Luther S. Bird
Texas Agricultural Experiment Station, College Station

The MAR (Multi-Adversity Resistance) Syster

Host resistance as a means of maintaining the health of domestic plants is paramount in considering the needs for renewable resources of food, fiber, and bioenergy as well as the need for reducing the use of pesticides and energy in crop production. The ultimate in biological efficiency and crop management can be approached most effectively by using host resistance that will neutralize or minimize losses caused by adversities in production. Ways to genetically improve plants for resistance to adversities are numerous when one considers the various aspects of host variability, gene action, genetic stability, pest variability, population dynamics of pests, environmental influences, and volume and quality of the product. The advantages and disadvantages of the available genetic systems have been reviewed (9,13) and the conclusion reached that developing cultivars with resistances to several diseases would be more difficult and nearly impossible with quantitative gene systems than with qualitative ones (9).

The utopia in crop improvement would be cultivars possessing the gene combinations giving resistance to all pathogens, nematodes, insects, and stresses and capable of producing a high volume and quality of the desired product. Furthermore, the resistance would be stable and persist against races and population levels within the many species of pests. The utopia resistance will probably never be obtained for a crop. The multi-adversity resistance (MAR) system for genetic improvement of cotton has such a goal, however, and it appears that the program will eventually produce cultivars approaching the ideal. The system makes it easier to produce cultivars with stable resistance to two or more adversities than to develop those with resistance to only one adversity.

Background Information

The techniques and procedures that evolved into the MAR program originated in information gathered on seed conditioning, bacterial blight resistance, and genetic interrelationships among genes for resistance to several diseases.

Seed conditioning. The level of conditioning and subsequent deterioration of cottonseed is determined by the period of time seed are exposed to moisture and heat. This has been standardized and expressed in days of equivalent exposure to 100% relative humidity at 50 C. Mature cotton in the field, seed cotton in trailers or modules, and seed in storage may be exposed to natural moisture and heat. Seed exposed for up to 1 day are unconditioned and are characterized by slower rates of germination and seedling emergence at reduced temperatures, seed coat resistance to mold, less seed rot, no abnormal roots, and optimum seed-seedling performance in cold soil; the seedlings are less likely to be killed by pathogens. Seed exposed to the equivalent of 2–3 days of moisture and heat are conditioned. Compared with unconditioned seed, conditioned seed germinate faster, the seed coat becomes susceptible to mold, seed rot may occur, seedlings have more abnormal roots, and stand establishment in cold soil is reduced; the seedlings are more sensitive to damage by pathogens. Seed exposed to the equivalent of 3–4 days of moisture and heat deteriorate; the germination and seedling emergence rates are low, the seed coat becomes very susceptible to mold, seed rot is high, the frequency of abnormal roots is high, poor stands result in cold soil, and seedlings are apt to be killed by pathogens or even some saprophytic organisms. Thus, genetic improvement for slow rates of conditioning would contribute to more seed that perform in cold soil and produce healthy seedlings. The above information led to choosing seed coat resistance to mold and a reduced rate of germination, when acid-delinted seed are held at 13.3 C for 8 days, for use in selecting for resistance to seed conditioning (2,3,12).

Bacterial blight resistance. Resistance to *Xanthomonas malvacearum* (E. F. Sm.) Dows. is one of the best understood traits in cotton. Sixteen independent major genes (B genes) for resistance have been designated. Eighteen races of the bacterial blight pathogen have been named. The main consideration in naming races has been to maintain order in breeding for resistance rather than to describe the broad variability of the pathogen.

The known major genes behave independently as quantitative genes in the presence of several races of the pathogen. Highly resistant genotypes can be recovered from segregating populations possessing at least three major and compatible modifying genes when these populations are challenged with a mixed inoculum of at least four races of the pathogen. Several varieties developed in this manner have been released and evaluated worldwide, and their high levels of resistance have held up for 15 years. This is an example of using the appropriate genetic variability of both the host and the pathogen to help establish gene combinations in the host that neutralize pathogen variability (2).

