



## Genetic Improvement of Cotton Utilising the Multi-Adversity Resistance (MAR) System

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### ABSTRACT

Maintaining the health of the cotton crop throughout the growing season will make it possible to approach the full genetic potential of quantity and quality. Increased levels of resistance to pests (insects and pathogens) and abiotic stresses are needed to maintain plant health. The MAR program has made steady progress in developing cotton germplasm with broad and higher levels of resistance to pests and stresses in addition to increasing lint yield, earliness, fiber and seed quality and stability over environments. The MAR concepts, procedures and techniques are based on continuous basic research in multi-disciplinary areas. The established MAR techniques involve extensive seed, seedling and plant screening and selection in the laboratory and greenhouse, followed by four stage field-testing and evaluation at 10 locations in Texas. Advanced strains are included in regional tests throughout the Cotton Belt. These procedures make it possible to identify new germplasm with genetic gains for more than 56 traits in cotton. Over 40 cultivars and 360 elite lines originating from eight MAR gene pools have been released. Averaged over 42 tests and 4-years, lint yield increased 31%, earliness 24% and fiber strength from 24.5 g/tex for the MAR-1 Tamcot SP37 to 30.2 g/tex for the MAR-7 Tamcot Luxor. Resistance levels have increased for eight pathogens and seven insects. The MAR program is active in genome mapping, fingerprinting and tagging, gene insertion and transformation and developing transgenic MAR cottons. Interfacing biotechnology with MAR breeding will enhance the programme in the 21<sup>st</sup> Century.

### Introduction

Maintaining plant health throughout the season to facilitate realisation of their full genetic potential for both quality and quantity is a key to sustainable agriculture. Estimates indicate that only about 60% of the genetic potential is realised due to pest (insects and pathogens) and abiotic stress losses (El-Zik and Thaxton, 1989). Yield and fiber quality are priorities in most breeding programs, but are constrained in the U.S.A. by insects (thrips, aphids, plant bugs, boll weevil, tobacco budworm, bollworm, beet armyworm, pink bollworm, whitefly), spider mites, pathogens causing diseases (seedling, bacterial blight, leaf spots, *Fusarium* and *Verticillium* wilts, *Phymatotrichum* root rot, boll rots nematodes, viruses), moisture, nutrients, and environmental stresses.

The primary objective of programmes for genetic improvement of resistance to pests and stresses is to develop cultivars that are resistant to one or more pests, while improving or maintaining their basic agronomic, yield, fiber and seed characteristics. Pest management is an important part of overall cotton production. The availability of adapted cultivars and gene pools with high levels of heritable resistance to multiple pests and abiotic stress is essential. Progress in the breeding of improved cotton cultivars has been particularly rapid during the past 15 to 20 years. Remarkable advances have also been made in breeding concepts and methodologies to develop pest resistant cottons.

### Breeding Approaches

Methods of improving crops genetically for resistance to pests are numerous when one considers various aspects of host variability and sources of resistance, pest variability, genetic stability and elasticity, environmental influences, and economic threshold and injury level of pests. Genetic improvement includes breeding for resistance to one pest (i.e., a single insect or plant pathogen), multiple species of pests, or multi-adversity (insects, pathogens, and abiotic stresses). Higher levels of resistance to pests and stresses should parallel improve yield, fiber and seed quality. Improvement of cotton for resistance to only a few pests or adversities is insufficient for sustainable agriculture.

Comprehensive reviews on the genetics, mechanisms, and sources of resistance and breeding cotton for resistance to pathogens and insects were provided by Bird (1973, 1982), Dahms (1943), El-Zik and Frisbie (1985), El-Zik and Thaxton (1989), Jenkins (1982, 1986), Jones (1972, 1982), Maxwell *et al.* (1972), Maxwell (1980), Niles (1980), and Russell (1978).

### Multi-Adversity Resistance (MAR)

The MAR concepts, procedures, and techniques, based on continuous basic research for the past 35 years, are used to develop MAR germplasm (Bird *et al.*, 1968; Bird, 1982; El-Zik and Thaxton, 1989). It is a monumental task to select directly for resistance to

every pest and environmental stress affecting cotton. A system of selecting directly for few traits (4 to 6) that indirectly improves levels of the remaining traits would simplify genetic improvement. A key element in the MAR program is to determine which traits are positively correlated or linked. MAR utilises direct seed, seedling and plant selection in cotton for the simultaneous genetic improvement of resistance to pests and abiotic stresses in addition to higher yield potential, earliness, agronomic characteristics, drought tolerance and higher fiber and seed quality.

