Building Up Resistance to *Verticillium* Wilt in Cotton Through Honeycomb Breeding

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ABSTRACT

Honeycomb breeding as evolved and refined over 25 years, is based on three principles: First: insuring conditions that optimize single plant heritability; Second: genetic analysis of crop yield potential into three components, potential yield per plant estimated by the progeny mean (X), tolerance to stress estimated by progeny standardized mean (X/s) and responsiveness to inputs estimated by the progeny standardized selection differential $[(X_s - X)/s]$*, all evaluated in the absence of competition; and Third: the intrinsic property of the genome for self restructuring that insures in perpetuity genetic variation for the three components of crop yield potential and quality. Thus in each generation, selection among progeny lines is based on the three components of crop yield potential whereas selection within lines is on the quality of the product of individual plants selected to be parents (*X_s*). Three cycles of honeycomb selection for the components of crop yield potential and quality within cotton cultivar grown under stress of Verticillium wilt and susceptible to the disease, led to the development of Verticillium resistant lines although selection did not consider resistance. The results suggest that non-stop honeycomb selection over the target area of cultivation of cotton leads to a constant improvement, longevity and adaptation of cotton cultivars to the unforeseen and continually changing biotic and abiotic stresses.*

The Essence of Honeycomb Breeding

Honeycomb breeding emerged as a sequel to systematic studies on the factors affecting efficiency in plant breeding. The end result was the formulation of the principles on which honeycomb breeding is founded (Fig. 1). Firstly, if the objective is optimization of single-plant heritability, evaluation and selection should be conducted in the absence of competition (Fasoula and Fasoula, 1997). Furthermore, the conventional field plot should be replaced at all stages of the breeding program by the single-plant plot grown in the absence of competition. Secondly, additive alleles, the only ones responsible for genetic advance through selection and transgressive variation, should be fixed for optimal gene expression. Gene fixation is also essential for erasing the masking effects of heterozygosity on single-plant heritability and for transforming non-additive allelic variation into additive. Thirdly, if the objective is high, stable performance, entries should be exposed every generation to the biotic and abiotic stresses encountered in the target adaptation area. Fourthly, in order to expose the entries to the multitude of biotic and abiotic stresses, utilization of experimental designs that sample effectively for environmental diversity, like the honeycomb designs, are essential. Fifthly, efficient selection for crop yield potential requires that the components of crop yield potential are accurately defined and assessed by objective criteria in the isolation environment. This goal has been accomplished (Fig. 2).

There are three components of crop yield potential: (1) potential yield per plant, (2) tolerance to stresses, and (3) responsiveness to inputs. The potential yield per plant is determined by the mean of single-plant yields of the genotype and is responsible for extending the range of plant densities for optimal crop productivity. The higher the potential yield per plant, the wider the range of plant densities within which optimal productivity is achieved. Furthermore, the higher the potential yield per plant the faster the early cover of the ground for protection from weeds.

Tolerance to stresses is determined by the standardized mean of the genotype. The larger the $\overline{X} / s$, i.e., large $\overline{X}$ and small s, the more tolerant to stresses the plants of the genotype. Responsiveness to inputs is determined by the standardized selection differential, $(\overline{X}_{sel} - \overline{X}) / s$, of the genotype and it indicates how much a given genotype is capable of taking advantage of the improved growing conditions. The three components of crop yield potential are controlled by independent sets of genes that may nevertheless be selected jointly in the isolation environment. Finally, principle 6 states that selection for crop yield potential and quality must be continuous in order to fully exploit the capability of the genome for self-restructuring that insures genetic variation in perpetuity. This is accomplished even under the presence of stabilizing mechanisms such as cloning and selfing. The results presented testify to the capability of cotton genome for self-restructuring.

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Crop yield potential is analyzed into three components, assessed in the absence of competition by objective criteria. Once joint selection is practiced on the three criteria, all secondary traits contributing to these components are automatically incorporated (Fig. 2) (Fasoula and Fasoula, 1999).

Application of Honeycomb Breeding in Cotton

This paper presents data that result from honeycomb selection applied by the Dept. of Genetics and Plant Breeding within the cotton cultivar Sindos 80 of the Cotton Research Institute for an MS thesis (Kyriakou, 1984) and a PhD thesis (Kliafas, 1991).

In 1981, more than 10,000 plants of cotton cultivar Sindos 80 were grown in Central Macedonia, using a spacing of 125x125 cm to avoid competition. At the stage of full productivity, 200 plants were selected visually. Subsequently, they were quality tested and in 1982, the best 49 plants were grown in the University Farm in a replicated-49 honeycomb trial with 35 plant positions per progeny line and a spacing of 125x125 cm. Pedigree honeycomb selection based on potential yield per plant and quality was applied in 1982 and 1983. This selection led to the development of the experimental variety Macedonia that was evaluated extensively by the Variety Research Institute of Cultivated Plants at Sindos (Kyriakou, 1984). The results of tests in 1984 and 1985 at 16 locations are depicted in Fig. 3.

