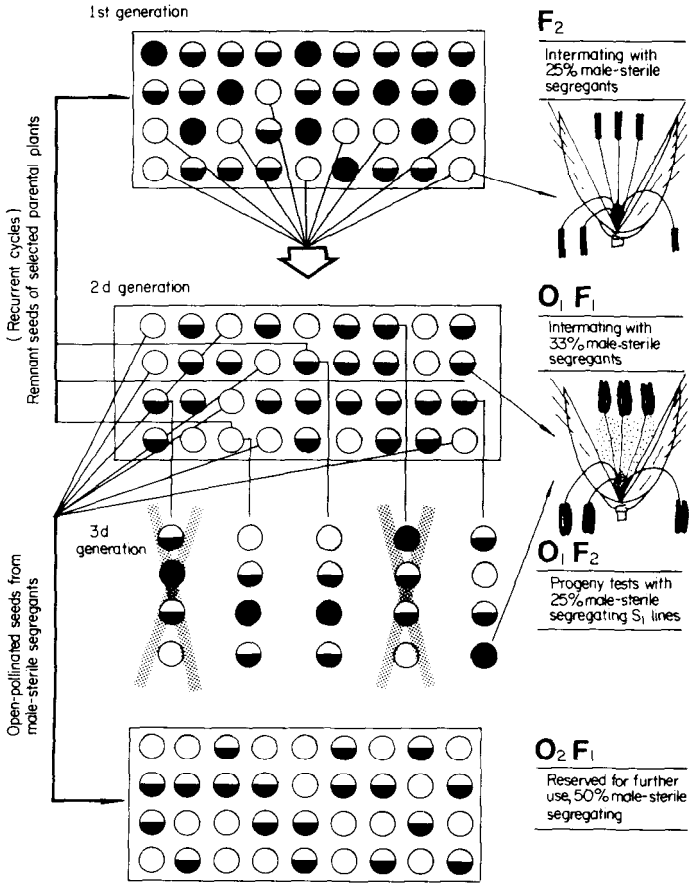
Male sterile-facilitated recurrent selection (MSRS) was first suggested by Gilmore (1964) for the improvement of naturally self-pollinated species. Application of genetic male sterility to recurrent selection was also proposed in the breeding of sorghum and soybean (Doggett and Eberhart 1968, Brim and Stuber 1973).

Backcross breeding may be regarded as a kind of recurrent selection in the sense that the recurrent operation of crossing and selection continuously increases the number of desirable genotypes. Breeding models of the male sterile-facilitated backcrossing (MSBC) technique as well as of MSRS were presented by Fujimaki (1978b, 1979).

For rice breeding, no useful allele for complete male sterility was available until male-sterile mutants were artificially induced by gamma ray irradiation by ^{60}Co sources or a chemical mutagen ethleneimine (Fujimaki et a1 1977). Male sterility of these mutants was found to be controlled by a single recessive allele that was used for establishing inter-mating or backcrossing populations. Such mutants are now available for indica rice (Singh and Ikehashi, unpubl. data) .

Description of MSRS for rice breeding. A model of recurrent selection for rice improvement by means of the recessive male-sterile allele is presented in Figure 2. A breeding program begins with composing a foundation population to which selection will be applied. The population, which is constructed to segregate for male sterility, is to be derived from various genetic sources such as biparental crosses, double crosses, polycrosses, or diallel crosses and grown in an isolated condition to advance outcrosses between male-sterile segregants and fertile ones.

In the crossing phase, random mating is attained by collecting open-pollinated seeds from male-sterile segregants. Mass selection may be applied to the segregating population, especially for days to heading to synchronize flowering time within the population. Generally speaking, however, intensive selection of highly heritable characters should be avoided because of the possibility of losing heterozygosity and opportunities for free recombination. In the selection phase of the recurrent cycle, selfed seeds are harvested from self-fertile plants to develop S_1 progenies. Some selfed seeds of each plant are reserved for later use and the others are used for S_1 progeny testing. Every S_1 line is supposed to monogenically segregate for male sterility. It is difficult, therefore, to evaluate agronomic traits relating to seed production such as yield. But the other agronomic traits such



2. Male-sterile-facilitated recurrent selection for rice breeding.

as grain quality, resistance to various diseases and insect pests, cold tolerance, plant type, culm strength, and leaf senescence can be evaluated by observing S_1 progenies.