### **MAR Procedures**

MAR genetic improvement procedures involve extensive laboratory and greenhouse screening and selection procedures, followed by a four stage field-testing and evaluation at 10 locations. This makes it possible to identify cotton strains with many desired traits. Fifty-six traits are considered simultaneously in the MAR program, from stand establishment and resistance to pests, to yield and its components and fiber quality. The 10 field testing locations include the major Texas cotton growing regions from the Rio Grande Valley in the south to Lubbock in the north (1,079 kilometres), representing a range of diverse environments including moderate to severe water stress and insect and disease pressures. Advanced strains are included in regional tests throughout the USA Cotton Belt.

The MAR cotton-breeding scheme is presented in Figure 1. Each year about 400 crosses representing 60 to 80 cross combinations are made in the field among the elite strains of the MAR germplasm and introduced parental types. Crosses are planted in the greenhouse in the fall, screened for resistance to the bacterial blight pathogen, and seed from the F<sub>1</sub> generation are planted in the field.

Direct laboratory and greenhouse selection is practised on F<sub>2</sub> selections for the following four traits: (1) Seed coat resistance to mould; (2) slow rate of radicle elongation when acid-delinted, non-treated seed are held at 13.3°C for 8-days in an incubator; (3) resistance to the bacterial blight pathogen; and (4) resistance to seedling pathogens caused by *Rhizoctonia solani* and *Pythium ultimum*. Direct selection for the four traits indirectly provides genetic gains for resistance to major pathogens and insects, in addition to higher yield potential, earliness, and improved fiber and seed quality. Application of the MAR system has been very effective and successful in developing new superior cottons.

Each year about 80,000 seeds representing 500 F<sub>2</sub> individual plant and progeny row field selections are processed in the laboratory and greenhouse during the winter. About 15,000 clean seeds with no mould growth and slow rate of radicle elongation are selected, planted in cups and placed in the greenhouse. About 10,000 seedlings are inoculated with a mixture of four

racess of the bacterial blight pathogen [*Xanthomonas campestris* pv. *malvacearum* (*Xcm*)]. Immune and susceptible reactions to bacterial blight are identified. Resistant seedlings are transplanted into pots (3,000). About 2,000 plants are selected and produce seed in the greenhouse. The other 1,000 plants are eliminated because of susceptibility to insects, fruit shedding, late maturity and undesirable agronomic characters (El-Zik and Thaxton, 1989).

Seed from the laboratory-greenhouse MAR individual plant selections are grown in single progeny rows in the field in the spring the same year (2,000 progeny rows). Seedlings are inoculated with a mixture of four races of the bacterial blight pathogen *Xcm*. Field evaluations are made for seedling establishment, resistance to bacterial blight, resistance to insects, fruiting patterns and plant type. We select for several characteristics of agronomic value such as close fruiting, short internodes and high boll retention, productivity, earliness, and higher fiber and seed quality. Seed produced by the selected F<sub>4</sub> progenies (50 to 60 strains) are adequate for planting MAR nurseries and increase rows the following season.

The MAR field testing procedure has four stages (Figure 1). The new strains are grown in 10 nurseries throughout Texas (Strain Test) for extensive insect, disease, drought tolerance and environmental stress performance evaluation. Selected promising strains (28 genotypes) are also evaluated in replicated Early Field Planting test (EFP) at six locations. The following year, the best 20 strains from the Strains Test and Early Field Planting test are included in a Uniform MAR (UMAR) test at 10 locations. The elite strains of this group are also entered in cultivar and demonstration tests in growers' fields. Strains are evaluated for their performance in each of the tests for: stand establishment, plant type, fruit load, boll type, resistance to plant pathogens and insects, drought tolerance, earliness, yield, fiber and seed quality, and stability over environments. Elite MAR strains are quantified for photosynthetic capacity and partitioning of biomass. Strains with a higher allocation of assimilate to fruiting organs and high lint conversion efficiency (harvest index) are identified and selected.

