



*Extracting anthers from a wheat spike to create a female plant before making a cross. The key to rapid development of hundreds of component lines for multiline varieties is CIMMYT's unequalled capability for making thousands of crosses each season (see page 9 for other steps).*

CIMMYT TODAY

## MULTILINES: SAFETY IN NUMBERS

*Multiline varieties—mixtures of genetically related lines that have different genes for disease resistance—offer farmers a way to tame outbreaks of rust, thus stabilizing annual wheat yields. CIMMYT is producing large numbers of component lines that national wheat programs can use in compositing their own multiline varieties.*

In the frustrating battle against rust diseases of wheat, victory is a forerunner of defeat. Success for a wheat breeder is developing a new rust-resistant variety and seeing it widely planted. But, at the same time, the new variety triggers its own destruction by destabilizing the population of rust. Changes occur in the rust population that, eventu-

ally, allow it to attack the new variety. The plant breeder's counterthrust is to produce a new resistant variety—which in turn forces new changes in the rust organism.

During the last decade or two, scientists have become increasingly interested in ways to strike a truce with rusts. The multiline concept offers

one way. CIMMYT began work on multiline varieties in 1970. Now hundreds of carefully matched and tested lines are available which national wheat programs can use to construct multiline varieties.

A multiline variety is created by mechanically mixing seed of several lines that are similar in appearance and genetic make-up, but that have different genes for resistance to rust. The lines are bred and selected to be nearly identical in height, maturity, plant type, grain quality, and other characteristics, except for rust resistance.

A field planted to a multiline wheat variety is genetically much like a field of open-pollinated (non-hybrid) maize. Plants of an open-pollinated maize variety look alike and have the same growth characteristics, but the genes of one plant are not 100 percent identical to the genes of the next plant. Although virulent rusts of maize exist and attack some plants in a field, they do not build up to epidemic proportions because of the diversity of resistance genes present in the plants in the field. Genetic diversity similarly protects stands of pine trees from rust epidemics although a single species of pine may cover several thousand square kilometers.

But when maize or pine trees are inbred, making all the plants genetically alike, rust becomes a serious problem. In self-pollinated small grains like wheat and oats, pure line breeding has had

many advantages, however the genetic uniformity within a variety has made rusts an unrelenting threat to farmers.

Since 1970 CIMMYT has been working towards a dwarf multiline variety based on the 8156 cross. This cross, grown under variety names like Siete Cerros, Mexipak, Kalyansona, and P.V. 18, has doubled and tripled yields of wheat farmers from Casablanca to Katmandu. The wide adoption of the 8156 cross has proved its capacity for stable high yields in many environments. But no pure-line variety can be expected to be safe from devastating attacks of rust forever. By creating an 8156 multiline, CIMMYT hopes to retain the best characteristics of the 8156 cross while building in insurance against rust attack.

The rusts are a collective term for fungus diseases that produce rust symptoms. The three major rusts of wheat are stripe rust, caused by *Puccinia striiformis*; leaf rust, caused by *P. recondita*; and stem rust, caused by *P. graminis tritici*. Different forms, or races, of each rust exist because the fungus pathogen is capable of changing through sexual reproduction, mutation, and other means. Thus while a conventional pure-line wheat variety may have genetic resistance to the prevalent rust race in an area, eventually a new race will arise to which the variety is susceptible (See box: "The drawbacks of purity").

Thousands of wheats representing germ plasm from throughout the world grow at CIANO, the regional experiment station of northwest Mexico, at which CIMMYT conducts research in the winter. By crossing Siete Cerros and diverse sources of disease-resistance genes, CIMMYT is creating hundreds of lines which may be used to composite multiline varieties.