The results of S_1 progeny evaluation indicate that S1 lines with desirable agronomic traits should be selected. Recurrent selection is terminated when some lines showing promise for practical use are obtained. Otherwise, S1 lines superior in some agronomic traits are marked and the reserved seeds of their parent plants are mixed to compose another foundation population for further recurrent selection.

Application of MSRS to multiple disease resistance. The MSRS technique seems suitable for improving complexly inherited multiple disease resistance. We have breeding projects under way to transfer polygene-controlled horizontal (or field) resistance to blast, major-gene resistance to bacterial leaf blight, and major-gene-affected stripe resistance of an upland cultivar into the most popular rice variety Nipponbare. A multiple-disease-resistant strain Imochi 314, a selection from a cross involving as one of the parents an upland cultivar was hybridized with a male-sterile mutant of the commercial cultivar Nihonmasari in 1975. A widely spaced F_2 population was raised in 1977. Male-sterile segregants appeared in a monogenic ratio. Open-pollinated seeds were collected from male-sterile plants to compose the first intermated 0_1F_1 population. This population was grown in 1978 and intermated again to obtain 0_2F_1. During the two intermating generations, mass selection was applied to heading date, not only to synchronize the flowering time within the population but also to make it easy to cross selected plants with the Nipponbare, which subsequently would be used as a parent. No selection was applied for the other agronomic traits. The undesirable characteristics of the upland cultivar were predominant in 0_1F_1. A few backcrosses to an adapted paddy rice cultivar seem desirable to converge the genetic variation of hybrids to a favorable direction.

The 0_2F_1 population will be planted between rows of the most popular paddy rice cultivar Nipponbare to introgress its useful germplasm to the hybrid population. Introgressed hybrid plants arising from open-pollinated seeds of male-sterile segregants will be selfed to recover recessive male sterility. The selfed population will segregate for various agronomic characteristics and the frequencies of multiple-disease-resistant genotypes are expected to be lowered by introgressive hybridization to Nipponbare. For this reason, selection for multiple disease resistance will be necessary at this stage of the recurrent breeding cycle. The selected hybrid population will again be subjected to another cycle of recurrent selection. This kind of cyclic selection procedure will be continued until the population is improved enough to obtain the Nipponbare-type promising segregants with multiple disease resistance.

An aggressive use of MSRS was reported in a barley program in Arizona, USA (Ramage 1977). To produce a short-straw barley

population, large numbers of four populations, each from a different source of short straw, were grown. Short-strawed plants (both male sterile and male fertile) were selected from each population and crossed with short-strawed plants in each of the three other populations by the use of male-sterile females. Crossed seeds from each of the six cross combinations were bulked and planted at Montana in the summer. The F_1 was grown and combine-harvested without selection. Then during the winter in Arizona, a large F_2 population was grown for intermating after the selection of short-strawed plants. Thus, male-sterile-facilitated recurrent-selection populations have been established for a number of characters, including earliness, shatter resistance, large awns, drought tolerance, resistance to powdery mildew and adaptation to being grown during winter in Arizona and during the summer in Montana.

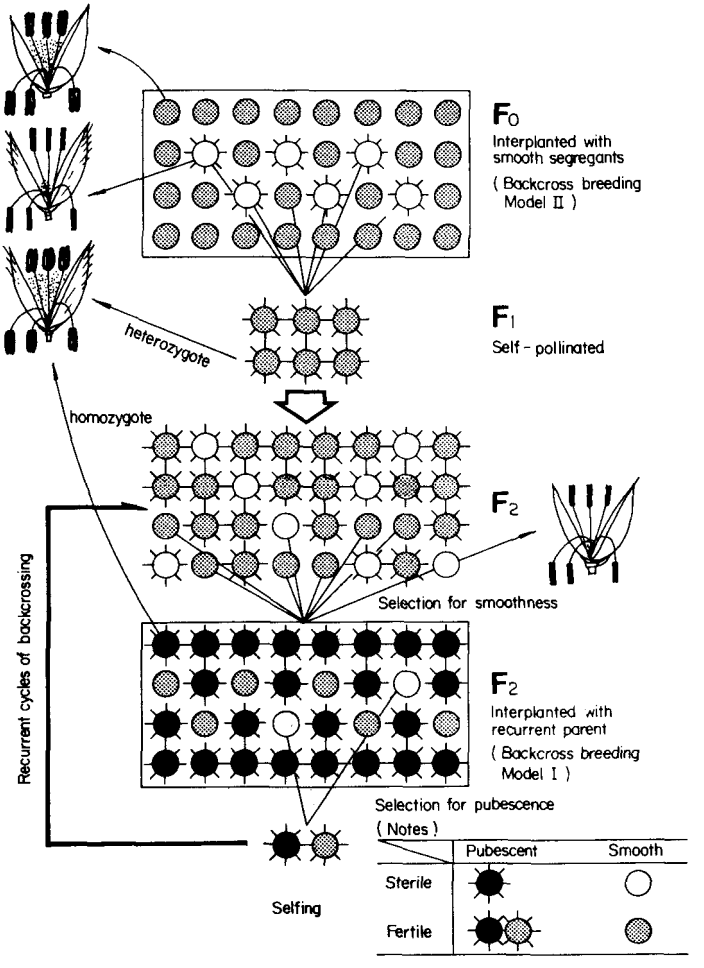
Ramage (1977) reported another way of exploiting the MSRS populations, which involves use of the male-sterile parent as a source of genetic materials for a particular character in a backcross program.