Genetic interrelationships among genes for resistance. Associations among genes causing resistance to several diseases have been measured in cotton. The genetic interrelationships suggest one of four possible situations: 1) genes causing resistance to two or more pathogens, 2) close linkages among genes conditioning resistance to several pathogens, 3) genes conditioning a mechanism effective against pathogens causing several diseases, or 4) a rather broad pleiotropic system.

The strongest association is between resistance to bacterial blight and the Fusarium wilt/root-knot nematode complex (Fig. 1). Lesser but significant associations exist between blight resistance and resistance to Verticillium wilt, Phymatotrichum root rot, and seed coat resistance to mold. The measured associations with bacterial blight are stronger when at least four races of *X. malvacearum* are used to evaluate and select for resistance. Similar associations exist between resistance to the Fusarium wilt/root-knot nematode complex, Verticillium wilt, and Phymatotrichum root rot. A reduced rate of germination at low temperatures (13–18 C) is associated with resistance to Verticillium wilt and seedling pathogens. Seed coat resistance to mold is strongly associated with high yield potential and early maturity. Small seed size is associated with rapid germination at reduced temperatures and susceptibility to Verticillium wilt. These associations form a complex model of interrelationships that have been measured in formal genetic studies and have held up under 15 years of use in the MAR genetic improvement system (Fig. 1) (1,3).

Other correlations that may complement the system are between the B_2, B_3, and B_6

or Genetic Improvement of Cotton

genes for bacterial blight resistance and resistance to a boll-rotting fungus (*Colletotrichum* sp.) and the cotton stainer, a boll- and seed-puncturing insect (8).

Techniques and Procedures

Seed coat resistance to mold, a reduced rate of germination after 8 days at 13.3 C, and cotyledon resistance to races of the bacterial blight pathogen are the key selection traits. Selection for these three traits takes advantage of the genetic interrelationships to obtain simultaneous improvement in resistance to the major diseases, higher yield potential, and earliness (Fig. 1). The MAR selection is performed each fall. Fuzzy seed are delinted with commercial grade sulfuric acid, rinsed in tap water, dipped in dilute sodium hydroxide, and rinsed again. The seed are dried in containers in a system of forced hot air (41 C), then held for 2 weeks at room temperature before being used.

Petri dishes (100 × 20 mm) with 1.5% water agar are prepared 16–20 hours in advance. Twenty-five seed per dish are placed on the agar surface (Fig. 2). The tops of the dishes are removed to allow the seed and agar to become naturally infested with fungal spores, primarily those of *Fusarium* spp. and *Alternaria*

spp. The dishes are covered with perforated plastic bags and placed in temperature-controlled chambers (13.3 C).

After 8 days the seed are evaluated for resistance to mold growth and for germination. Seed with no mold and only slightly emerging radicles are selected (Fig. 3). All seed of some cultivars may have varying degrees of mold; in these cases, seed with the least mold are selected. The degree of germination is handled differently because seed that have germinated quickly and whose radicles have formed crooks are never selected. If the choice is either fast or no germination, nongerminating seed are selected because their seedlings would have been the slow performers under the cold conditions.

Paper cups are filled to 80% capacity with soil infested with the seedling pathogens *Rhizoctonia solani* Kühn and *Pythium* spp., and the selected seed are placed on the soil in the center of the cups and covered 2 cm. deep. After 5 days, the selections are evaluated and cups with abnormal seedlings are discarded. The remaining seedlings are inoculated with the bacterial blight pathogen. Growth for each of four *X. malvacearum* races is mixed equally with enough distilled water to give an inoculum with a viscosity slightly higher than water. Mixtures of

races 1, 2, 7, and 13; 1, 2, 7, 12, and 13; 1, 2, 7, and 14; 1, 2, 7, and 18; and 1, 2, 6, and 18 have been used. Round wooden toothpicks are dipped into the inoculum and used to scratch the lower epidermis of each cotyledon (Fig. 4). Ten days after inoculation, the scratch lesions are examined and seedlings showing the immune reaction (no blackening of the lesion or extensions along the margin of the scratch into the unwounded tissue) are retained. Each seedling hypocotyl is examined at the soil line and those with evidence of soreshin lesions are discarded (1). The remaining seedlings are MAR selections and are transplanted into larger pots and managed for production of two to three bolls per plant.