Final selection of the best MAR strains is made based on this extensive performance testing and evaluation over years, and periodically released as new elite MAR germplasm lines. When a strain shows exceptional performance for all traits, it is increased as breeder seed and released as a new MAR Tamcot cultivar (Figure 1). New Tamcot cultivars will have the desired characteristics to all 56 traits. It takes 7 to 8 years from the time a cross is made to releasing germplasm in the MAR program, compared to 11 to 12 years in conventional breeding programs, thus reducing by 1/3 the time needed to develop new cultivars.

## Genetic Gains and Performance of MAR Germplasm

Since the mid-1960's, the MAR program has created, processed and evaluated eight gene pools, and the MAR procedures were used to make selections from each pool. Gene pools were established by making crosses between parental breeding lines and advanced MAR strains to provide genetic variability and diversity, followed by evaluation and selection. The sequential gene pools are referred to as MAR-1 to MAR-8. Progress is achieved when a new gene pool has an increase in average levels of multi-adversity resistance in addition to higher yield potential, earliness, and improved fiber and seed quality than previous gene pools.

The MAR germplasm includes several morphological traits known to be associated with resistance to pests. These traits include nectariless, okra-shaped leaf, frego bract, red plant colour, and their combinations, in both glabrous and pubescent plant types (Thaxton and El-Zik, 1995).

### Resistance to Biotic Stresses

Genetic resistance is a heritable characteristic that suppresses pest populations or reduces pest damage. Defence of host plants against pests may be due to avoidance, non-preference, antibiosis, or to resistance mechanisms. Resistance can range from very small to very large; plant reaction ranges from highly susceptible to immune. Resistance levels are based on comparisons with reference cultivars and breeding lines with known levels of resistance and susceptibility to pests.

Resistance levels have increased for eight pathogens and seven insects from the MAR-1 to the MAR-8 gene pools. A companion paper (Thaxton and El-Zik, 1999) reported on genetic gains obtained for preservation of seed quality and seed deterioration, resistance to pathogens causing diseases: seed rot (internal and external mould growth on seed coat), seedling disease complex, bacterial blight, *Fusarium* and *Verticillium* wilts, *Phymatotrichum* root rot, root-knot and *Reniform* nematodes, and leaf spots. The MAR program maintains high levels of resistance in its germplasm to the 19 USA races of bacterial blight *Xcm*. Progressive improvements in resistance to vascular wilt pathogens, *Phymatotrichum* root rot, and nematodes have been made, although no direct selection for these pests was practised. However, the rate of genetic gain in resistance to these pathogens was slower than the rate for the four traits for which direct selection was employed.

Levels of insect resistance are based on grades and observations made at each of the 10 test locations several times during the season for the Strains, EFP and UMAR Tests, knowing insect pressures and damage, and based on lint yield and earliness. Levels of resistance to pests are determined in comparison

with strains and cultivars with known levels of resistance and susceptibility to those pests. In addition, progressive increases in levels of resistance to insects in the MAR germplasm are measured and quantified by plant mapping at three tests conducted under no-insecticide and insecticide treatments. Plants are mapped at the end of the season to assess boll retention and distribution of bolls on fruiting branches and nodes.

Initially the MAR program dealt with diseases. However, there was a simultaneous gain in resistance to insects. The MAR-1 germplasm was susceptible to most insects. Partial and intermediate levels of resistance to certain insects were obtained in the MAR-2 to the MAR-4 germplasm. In the MAR-5 to MAR-8 germplasm resistance levels increased to intermediate resistance and resistance (El-Zik and Thaxton, 1989, 1990, 1992, 1996; El-Zik *et al.*, 1991; Thaxton and El-Zik, 1994, 1996).