The 10% average superiority of Macedonia over Sindos 80 over locations and years testifies to the presence of genetic variation and the ability of honeycomb breeding to exploit latent diversity. Honeycomb selection for potential yield per plant and quality in Macedonia continued in 1984 and 1985.

Although the variety Macedonia developed using very high selection pressures (1.5%), the progeny lines retained high genetic diversity as evidenced when the best 49 lines were grown in 1986 in two contrasting fields. One in the University Farm, free from *Verticillium wilt*, and the other in a heavily infected field, in the county of Imathia (Kliafas, 1991). The lines in the two fields showed strong genotype by site interaction, with the first line in the non-infected field occupying the fortieth position in the infected field, and the first line in the infected field occupying the thirtieth position in the non-infected field. In 1987, the best selections over the two sites as well as selections with good performance across sites and four checks were compared in two honeycomb trials. One trial was grown in a heavily infected field in Thessaly and the other in a non-infected field in the University Farm. Fig. 4 shows the degree of infection (average of 35 plants) in the first trial using a scale from 0 to 4 and a plant spacing of 150x150 cm.

These results may be summarized as follows:

1. One year of honeycomb selection for potential yield per plant in an infected and a non-infected field allowed the isolation of lines 7E and 2B with a similar degree of resistance to *Verticillium* as the resistant checks Acala S.J.2 and Acala S.J.5. As to potential yield per plant, line 7E outyielded the best check Acala S.J.5 by 140%.
2. The two low-yielding lines of Macedonia, 4F and 5F, were more susceptible compared to the original variety Sindos 80, indicating the plasticity of the cotton genome to generate diversity for resistance and susceptibility to *Verticillium wilt*.

The degree of infection to *Verticillium wilt* using a scale of 0 to 4 shows that lines 7E and 2B, derived from the susceptible cultivar Sindos 80, had similar resistance but higher potential yield per plant compared to Acala S.J.2 and Acala S.J.5 (Fig. 4.) (Data from Kliafas, 1991).

The results of Fig. 4 verify the conclusion reached by Pryor and Ellis (1993) that resistance's are specified at complex loci that generate new resistance genes through mechanisms of genetic re-assortment.

Genetic diversity for potential yield per plant within the cotton cultivar Sindos 80 is so large that lines outperforming those of the previous years may be constantly obtained through non-stop honeycomb selection (Fig. 5) (Data from Kliafas, 1991).

Varieties Acala SJ-2, Acala SJ-5, 4S, Sindos 80, Macedonia and the two best lines (7E, 2A) out of 45 lines representing Macedonia, are compared for potential yield per plant in the two contrasting sites (Fig. 5). The results may be summarized as follows:

1. In the infected field, Macedonia outperformed Sindos 80 due to better resistance to Verticillium wilt and was comparable with variety Acala SJ-2. In the noninfected field, Macedonia and Sindos 80 gave similar performances, while the two checks Acalas dropped off sharply.
2. The two best lines derived from Macedonia, 2A and 7E, outyielded all varieties in the two environments, showing that nonstop honeycomb selection across environments for the three components of crop yield potential and quality avoids degeneration and insures constant improvement of cultivars. This permanent genetic upgrading of the genome prolongs the life span of cultivars and improves their performance on a constant basis.

General Conclusions

1. Once selection is performed in the isolation environment synchronously for the three components of crop yield potential and quality, all

secondary traits contributing to the four key traits are automatically incorporated.

2. Seed companies can constantly improve inbred line cultivars, so that every season, new inbred seed with constant improvement that makes its purchase obligatory, can be produced at much lower cost than the cost-ineffective hybrid seed.

References

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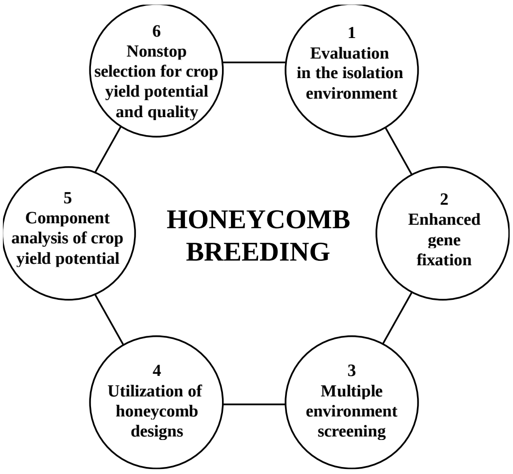
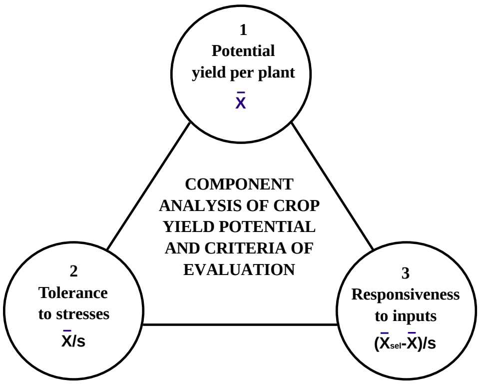
Figure 1. The six principles on which honeycomb breeding is founded.