Expectations and Precautions

Expectations within the MAR system are based both on the genetic interrelationships and on past achievements. Direct selection for seed coat resistance to mold, reduced rate of germination, and cotyledon immunity to the bacterial blight pathogen indirectly gives simultaneous improvements for resistance to diseases, insects, and stresses. About 5% of each new series of MAR progenies have the simultaneous broad improvement. Consequently, each population must be evaluated in nurseries and test plantings to identify the progenies with higher levels of multi-adversity resistance.

A major precaution is to avoid applying direct selection for a trait unless its genetic interrelationships within the MAR system are known. For example, susceptibility to Verticillium wilt is associated with small seed and slow germination. Small seed are associated with establishing stands and higher yields. Selection for seed size is avoided within the system. As a test, direct selection for resistance to Verticillium wilt is practiced within MAR strains each year. In the past, this selection had resulted in reductions in yield potential and earliness just as the genetic interrelationships indicate (Fig. 1). In recent years, the selections for Verticillium wilt resistance within some advanced MAR types have not lost yield potential or earliness. Thus, by understanding the system and being patient, one may change unfavorable genetic associations; more likely, levels of MAR genes have been obtained that are strong enough to mask other genes that condition

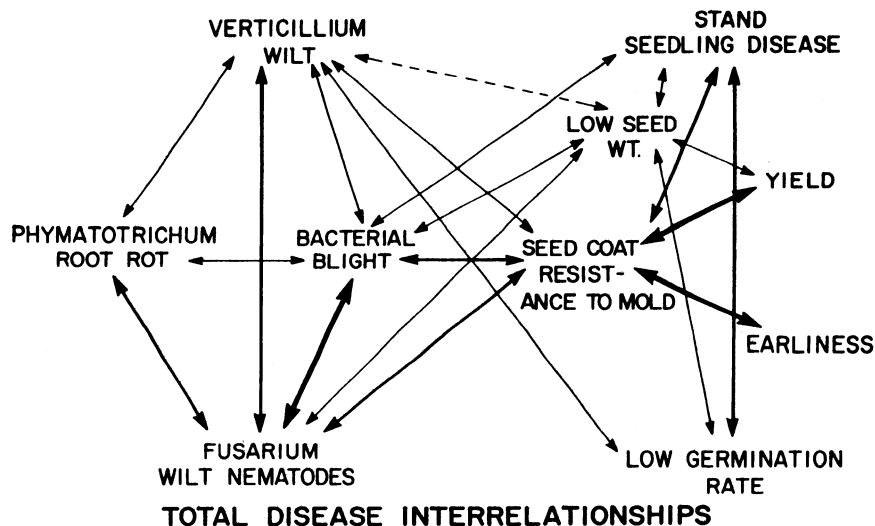**TOTAL DISEASE INTERRELATIONSHIPS**

Fig. 1. Genetic interrelationship pathways among genes for disease resistance and performance traits in the MAR system. The width of the pathway line indicates the relative strength of the relationship. Favorable gains for all traits in the system are aided by selecting for seed coat resistance to mold, low germination rate, and bacterial blight resistance in the laboratory and greenhouse.

Table 1. Changes in levels of resistance to adversities within TAM-MAR germ plasm during 1967–1968 and 1976–1978

	Level of resistance[a]	
Adversity	1967–1968	1976–1978
Diseases		
Resistance to seed deterioration	40	60
Seed-seedling cold resistance	40	60
Seedling pathogens	0	50
Bacterial blight (*Xanthomonas malvacearum*)	95	99
Verticillium wilt	60	80
Fusarium wilt root-knot nematode complex	70	85
Phymatotrichum root rot	0	40
Insects		
Fleahoppers	0	40
Boll weevil	10	50
Heliothis spp.	0	50
Pink bollworm	0	60

[a] From 0 = no resistance to 100 = high resistance.

Fig. 2. Acid-delinted seed are placed on water agar in petri dishes before being placed in temperature-controlled chambers for 8 days.

Fig. 3. Seed with no mold growth and only slightly emerging radicles (top right) after 8 days at 13.3 C are selected. Not selected are seed with radicles extending into the crook (top left) or with mold growth or damaged radicles (middle and bottom).