Bird (1979) provided evidence that Tamcot CAMD-E (MAR-2) has intermediate resistance to the boll weevil. It was later confirmed that Tamcot CAMD-E has intermediate resistance to *Heliothis* spp. (McCarty *et al.*, 1983; Zummo *et al.*, 1983) and fleahopper (Lidell *et al.*, 1986). Tamcot CAMD-E was the first Upland cultivar with significant levels of resistance to five plant pathogens and three insects (Bird, 1979). Tamcot HQ95 (MAR-5) and Tamcot Sphinx (MAR-6) have high levels of resistance to insects and water stress; they are resistant to six plant pathogens and four insects (El-Zik and Thaxton, 1990, 1996). New MAR-5 and MAR-6 strains with higher levels of resistance to early and late season insects subsequently have been identified and released (El-Zik *et al.*, 1991; El-Zik and Thaxton, 1989, 1992; Thaxton *et al.*, 1991; Thaxton and El-Zik, 1994). The MAR-7 and MAR-8 germplasm has the highest levels of resistance to seven insects (aphids, thrips, fleahopper, boll weevil, tobacco budworm, bollworm, whitefly) than previously released MAR germplasm (Thaxton and El-Zik, 1996). The MAR system is making progress with genetic variability provided by adapted cottons.

### Resistance to Abiotic Stress

#### Cold Tolerance

Stand establishment and vigour are the cornerstone of cotton production because the expression of the genetic potential of a cultivar is determined in the early developmental stages of the plant. Planting seed quality and the ability of cottonseed and seedlings to perform in cool, wet soil with minimal damage from soil fungi are key MAR germplasm traits. MAR screening procedures select seed for cold tolerance (13.3°C for 8-days) and resistance to seed/seedling pathogens under laboratory, greenhouse and field conditions (Thaxton and El-Zik, 1998).

### *Drought Tolerance*

Water stress is the most important adversity affecting cotton fruit production, square and boll shedding, lint yield and fiber quality. Genetic variation exists among cotton cultivars for flower and boll production, lint yield, earliness, and water use efficiency (Cook and El-Zik, 1993; Gerik *et al.*, 1996). Selection for and incorporation of increased seedling vigour, rapid root system establishment and lower root-to-shoot ratios in future cotton germplasm could improve drought tolerance and lint yields (Cook and El-Zik, 1992).

Under both well watered and water stressed conditions, Tamcot CD3H (Cook and El-Zik, 1993), and Tamcot HQ95 (Gerik *et al.*, 1996) produced more bolls, had higher yields and greater water use efficiency than other cultivars studied. These studies suggest that the intrinsic photosynthetic capacity of the two Tamcot cultivars may be greater than the other cultivars.

### *Earliness*

Since the 1970's, most breeding programs have emphasised early crop maturity. Earliness is expressed as the ratio of first harvest to total lint yield. The percentage of acreage planted in early maturing cultivars in the USA mid-south increased by an average of 19% in 1978 and 90% in 1986 (Bridge and McDonald, 1987). In contrast, only small increases in the percentage of early maturing cultivars occurred in the Southeast and Western cotton growing regions during the same period. The Southwest (Texas and Oklahoma) grows predominantly early short-season cultivars. El-Zik and Frisbie (1985) and Bridge and McDonald (1987) reviewed earliness and discussed the advantages of early maturity and short season production systems. The MAR germplasm has a distinct advantage in early fruit set and crop maturity.

In the 1997 UMAP Tests, most MAR-7 strains were as early as Tamcot Sphinx and Tamcot CAB-CS. Averaged over the three locations where earliness data was assessed, it ranged from 60.7% for CHGUA7QCUH-1-95 to 37.9% for the okra-leaf strain OSIKRHQWIH-2-94 compared to Tamcot Sphinx at 50.3% and Tamcot CAB-CS at 50.2% with an overall mean of 50.9% (Table 1). Averaged over two EFP Test locations, earliness ranged from 70% for CIQBCHGC8H-1-96 to 34% for Deltapine 50, with an overall mean of 55% (Table 2). MAR-7 strains matured earlier than check cultivars.

### *Lint Yield*

The objective of cotton genetic improvement programs is to develop cultivars with high yielding ability and superior fiber and seed quality. Incorporating high levels of resistance to pests and abiotic stresses into adapted high yielding cultivars is a major challenge.

The superior performance of MAR germplasm and cultivars has been documented (Bird, 1975, 1982; El-Zik *et al.*, 1991; El-Zik and Thaxton, 1989, 1992; Thaxton *et al.*, 1991; Thaxton and El-Zik, 1994, 1996). Data gathered over 35 years show MAR cultivars have high yield potential in both the presence or absence of adversities.