*With patience and discerning eye, Sanjaya Rajaram, CIMMYT bread wheat breeder, selects only lines that have the appearance of Siete Cerros to be retained as components of multiline varieties.*

### **Bringing the genes together**

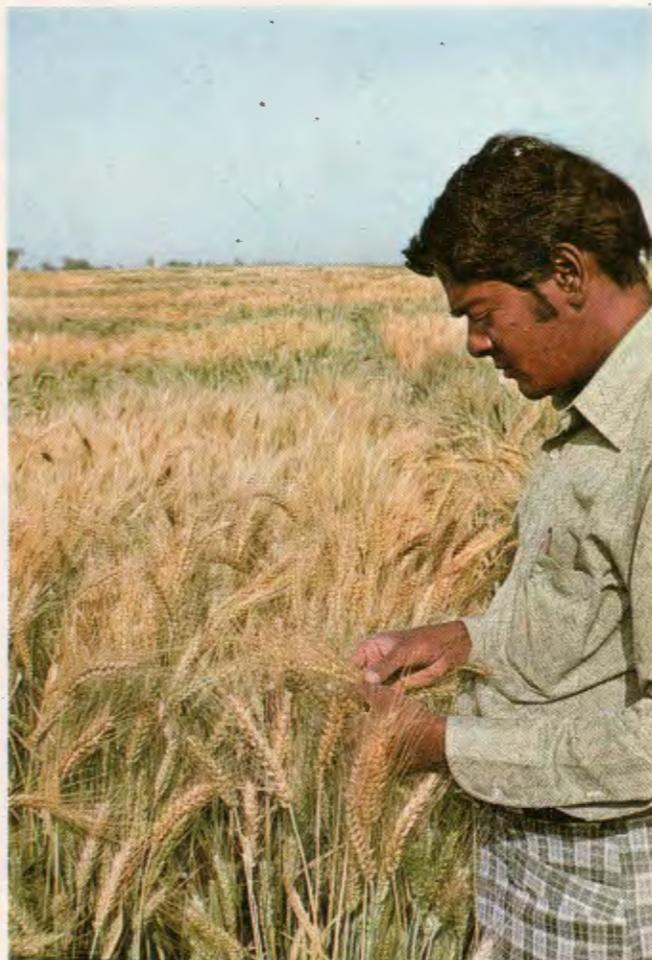
To create the individual lines that go into the multiline variety, CIMMYT breeders make thousands of crosses between Siete Cerros (the Mexican name for the 8156 cross) and varieties from throughout the world that are believed to have different genes for rust resistance than Siete Cerros. Then to further broaden the rust resistance they intercross the progeny of the crosses—a double crossing system. Such a double cross can be represented:  $(A \times B) \times (A \times C)$ , where A stands for Siete Cerros, and B and C for other varieties. Each double cross is grown twice a year at experiment stations in Mexico. Plants that do not look the same as Siete Cerros are discarded.

This system is not the full backcross approach originally conceived for developing multiline varieties. Repeated backcrossing would make the component lines almost identical genetically except for their rust resistance and would be a slow process.

Using double crosses to produce multiline components is more rapid and provides a degree of genetic variation aside from the differences in genes for rust resistance.

CIMMYT has used over 500 different varieties in crosses with Siete Cerros to create suitable lines for multiline varieties. The varieties crossed with Siete Cerros were chosen for their diverse origins and their proven resistance to stem rust, stripe rust, leaf rust, and septoria, the major fungus diseases of wheat. The lines being developed for multiline varieties must survive the same selection procedure as other lines in the CIMMYT breeding program. They are grown in Mexico in the winter at a sea-level desert location at 27° N and in the summer in a high (2600 m), humid plateau at 19° N. Any line that shows susceptibility to rusts or other diseases or that exhibits agronomic weakness is eliminated.

In 1974, CIMMYT had refined the first group of double-cross multiline components enough to yield test them in various combinations—that is, as experimental multiline varieties. The yield tests were a critical stage for the multilines. Farmers will not accept multiline varieties for their disease resistance alone. The multilines must yield as well as the best alternative variety the farmer has available. That they do was proven in yield trials harvested in Sonora, Mexico, in 1975. The six check varieties—



mostly recently released dwarf wheat varieties for the area—averaged 8.4 t/ha while one experimental multiline variety yielded 9.4 t/ha and another yielded 8.6 t/ha.