Verticillium wilt resistance in cotton. As another example, elimination of seedlings with soreshin lesions applies direct selection for resistance to seedling pathogens. This has been done, although genetic interrelationships for seedling resistance with other traits in the model (Fig. 1) have not been determined. Careful monitoring for three successive selection cycles revealed no unfavorable changes. Consequently, it appeared that direct selection for resistance to soreshin could be continued within the MAR program without disrupting the system.

Indirect selection has resulted in increasing resistance to the major insects within the MAR germ plasm. Even so, direct selection as a means of enhancing levels of insect resistance has been purposely avoided. So long as the MAR system gives favorable results, gambling with upsetting the genetic inter-

relationships that give favorable genetic improvement of multi-adversity resistance is not recommended.

Managing the MAR program should allow for adequate populations and means of evaluating each set of new selections. About 60,000 seed each fall are processed and about 2,500–3,000 selections produce seed in the greenhouse during the winter. Progenies of the greenhouse-grown plants are planted in single row plots in a field nursery. The main evaluations are stand establishment, agronomic performance, bacterial blight resistance, and boll, fiber, and seed traits. Seed produced by selected progenies are adequate for planting 12–15 adversity nurseries and increase rows the following season.

The Germ Plasm Pool

When the MAR system was begun in 1963, stocks on hand had been developed with emphasis on resistance to bacterial blight and the Fusarium wilt root-knot nematode complex. The B_2, B_3, B_4, B_6, and B_7 major genes and the B_{Sm}, B_{Dm}, and B_{Im} minor gene complexes for resistance to bacterial blight were in the germ plasm.

To respond properly to MAR selection, a germ plasm pool must have adequate variability for bacterial blight resistance. The B_2, B_3, B_4, and B_6 genes were transferred to *Gossypium hirsutum* L. from Uganda BAR Sakel *G. barbadense* L. strains. The B_4 gene in the Sakel types had been transferred from *G. arboreum* L. Consequently, the germ plasm pool has genetic variability that evolved from three species of cotton. In addition, variability within *G. hirsutum* is provided by the following variety groups: Empire WR, Deltapine SL, Lankart 57, Texacala, Paymaster 105, Blightmaster, glandless genetic stocks, stocks with genes for resistance to southwestern cotton rust, and genetic stocks with okra-shaped leaves. The germ plasm pool has the traits of glandlessness, glabrousness, nectarilessness, okra-shaped leaves, and frego bract. It has a tremendous potential for obtaining recombinations of genes from a broad spectrum of the variability within the genus. The MAR indirect selection makes effective use of the variability.

Gains in the Program

To date, three hybrid pools have been produced and processed in the MAR program. Selections from the first pool were progenies during 1967–1968 and they were released as varieties (Tamcots SP21 and SP37) in 1972. Areas of Texas, such as the Coastal Bend, actually doubled the average yield per hectare by converting a high percentage of cotton plantings from older varieties to the new Tamcot SP varieties (Fig. 5). Comparable results were obtained by farmers in the Rolling Plains, and similar but not as impressive results were obtained in the Blackland and the Lower Rio Grande Valley regions of Texas. Three varieties (Tamcots SP21S, SP37H, and CAMD-E) were released in 1975, and cultivars LEBO and CDPS, representing material from the third hybrid pool, became progenies during 1976–1978. Comparisons made in the Coastal Bend area (12) among cultivars representing each hybrid pool show that each sequence of MAR improvement resulted in higher yield and earliness potentials (Fig. 6).

Resistance to diseases and insects has improved simultaneously with increased yield and earliness (Table 1). The greatest improvements have been for resistances to various adversities that were low or nonexistent during 1967–1968. Probably, the rate of progress slows as the high level of resistance is approached. As more MAR genes are accumulated, resistance increases for some adversities, resistance to other adversities occurs where none could be measured earlier, and production potentials increase. This indicates that a weak MAR gene complex is adequate for some adversities but a stronger one is necessary to influence others (4,6,12).