Averaged over five UMAP Tests in 1997, lint yield ranged from 704 to 1081 lb./acre, with a mean of 862 lb./acre (Table 1). Six MAR-7B strains and Tamcot Sphinx gave yields above 900 lb./acre. In the EFP Tests averaged over four locations lint yield ranged from 650 to 943 lb./acre, with a mean of 808 lb./acre (Table 2). Seven MAR-8 strains gave lint yield higher than 900 lb./acre, and significantly higher than the cultivar checks Paymaster 330, Deltapine 50 and Tamcot CAB-CS. Yield of Tamcot Sphinx was not significantly different from the seven strains except LBCHGPI2KS-1-96.

The progressive genetic gains in lint yield in the MAR gene pools are shown in Figure 2. The incremental increases in yield paralleled the increases in levels of resistance to insects, plant pathogens, and abiotic stresses. Averaged over 4-years and 42 tests, lint yield increased 31% and earliness 24% from the MAR-1 Tamcot SP37 to the MAR-7 Tamcot Luxor (Figure 2).

### *Fiber Quality*

Generally, as yield increased from the MAR-1 to MAR-8, fiber quality traits also improved. In the MAR-8 germplasm, fiber length ranges from 1.10 to 1.28 inches, uniformity 83.3 to 88.2, strength 28.5 to 35.4 g/tex, elongation 5.8 to 7.4, micronaire 3.7 to 4.8, maturity 85.6% to 95.1%, and fineness ranges from 142 to 187.

A substantial improvement in fiber strength has been achieved in MAR germplasm. MAR-5 Tamcot HQ95, averaging 27.6 g/tex, is 3 g/tex higher than the MAR-1 and MAR-2 germplasm. MAR-6 Tamcot Sphinx has high yielding ability and fiber strength of 28.8 g/tex, averaging 4.3 g/tex higher than MAR-1 and MAR-2 releases and 2.3 g/tex higher than Tamcot HQ95. Tamcot Luxor is a new MAR-7 genotype with the highest fiber strength (30.1 g/tex) and yielding ability. It averages 5.6 g/tex higher than MAR-1 and 3.6 g/tex higher than the MAR-5 germplasm. The MAR system is pyramiding and identifying favourable recombination of genes for high yield potential, earliness, resistance to insects, pathogens and abiotic stresses, and improved fiber and seed quality.

### *Stability in Performance*

Regression analyses establish stability of traits over environments (locations and years) and compare the response of individual cultivars (dependent variable) with the mean linear response of all genotypes (strains and cultivars) in the test (independent variable). Fig. 3 shows the regression lines for fiber strength of MAR Tamcot cultivars from MAR-1 to MAR-7 gene pools

and Deltapine 50 over 36 locations and 3-years. Tamcot Sphinx (MAR-6) has the highest fiber strength in all environments and is the most stable based on slope of the regression line.

### **Impact of the MAR Germplasm**

Application of the MAR breeding system has been effective in developing superior cotton germplasm and cultivars that minimise growers' risk, reduce production costs, and increase profit. The programme has released 11 Tamcot cultivars and more than 360 elite MAR breeding lines. Commercial seed companies and breeders have released 29 cultivars by selecting from released MAR germplasm. For the last 15 years, MAR germplasm and Tamcot cultivars have been grown in 35 to 50% of the cotton acreage in Texas and Oklahoma. This represents four million acres or 25% of the total USA cotton acreage. MAR germplasm is also used extensively in many countries of the world. MAR germplasm narrows the gap between actual and potential yield through host-plant resistance to pests and abiotic stresses.

### **Biotechnology**

Despite breeding progress achieved, additional gains in cotton productivity, quality and resistance to pests and abiotic stresses are needed. Agricultural biotechnology offers opportunities to enhance the development of new cottons with higher levels of resistance to multi-pests and other important traits. Important genes need to be mapped and cloned. Gene transformation is a valuable tool in inserting pest and herbicide resistance genes and other traits from other species. Regeneration of transformed cells through somatic embryogenesis, shoot tip, or other means, is necessary to recover transgenic cells and plants. Transgenic plants should carry the inserted gene(s) in addition to the many genes controlling crop productivity, quality, stability and adaptability.