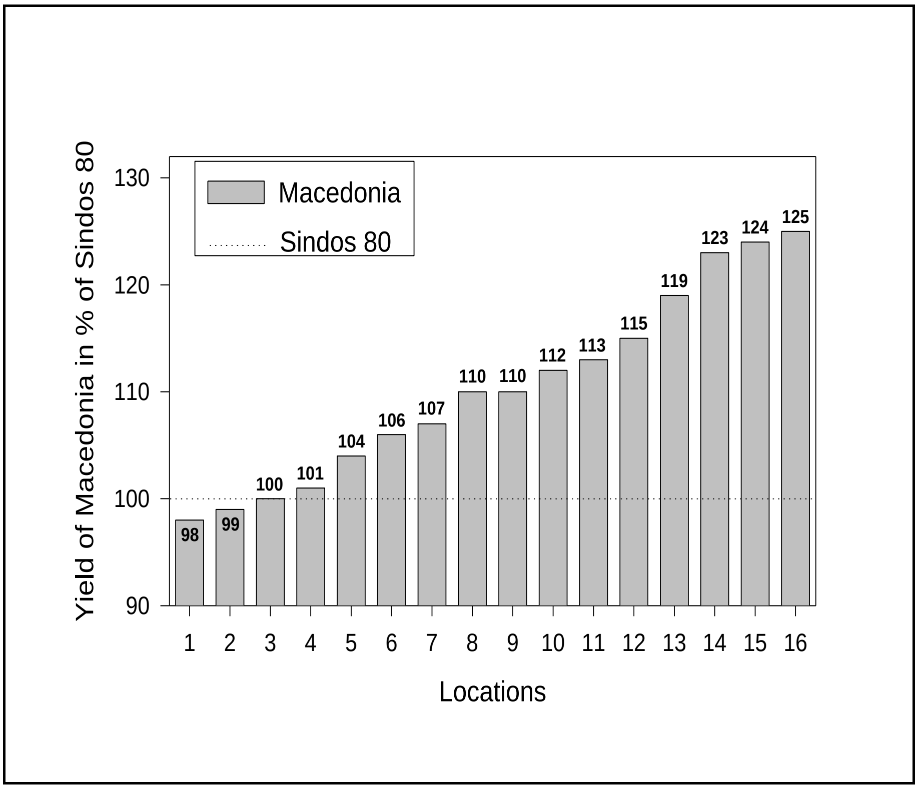
(Fasoula and Fasoula, 1999).

Figure 2. Component Analysis of Crop Yield Potential.



(Fasoula and Fasoula, 1999).

Figure 3. The average seed-cotton yield of the variety Macedonia across sixteen locations and two years was 110% of Sindos 80.



Source: Variety Research Institute of Cultivated Plants - Sindos.

Figure 4. Degree of *Verticillium* infection and potential yield of seven cultivars.

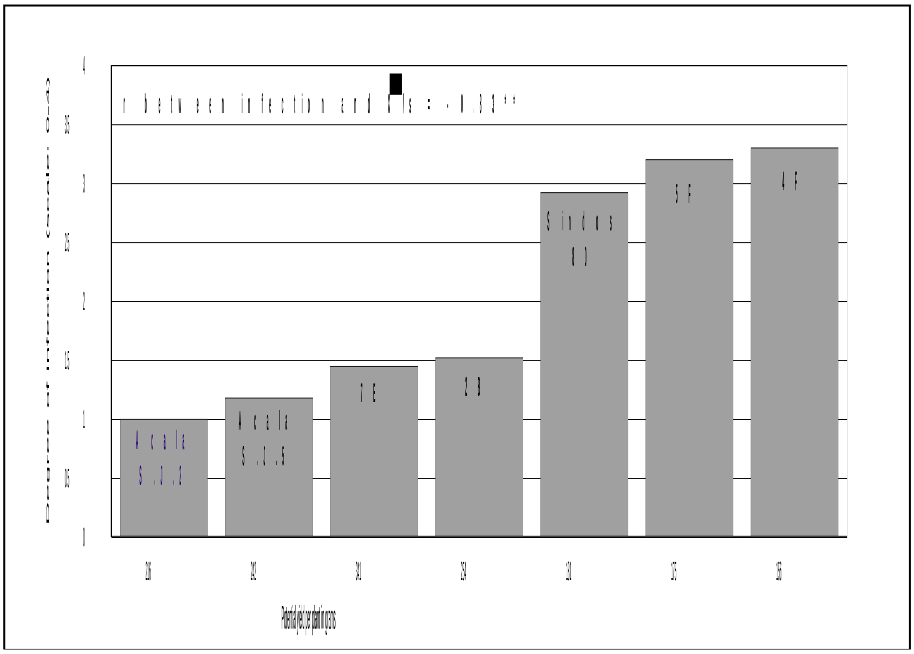


Figure 5. Genetic diversity for potential yield per plant in *Verticillium* infected and non-infected conditions.