Within a plant species, genes for viability, adaptation, fitness, and some

morphological traits tend to be linked or associated (10,11). Probably, many of these genes are not dealt with in domestic plant improvement. If this is the case, the MAR system may have evolved a bioassay procedure that identifies little used but important genes for survival and fitness. Such genes, having a small genetic but a broad biological effect for resistance, may be accumulated under natural selection. But, these genes may be bypassed or diluted by domestic procedures that deal more with specific traits. We believe that within this general concept lies the explanation for the genetic events occurring in the MAR program. The program offers a system in which quantitative genes are easier to use than qualitative ones in developing cultivars with resistances to several diseases.

Nature of Resistance

The base hypothesis is that cotton has the genetic potential to alter its natural symbiotic microflora, which occur on seed and root surfaces and in tissues, to organisms that are unfavorable to pathogens and insects. The system is under the control of MAR genes. Further, the change is accomplished by genetic alteration of the quality and quantity of components of exudates from seed coat, seedling and plant roots, and fluids in tissues. The altered fluids are nutritionally unfavorable for pathogens and insects and selectively favorable for bacteria, actinomycetes, and fungi that are highly competitive with pathogens and insects. Unfavorable nutrition and microorganisms function together to provide a mechanism of multi-adversity resistance.

Several models were developed in a sequence of thesis and dissertation investigations (7). Measurements of leachate constituents and rhizosphere-rhizoplane bacteria and actinomycetes from sets of MAR cultivars were used. Differences were significant among sets of MAR cultivars for concentration levels of constituents of the leachate and population levels of rhizosphere organisms. The first model indicated that variability in concentration of exudates from seed coats could influence resistance to diseases as well as yield potential and earliness. The second model indicated that exudates from seed coats and roots of 9-day-old seedlings could influence the rhizosphere-rhizoplane bacteria and actinomycetes. The exudate components and organisms had the potential for influencing resistance to root diseases. The third model indicated that exudates from roots of 18-, 30-, and 55-day-old plants could influence rhizosphere-rhizoplane bacteria and actinomycetes. The exudates and microorganism variability in turn could influence resistance to various root diseases as well as yield potential and earliness.

In initial cause and effect investigations based on the models, bacteria and fungi (*Bacillus* spp., *Pseudomonas* sp., and *Fusarium* spp.) from seedling roots of MAR cultivars were used as treatments and reduced damage by root pathogens. The bacteria and fungi together were more effective than either alone. Two bacterial (*Bacillus* spp.) types from leaves and squares of Tamcot CAMD-E (high resistance to bacterial blight and partial resistance to the boll weevil) induced resistance to the bacterial blight pathogen in leaves of a susceptible cotton. The same bacteria applied to a susceptible cotton cultivar made it as resistant as Tamcot CAMD-E to the boll weevil. Thus, bacteria that can influence host responses to diseases and insects do exist in tissue of MAR cottons (5).

These investigations are by no means complete. The results, however, indicate it may be more meaningful to focus future research on the complex interactions of host-pathogen-symbiont-environment rather than on the simpler classic interaction of host-pathogen-environment. The results obtained thus far give general support for the hypothesis on the nature of MAR resistance and by no

Fig. 4. Seedling cotyledons are scratch-inoculated with a wooden toothpick 5 days after emergence. A mixed inoculum containing four races of the bacterial blight pathogen, *Xanthomonas malvacearum*, is used.
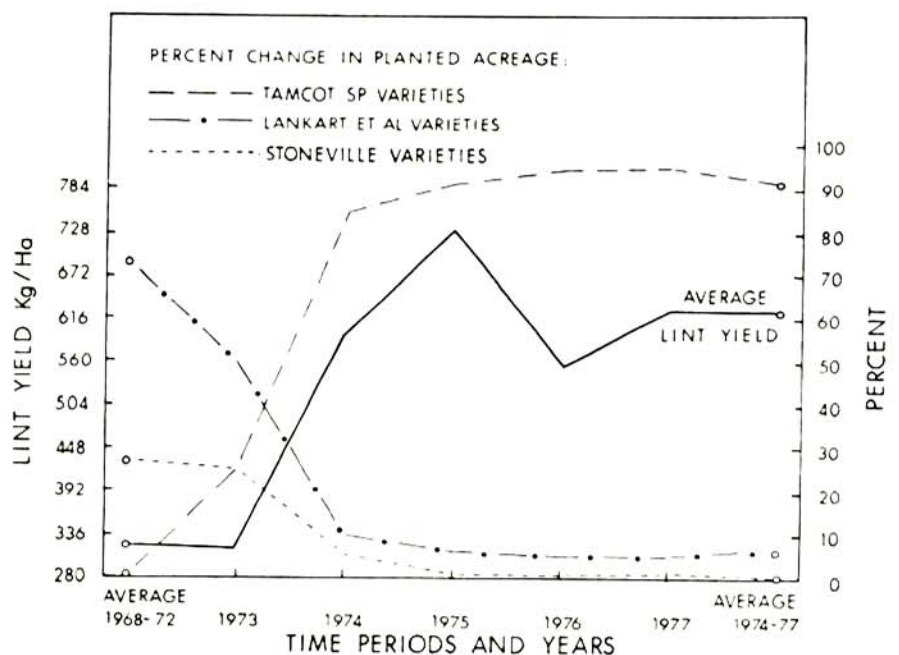