### **Biological fire bomb**

The promise of the multiline concept is that it combines diverse sources of resistance with an increased possibility of "escape" from damage by rusts. To understand why, compare how rust spreads in a field planted to a pure-line variety with the way it spreads in a field planted to a multiline variety, when conditions are right. The right conditions are a film of moisture on the leaves from dew or rain and a rather specific range of air temperature which is different for each of the rust diseases.

When a new virulent race of a rust develops in a field planted to a pure-line variety, and the proper weather conditions exist, the rust spreads rapidly, even explosively, because every plant is genetically identical. Spores that develop on one plant blow on to surrounding plants, all of which are susceptible, too. A fairly heavily rusted 1-hectare field may contain 100 trillion spores. With a steady wind those spores can be scattered to wheat fields thou-



*With the next planting season just a few weeks off, backs bend from dawn to dusk as the wheat staff rushes to harvest new lines.*



*Careful inoculation with rust spores ensures that susceptible plants can be spotted and thrown out.*

sands of kilometers away in a few days. Wheat breeders realize that when they release a new resistant pure-line variety, its resistance is likely to break down within 5 or 10 years. They must continue to develop new resistant varieties to stay ahead of changes in the rust.

In contrast, although plants in a field planted to a multiline variety are similar, they have different genes for rust resistance. So when a new virulent race of rust develops, only a small percentage of the plants are susceptible to it. And because most plants are not susceptible, the population of spores will not explode: spores produced on susceptible plants tend to fall mostly on resistant plants where they are unable to multiply. As a result, the growth of the spore population in the field is suppressed and even susceptible plants are less affected. Many reach maturity and are harvested before they are destroyed by the disease. That is, they "escape". The combination of resistant plants and "escapes" protects the farmer from severe yield losses when a new race occurs. As Norman Borlaug, director of CIMMYT's wheat program and inventor of the multiline idea, puts it: "An analogy can be drawn between the rate of spread of stem rust in a large

area where one uniformly susceptible group of wheat varieties is grown, and the rate of spread of fire in a vast expanse of dry prairie grass. Both are explosive. If, however, only half the grass plants are dry and the rest green, the fire will spread much more slowly; if the proportion of dry plants is dropped to 6 to 12 percent of the population, the fire will spread very slowly or not at all."

#### **Multilines in tall wheats**

In the 1950's Borlaug thought up the idea of creating a multiline variety through backcrossing. And he and colleagues in Mexico's Oficina de Estudios Especiales began making the crosses leading to a multiline. But the parents involved were tall wheats, and by the time the experimental multilines were ready for testing, the far greater yield potential of dwarf wheats had been recognized, making the tall multilines obsolete before they reached farmers' fields.

In 1963, however, Rockefeller Foundation scientists developed and released a tall multiline variety in Colombia, one of the world's most severe stripe rust areas. The multiline, called Miramar 63, was a mixture of 10 lines resulting from crosses of Frocor with 600 other varieties.