Biotechnology will not replace or be used independently from conventional breeding. Conventional breeding is essential for developing new and improved germplasm and cultivars for the future. Biotechnology will provide new tools for selection in segregating populations. DNA marker-assisted selection can be used in early generations (F2 or F3) to screen large numbers more efficiently.

The MAR program collaborates actively with genome mapping, fingerprinting and tagging, gene insertion and transformation, and developing transgenic MAR cottons. Interfacing biotechnology with MAR breeding will enhance MAR cotton breeding for the 21st century.

### **The Future**

There have been many advances in the past 15 years in breeding cotton for resistance to pests and abiotic stresses. Resistant sources have been identified for most cotton pests. The development and release of multi-pest and multi-adversity resistant cultivars is

particularly significant. However, higher levels of resistance to all adversities affecting cotton production (insects, plant pathogens, nematodes, and abiotic stresses) are needed to reduce production cost and risk and to increase grower's profit.

Maintaining crop health is essential for profitable and sustainable cotton production. Breeding programs must focus on developing adapted cottons with multi-adversity resistance, in addition to high yielding ability, earliness, and improved fiber and seed quality. In addition, the cotton plant of the future must be highly efficient in fruit retention, and its use of solar radiation, water, and nutrients in both irrigated and dryland production. The MAR system, differs from conventional breeding procedures, being efficient in simultaneously pyramiding favourable and compatible genes for resistance to pests and abiotic stresses, earliness, yield, and quality. Continued progress will be made with the MAR genetic improvement system, benefiting cotton growers and industry.

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**Table 1. Mean lint yield and earliness for MAR strains and cultivars in the 1997 Uniform MAR (UMAR) tests averaged over five locations<sup>1</sup>.**

MAR Strain/ Cultivar	MAR Gene Pool	Lint Yield lb./acre	Earliness %
SPNXCDUG8H-1-95	MAR-7B	1081**	48.9**
HQCULHQPIH-1-95	MAR-7B	944	46.9
HGPIHQBPIH-2-94	MAR-7B	941	53.3
SPNXCBCGP6H-1-95	MAR-7B	932	51.6
PD22CUBQWS-1-95	MAR-7B	926	50.8
CABU2HGC8H-2-91	MAR-7A	919	53.5
Tamcot Sphinx check	MAR-6	913	50.3
CIQUBCHGBS-1-95	MAR-7B	890	51.5
Paymaster 330 check	----	852	42.5
Tamcot CAB-CS check	MAR-4	775	50.2
CHGUA7QCUH-1-95	MAR-7B	704	60.7
MEAN		862	50.9
LSD (P=0.05)†		94	8.5
C.V. %		17.5	20.8

<sup>1</sup>Weslaco (2), Corpus Christi, Brazos Valley and Temple, Texas.

\*\* Significant at the 0.01 probability level.

† Least significant difference between two means within a column.

**Table 2. Mean lint yield and earliness for MAR strains and cultivars in the 1997 Early Field Planting (EFP) tests averaged over locations<sup>1</sup>.**