Practical use of MSBC. We may place the backcross breeding technique under the category of recurrent selection in the sense that frequencies of desirable genotypes are gradually increased by repeating recurrent cycles of crossing and selection. Models of a backcross breeding technique in which a recessive male-sterile factor is used have been proposed by Fujimaki (1978a) (Fig. 3).

The character smoothness of rice plant is known to effectively reduce dust generated in harvesting, threshing, and hulling operations. This useful character is governed by a recessive allele and seems to be transferred by MSBC.

We are attempting to transfer a recessive allele for smoothness from a foreign rice variety to a domestic one by MSBC. Genetic variation in the hybrid population has been. remarkably reduced through the last two backcrosses. The present hybrid population closely resembles the recurrent parent Nipponbare in days to heading, plant stature, grain shape, and other morphological traits. But smooth segregants have smaller grains and poorer tillering ability than pubescent ones.

Utilization of RGA with MSRS or MSBC. Although MSRS is a powerful breeding technique, it has the drawback of prolonging the breeding cycle by inserting intermating phases, during which no fixation is advanced. To advance MSRS to an equivalent basis with conventional breeding techniques, RGA should be effectively employed to shorten the breeding cycle. For instance, after several intermatings or recurrent selections, RGA should be



3. Transfer of smoothness using male-sterile-facilitated backcrossing.

applied to the recombinant—rich hybrid population to accelerate fixation.

MSBC, a modified form of MSRS, is an excellent technique for skipping laborious artificial backcrosses in all recurrent cycles. When we use a recessive allele for male sterility,

however, two generations are required for a single backcross to recover homozygous segregants for the recessive male sterility in a hybrid population. It is easy to complete one backcrossing cycle in a year by raising a selfing generation in a greenhouse during winter. Thus, we can make the best use of MSRS or MSBC in rice improvement by linking either of them with RGA.

BULK METHOD INNOVATIONS FOR INTERNATIONAL COLLABORATIVE PROGRAMS

The bulk method has been refined for application to composite populations and shortcut breeding methods such as rapid generation advance. The flexibility and volume that the bulk method accommodates should be fully explored to cope with the breeders' task, particularly in developing countries where breeding objectives are manifold despite limited resources.