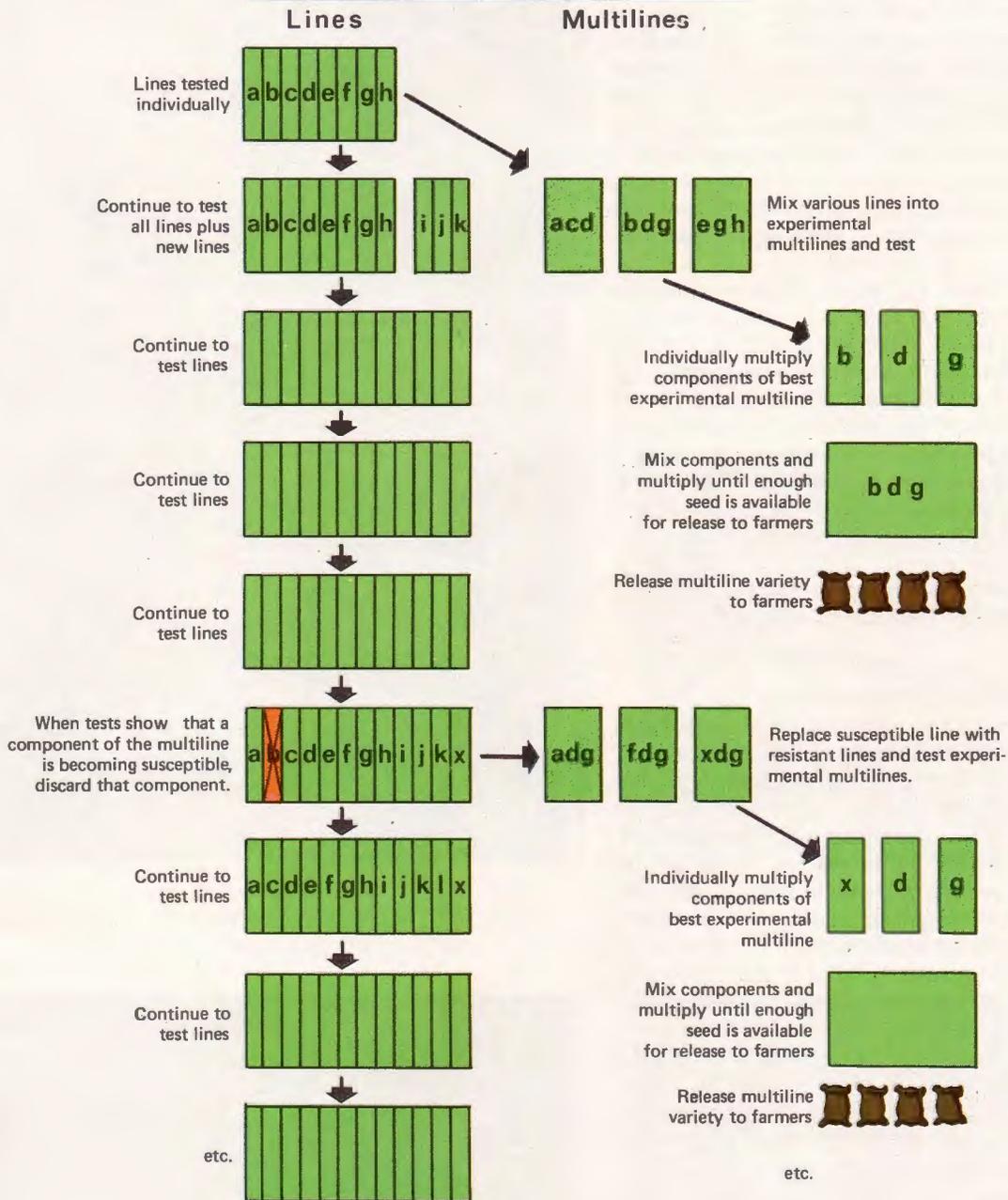
Two of the component lines became susceptible to stem rust within 2 years. Those two lines and two others were replaced and the multiline Miramar 65 was released in 1965. Although today's dwarf wheat varieties far outyield Miramar, it is still cultivated by a few farmers in Colombia and its resistance is still holding up.

#### **International distribution and testing**

Since 1973 after each harvest at its Mexico stations, CIMMYT has sent 200 lines suitable for the 8156 multiline to 20 or 30 locations around the world. Although CIMMYT is able to draw on the resources of its huge breeding program to produce many component lines, it is unable to produce finished multilines for areas outside Mexico. An experimental multiline variety that is successful against the predominant races in Mexico is unlikely to be the best multiline in another country where the predominant races are different. The race complex may even differ from area to area in countries like India that have several large wheat-growing regions.

# TESTING

# MULTIPLICATION



To decide which lines should be mixed to form a multiline, local scientists must test the lines to determine which have the greatest resistance for their area. But in some areas, such as the humid coasts of North Africa and Turkey, the fungus disease septoria is more important than any of the rusts, so breeders must test primarily for septoria resistance and only secondarily for rust resistance.