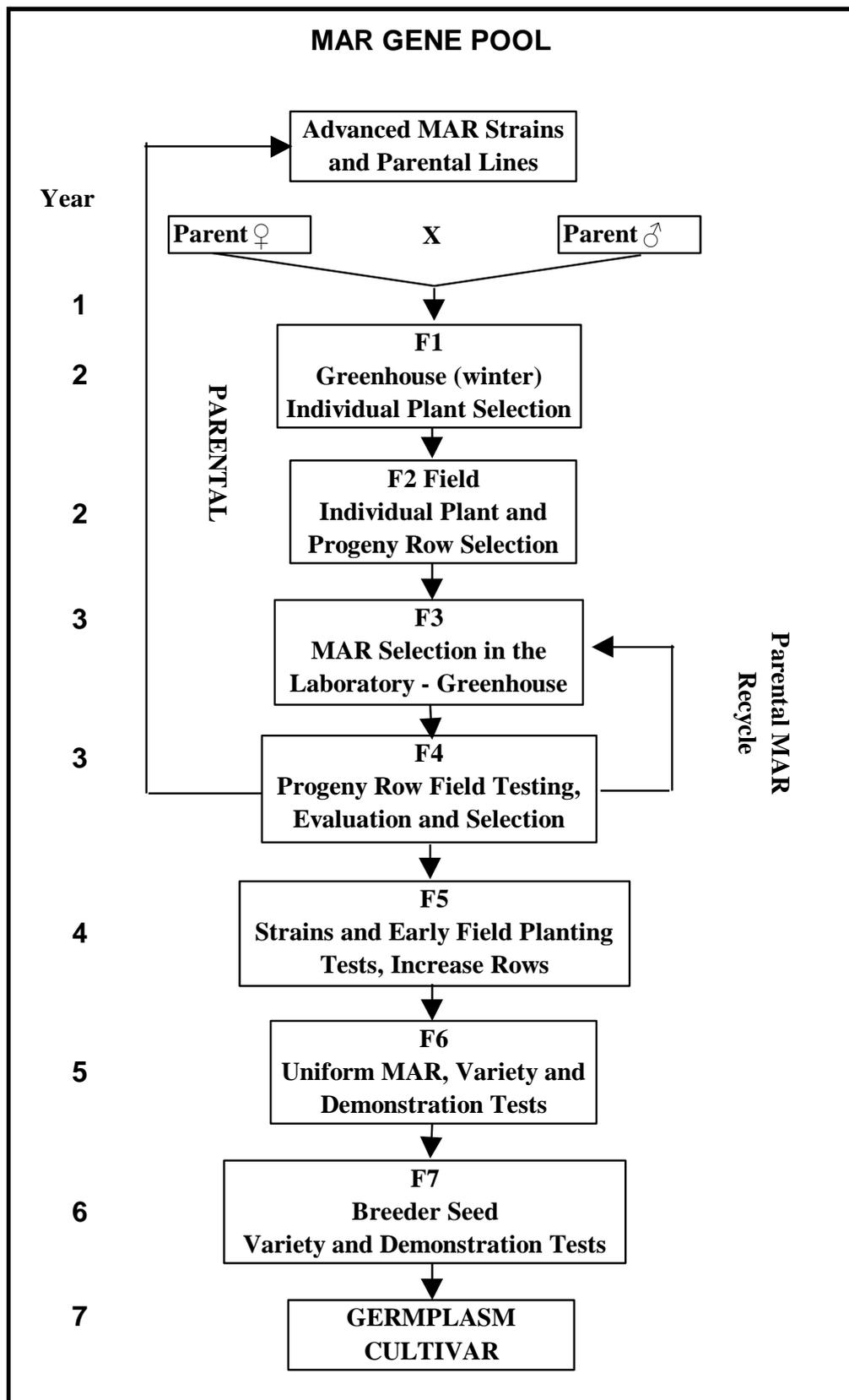
MAR Strain/ Cultivar	MAR Gene Pool	Lint Yield lb./acre	Earliness %
LBCHGPI2KS-1-96	MAR-8	943**	50**
CHGBCHGC8H-1-96	MAR-8	920	57
CIQBCDULBH-1-96	MAR-8	917	62
LBQWICQPIS-2-96	MAR-8	916	53
CIQBCHGC8H-1-96	MAR-8	916	70
LBQWICQPIS-1-96	MAR-8	909	56
CSHLBCLBGH-1-96	MAR-8	901	42
CIQBCDUG8S-1-96	MAR-8	883	67
Tamcot Sphinx check	MAR-6	832	45
Paymaster 330 check	----	796	35
Deltapine 50 check	----	753	34
Tamcot CAB-CS check	MAR-4	661	53
CSHLBLGBQS-2-96	MAR-8	650	63
MEAN		808	55
LSD (P=0.05)†		100	9.3
C.V. %		17.8	17.1

<sup>1</sup> Weslaco, Corpus Christi, Brazos Valley and Temple, Texas.

\*\* Significant at the 0.01 probability level.

† LSD between two means within a column.

Figure 1. Multi-Adversity Resistance (MAR) cotton breeding scheme.



**Figure 2. Mean lint yield and fiber strength of Tamcot cultivars representing MAR 1 to MAR 7 gene pools averaged over 42 tests and four years (1993 – 1996).**

