COMPARISON OF BC_1 AND F_2 MAPS OF AN INTERSPECIFIC HYBRID (*Gossypium darwinii* X *Gossypium hirsutum*)

Juliana Osorio and J. McD. Stewart

Crop, Soil and Environmental Sciences Department. University of Arkansas, Fayetteville, AR.

INTRODUCTION

Crosses between cultivated cotton (*Gossypium hirsutum x G. hirsutum*) show low level of polymorphism, thereby limiting the construction of molecular maps. In contrast, a high level of polymorphism is expected in crosses among different *Gossypium* species (e.g., *G. hirsutum X G. barbadense* or *G. hirsutum X G. darwinii*) because of the wider range of genetic diversity. Furthermore, crosses between wild and cultivated cotton are developed in order to introduce specific traits of interest, such as drought tolerance (*G. darwinii*) and resistance to reniform nematode (*G. arboreum*). Several linkage maps have been developed for the *Gossypium* tetraploid species with different molecular markers (RFLP, AFLP, SSR).

The use of molecular markers has increased in recent years. They are especially useful in genetic analysis, diversity studies, genetic mapping and DNA fingerprinting. Among the molecular markers available, the AFLP technique (Vos et al., 1995) produce a large number of polymorphic loci at low cost. Its attractiveness lies in its high reproducibility, capacity to generate a large number of loci and its wide applicability in measuring diversity. In addition, with AFLPs it is possible to amplify many sequences, which makes possible the construction of high density linkage maps (Thomas et al., 1995).

The purpose of this study is to compare BC1 and F2 molecular maps from a cross between *G. darwinii X G. hirsutum*. AFLP markers are being used to construct the maps. The comparison will give a better understanding of the genetic arrangement within the genomes and determine which population gives a more reliable map.

OBJECTIVES

- Compare protocols and commercial kits for DNA isolation in order to determine the best method for utilization in AFLP fingerprinting
- Construct AFLP genetic maps based on BC_1 and F_2 populations from a single *Gossypium darwinii* X *G. hirsutum* F_1 hybrid.
- Compare the developed maps to determine the more suitable population (BC_1 or F_2) for genetic mapping.

MATERIALS AND METHODS

1. Plant Material: Two populations (BC1 and F2) of 100 plants each from a single F1 hybrid of *Gossypium darwinii* (AD5) x *G. hirsutum* (cv. DP 33B) are being used in the construction of molecular genetic maps. (Figure1.)
2. DNA extraction: Young, unfolded leaf tissue was collected and processed with three protocols for DNA isolation. 1-A rapid and high yielding DNA miniprep for cotton (*Gossypium* spp.) (Li et al., 2001)(AR). 2-Economical and rapid method for extracting cotton genomic DNA (Zhang and Stewart, 2000) (ER). 3- DNA easy Plant Mini Kit from QUIAGEN® (Valencia, CA) (QI).
3. AFLP:AFLP fingerprinting uses by a method similar to that reported by Vos et al. (1995). The fragment resulting from selective amplification reactions were resolved by denaturing polyacrylamide gel electrophoresis and resolved by silver staining.

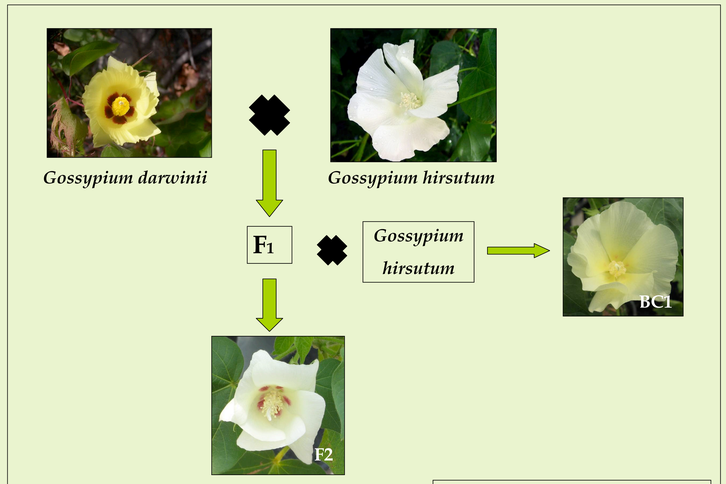
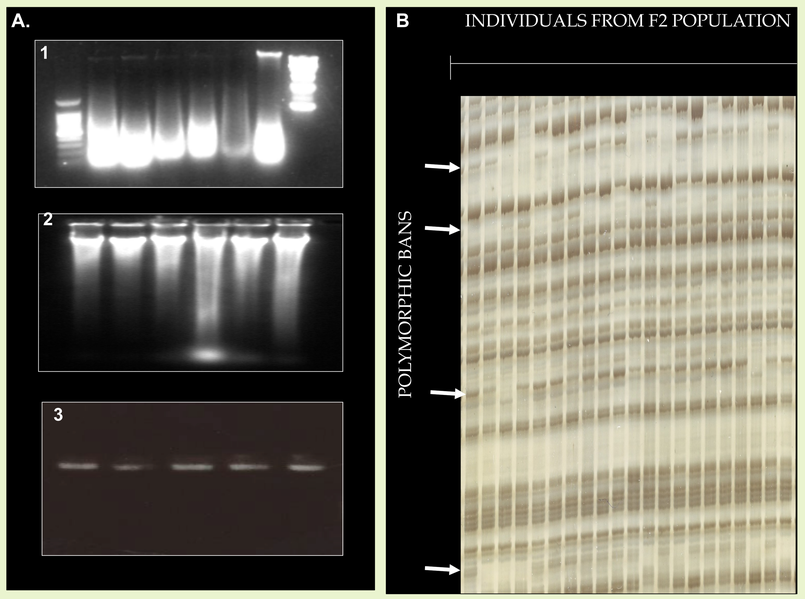