Also, while the components are nearly identical in appearance in Mexico, certain local conditions may change the maturity and height of some lines. Local breeders must grow and classify the lines by

**Schematic diagram of the steps in testing multiline varieties and releasing them to farmers. The main features are (1) the continual testing of the lines individually to detect the onset of susceptibility to a new race of rust, (2) testing mixtures of lines to find the most agronomically satisfactory combination, and (3) multiplication of the multiline and release to farmers. Actual multilines would be mixtures of 10 to 20 lines.**

length of maturity and height to be sure that the assembled multiline variety will be uniform for these characters.

Moreover, when the most resistant and uniform lines have been identified they must be mixed in various combinations and tested as experimental multiline varieties. Certain combinations of lines seem to work well together and certain other combinations don't. In fact some combinations "mesh" so well that the multiline variety yields better than the average of its component lines when they are grown separately. (See box: "Meshing and genetic drift.")

Ideally, says Glenn Anderson, deputy director of CIMMYT's wheat program, a multiline variety should be a mixture of 15 to 20 lines. If one line becomes susceptible in a multiline containing 15 lines, the farmer's yield should be reduced by, at most, 6 percent. But if the multiline contains only 4 lines and 1 line becomes susceptible yield might be reduced by 25 percent. Similarly, if one line out of 15 is several centimeters shorter than the rest it will be hardly noticeable in the field. But if one line out of four is shorter, a field planted to a multiline variety will look ragged and this may retard the willingness of farmers to adopt the multiline.

#### **Multiplying and renewing the multiline.**

When the best lines for a multiline variety have been chosen each of the lines is multiplied separately. For example, a half hectare might be planted to each of the 15 lines that will be components of the multiline. From each half-hectare plot up to 3 tons would be harvested. Some of the seed of each line would be held in reserve, but the rest would be mixed together in equal proportions giving about 40 tons of seed of the multiline variety. This "foundation seed" of the multiline would be planted again to produce seed for release to farmers.

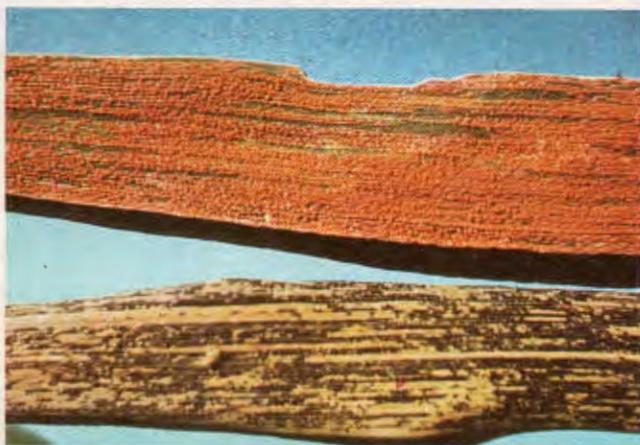
In each season some of the reserve seed would be planted by scientists along with seed of other lines that have not been included in the multiline variety. By observing the performance of the multiline variety and of its component lines, plant scientists will be warned when a new race arises that is virulent to one or more lines of the multiline. At the same time, they will be able to see which of the many lines in their experimental fields are resistant to the new race. They thus can immediately replace susceptible lines in the multiline with various combinations of resistant lines and begin to determine which new combination "meshes" best. When they have found the best combination, the lines are multiplied and a modified multiline is released to farmers.

While CIMMYT cannot create multiline varieties suitable for areas outside Mexico, it is able to offer





*Components of multiline varieties being bred by CIMMYT have resistance to the three most costly diseases of wheat, stripe rust, stem rust, and leaf rust. Clockwise from left: stripe rust, also called yellow rust, attacking the spike; stripe rust on the leaf; stem rust; leaf rust, closeup; leaf rust in the field.*



other countries massive production of 8156 lines. CIMMYT's breeders have access to numerous varieties from throughout the world that are potential sources of different genes for rust resistance. And CIMMYT has the personnel, experiment fields, and laboratories to make and test thousands of crosses annually between these varieties and Siete Cerros. At the end of 1975, Sanjaya Rajaram, CIMMYT's bread wheat breeder, had 150 lines, selected from a multitude of crosses, which were thoroughly tested and matched for appearance, and hence from which multiline varieties could be composited.