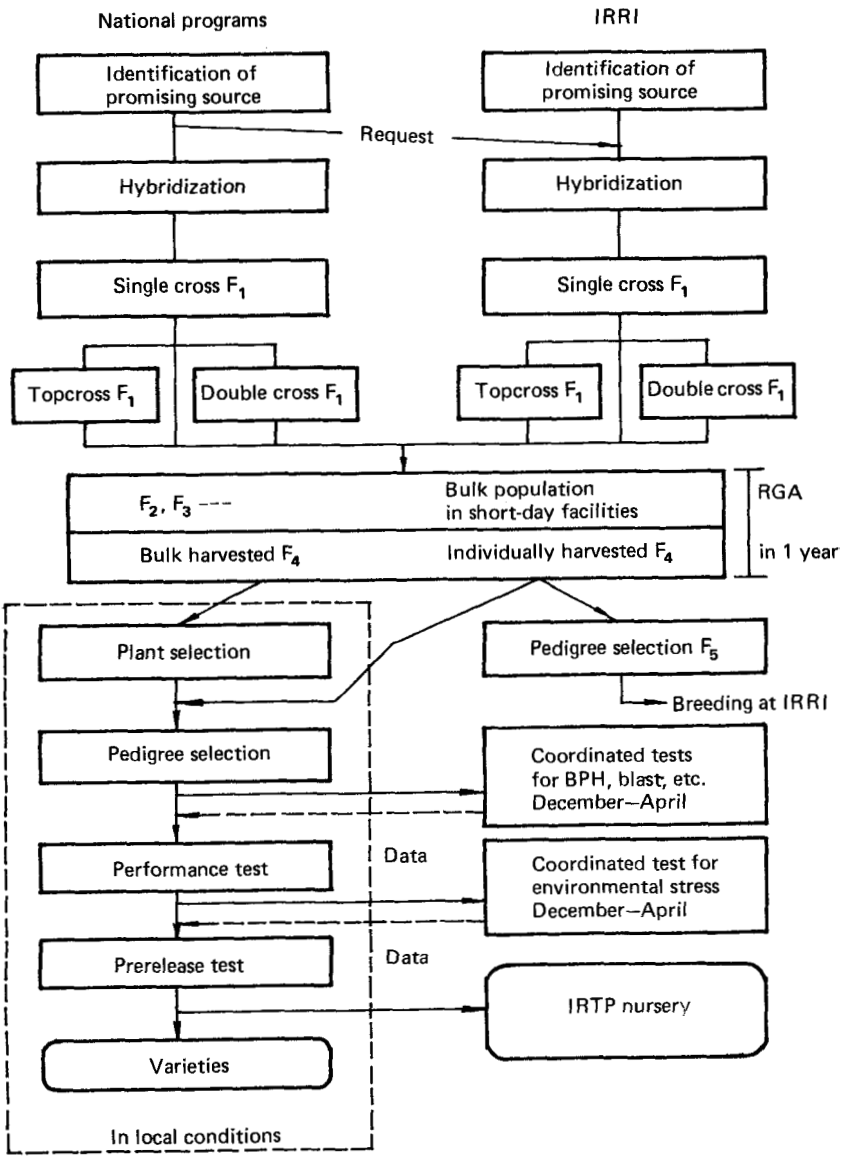
Bulk method for extensive implementation of RGA

During the past decade substantial progress in rice breeding has come about through the shift from traditional, photoperiod—sensitive rice to photoperiod-insensitive semidwarfs. However, half of the rice-growing area in South and Southeast Asia is still planted to photoperiod-sensitive rices, for they are adapted to varying water regimes. Breeding progress with these types of rices is unremarkable, partly because of their long growth duration which requires a longer breeding cycle than that for photoperiod-insensitive rices.

Under these circumstances, SSD as a modification of the bulk method can be employed very efficiently as rapid generation advance is of particular merit for these types of rices. An integrated international program for the use of RGA, proposed by Ikehashi and HilleRisLambers (1979), has been implemented at IRRI (Fig. 4).

Another area of RGA use is in support of rice breeding programs in temperate zones where short-duration varieties with adequate cold tolerance are urgently needed, but climatic conditions limit breeders to only one generation a year. After hybridization, two or three generations of segregating populations could be grown in the tropics in RGA facilities. An obvious advantage of this scheme is that by avoiding pedigree selection in the tropics undesirable selection pressure can be eliminated.

In all of the schemes it is vital that the selection be done on nearly fixed materials *in situ*, while the maximum potential of the population is maintained during bulk growing. Thus,



4. Integrated international breeding program for photoperiod-sensitive rices (Ikehashi and HilleRisLambers 1979).

generating the most diverse materials and selecting in a specific location can be integrated.

Perspectives of male-sterile-facilitated composite

In any breeding project it is noteworthy that only a few of the source materials produce most of the leading varieties in a given area. Breeders also tend to rely on relatively limited source materials as they know elite breeding lines can be bred from excellent sources. Today many rice breeders, particularly those in rainfed wetland areas, are making crosses among rices from their own areas. This is a wise approach to obtaining well-adapted lines for their specific areas with least risk.

However, these situations may lead inevitably to a convergence of the genetic variation. Obviously, a single cross with some exotic varieties would not satisfy the breeders when they try to magnify variations, as many poor recombinants would dominate the progeny of such crosses. Frequent recombination should be ensured in the conscious effort to utilize diverse sources.

To incorporate diverse materials into breeding programs despite their sometimes inherent conservativeness, and to exploit maximum chances for recombination, it would be of great benefit to develop an international scheme for the use of the male sterile-facilitated composite. Fortunately, the monogenic male-sterile lines are now available in japonica and indica rices. An excellent model for this is in barley breeding by Ramage (1977).

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