Fig. 5. When older cotton varieties were replaced with the original Tamcot SP varieties in San Patricio County, Texas, average lint yields per hectare doubled, illustrating how MAR cultivars with resistance (primarily cold tolerance and resistance to seedling disease and bacterial blight) to key adversities helped farmers in the Texas Coastal Bend area reestablish a viable cotton industry.

means discourage continuing the investigation.

The gains made in yield and earliness in the multi-adversity resistance cultivars parallel gains in resistance to diseases and insects. The MAR cultivars provide a good example of improved performance by plants that remain healthier under many different circumstances.

Acknowledgments

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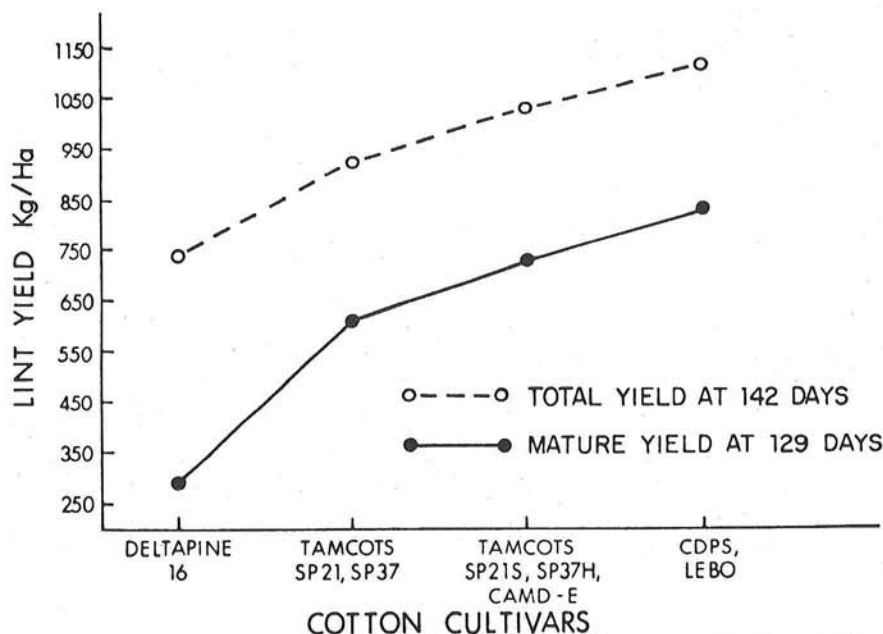
Fig. 6. Total lint yield at 142 days and mature yield at 129 days (a measure of earliness) after planting for Deltapine 16 (an older variety) and cultivars representing sequential hybrid pools 1, 2, and 3 of the MAR program. Performance of the MAR cultivars shows how yield and earliness potentials improve with each increment in level of disease resistance conditioned by MAR genes.

Luther S. Bird

Dr. Bird is professor in the Department of Plant Sciences at the Texas Agricultural Experiment Station of the Texas A&M University system at College Station. He received his B.S. degree from Clemson University in 1948 and his Ph.D. from Texas A&M University in 1955. His research focuses on host plant resistance for controlling all the major diseases of cotton.