But the job of deciding which lines to include in a multiline variety, multiplying and mixing the seeds, and distributing seeds to farmers falls by necessity on the personnel of national wheat programs.

#### **Other multilines**

The expense and research time needed to develop a multiline can only be justified when a variety exists that has proved to have high yield in many widespread locations.

CIMMYT is not alone in recognizing the potential advantages of an 8156 multiline. In India, M.V. Rao has a crossing program under way leading to an 8156 multiline, too. India will probably be the first nation to release a dwarf multiline wheat variety. CIMMYT and the Indian program are cooperating by exchanging the lines they develop. India is also breeding components for multiline varieties of Sonalika, Sharbati Sonora, and other successful Indian varieties.

At CIMMYT in addition to an 8156 multiline, an "Anza" multiline is being developed. Anza is a U.S. name given to the Mexican cross Lerma Rojo-Norin 10 x Andes E. This cross is known as Mexicani in Sudan, WW15 in Australia, T4 in South Africa, Karamu in New Zealand, and Moghan I in Iran. The widespread use of this cross is evidence of its adaptability and good yield potential. The Anza multiline project is a cooperative undertaking. Breeders in the USA, South Africa, and Australia are making the basic crosses and CIMMYT scientists are evaluating and selecting the progeny.

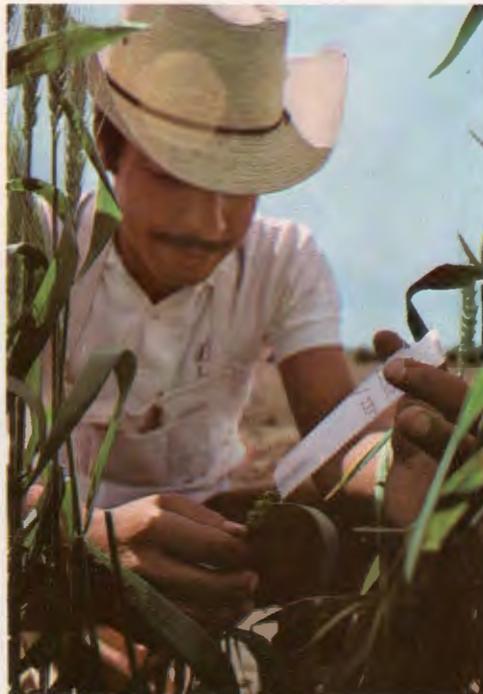
*Steven A. Breth*

*Each year CIMMYT sends new lines to dozens of scientists for observation and testing as potential components of a local multiline variety. Max Alcalá is in charge of CIMMYT's international nursery program.*





*Clipping the bracts of a wheat spike to facilitate removing the anthers.*



*A clipped and emasculated spike, left, ready for pollination. Right, covering the spike with a glassine bag after hand pollination.*

## MESHING AND GENETIC DRIFT

Determining which lines have high yield and good rust resistance is not the end of the testing needed for developing multilines. Scientists in each country must grow various mixtures of the lines to determine which combinations "mesh" best under local conditions. Sometimes, in fact, the multiline mixture may yield more than the average of the component lines grown separately. For example, in trials harvested in 1975 when the component lines of one of the experimental multilines were grown in individual plots the yields ranged from 7.72 to 7.94 t/ha. But the experimental multiline itself (the mixture of the same lines) yielded 7.96 t/ha.

Why a multiline may yield better than the average of its components is not well understood. Speculation centers on the small variations in the architecture of individual plants

in a multiline variety. Possibly because of slight differences in the leaf angle, root length, and root feeding area of neighboring plants of a multiline variety, there may be less competition for space in the crop canopy and root zone than occurs in a stand of genetically identical plants. In addition, yields of multilines may benefit by suppression of some diseases such as *Helminthosporium* and *Alternaria*, which are usually present but not severe in pure-line stands, but which nevertheless can affect yield.