Figure 1. Hybridization scheme

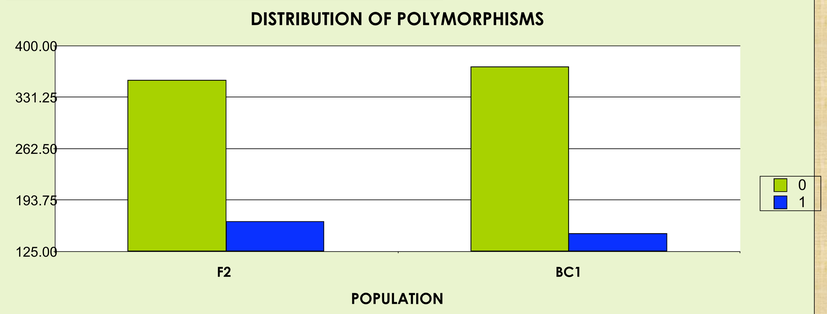
RESULTS AND DISCUSSION

A. DNA from the three protocols used for the extraction: 1-AR, 2-ER, 3-QI. DNA degradation was observed when using protocol 1 and 2. DNA extracted with a commercial kit (3) gave good quality DNA, although the yield was considerably less. Because AFLPs require good quality DNA, the commercial kit was chosen to isolate DNA from the populations.

B. AFLP gel from individuals of the F2 population. The polymorphisms were obtained with primers having two selective nucleotides (beyond the pre-amplification reaction). The primers used here were EcoRI+A(GC) and MseI+C(AT).



DISTRIBUTION OF POLYMORPHISMS



0= number of common bans 1= number of polymorphic bands

Each primer set used in the generation of AFLP fingerprints generates a different number of polymorphic bands. The polymorphisms are analyzed by scoring for the presence (0) vs. absence (1) of amplified fragments on the resolved gels. The resulting data will be processed in JOINMAP 3.0 (Stam, 1993) softwares for the construction of the genetic maps.

SUMMARY AND PROJECTED WORK

- The commercial DNA extraction kit, although it yielded less DNA, was more suitable for obtaining the high molecular weight DNA required in the AFLP technique.
- For the quantity of markers used (six, so far), the F_2 populations show a higher percentage of polymorphisms, as expected. Surprisingly, the BC_1 population also showed a high level of polymorphism.
- In order to develop the genetic maps, additional primer sets will be utilized to detect additional polymorphic loci.
- A BC_3 population is being advanced in order to screen for introgression of specific traits of interest, e.g., drought tolerance.

LITERATURE CITED

Li H., J. Luo, J.K. Hemphill, J.T. Wanh, and J.H. Gould. 2001. A rapid and high yielding DNA miniprep for cotton (*Gossypium* spp.). Plant Molecular Biology Reporter 19:183a-183e.

Lincoln, S E., M.J. Daly, and E.S Lander. 1993. Constructing Genetic Linkage Maps with MAPMAKER/EXP Version 3.0. Whitehead Institute.

Stam, P. 1993. Construction of an Integrated genetic linkage maps by means of a new computer package: JOINMAP. The Plant Journal. 3:739-744.

Thomas, C.M., P. Vos, M. Zabeau, D.A. Jones, K.A. Norcott, B.P. Chadwick, and J. Jones. 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to *Cladosporium fulvum*. The Plant Journal. 8:785-794

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijter, J. Pot, J. Paleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research. 23:4407-4414

Zhang, J., and J McD. Stewart. 2000. Economical and Rapid Method for Extracting Cotton Genomic DNA. The Journal of Cotton Science 4:193-201.