On the other hand once the multiline variety has been composited and is grown season after season a degree of genetic drift will occur. That is, plants of some component lines will not compete for moisture, nutrients, and sunlight quite as well as others. The more competitive plants will over the years tend to produce more seed and thus the composition of the multiline will tend to "drift" slowly.



*CIMMYT pathologists, Santiago Fuentes, left, and Jesse Dubin, below, help breeders make the final choice of lines that have high-level resistance.*



## THE DRAWBACKS OF PURITY

Many of the milestones in agricultural history have led to increase crop uniformity or purity. When man began to cultivate crops, he tried to help his crop plants and eliminate competition from other plants. Pulling weeds made the farmer's field more uniform. It might contain numerous "varieties" but they were all of the same species. Fields became even more uniform when some farmers recognized that in self-pollinated crops like wheat the seed from one outstanding plant could be multiplied until enough seed was available to plant an entire field to identical outstanding plants. More recently, pure-line breeding by scientists began. During the past hundred years, superior varieties selected by plant scientists started to displace "farmer" varieties so the number of varieties planted within an agricultural region began to decrease.

The advent of scientific plant breeding—crossing two varieties to combine their best features—led to substantially higher yields, and to greater uniformity. Varieties that resulted from crosses and careful selection and testing of the progeny were so much better than older varieties that farmers eagerly switched into new varieties.

Since disease resistance was an important breeding objective, a variety that proved to be resistant was often used in many crosses as a source of that resistance. Thus different varieties often had the same gene for resistance to a specific disease. Moreover, vast areas were sometimes planted to just one variety. For example, the variety Clinton occupied 75 percent of the land planted to oats in the USA in 1950.

Agricultural progress has led to yields unimaginable even 50 years ago. But the increased uniformity and, ironically, the use of resistance genes in self-pollinated crops has continued to leave the farmer open to the threat of rust attacks. Each step toward uniformity has upset the stability of relations between rust diseases and their host plants as well as between individual races of rust.

Breeders look for genes that will protect a variety from the predominant virulent races of rust. Usually one gene gives resistance to one race. When the variety is widely planted, the race to which the variety is resistant can

not multiply and thus that race is less able to compete with other races. With the equilibrium between races upset, some previously minor race, or a new race, may arise which is capable of attacking the "resistant" variety. The susceptibility of the variety enhances the ability of the new race to reproduce, and as a result the population of rust in the environment shifts almost exclusively to the new race. When weather conditions are right, enough spores of the new virulent race are in the air to set off the lightning fast cycle of multiplication and destruction known as a rust epidemic.

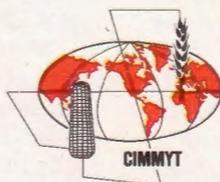
For wheat breeders today, the race against rust is an endless chore. They must continually turn out new varieties to replace those whose resistance to rust is being overcome.

Forty years ago breeders and pathologists thought that incorporating a single resistance gene in a variety would solve the problem of rust diseases. They now know otherwise. To ensure that new varieties do not all have the same genes for rust resistance, hundreds of different varieties are used as sources of resistance genes.

Breeders are also ardently pursuing a little understood phenomenon called generalized, or horizontal, resistance. Certain wheat varieties have remained resistant for over two decades. These varieties apparently have a number of genes which have a cumulative effect in giving general resistance to all or many races.

Multilines are a combined mechanical-biological way of achieving generalized resistance. While a new race may arise that is capable of attacking one of the component lines it is unable to multiply rapidly because each susceptible plant tends to be surrounded by plants that have different genes for resistance. As a result the new race has little competitive advantage over the existing races in the area and the population mix of races doesn't shift.

The fungus pathogen is so inherently variable that over time the race make-up will of course change. In solid-stand test plantings of the individual lines incorporated in the multiline variety, the change in virulent rust races will be reflected by the sudden susceptibility of one of the lines. With this warning, breeders can take steps to substitute a resistant line in the multiline variety long before the new virulent race has greatly affected farmers' yields.



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