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شبكة المعلومات الجامعية
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Genetic Diversity and Potentialities of Improving Egyptian Cotton through AFLP- DNA Profiling and Hybridization with Some Imported *barbadense* Stocks.

By

۳۱۵۴

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M. Sc. Agric. (Agronomy), Cairo university, 1994

THESIS

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In

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INTRODUCTION

resistant. Such loss of current popular cultivars would cause a temporary disruption in the industry as necessary adjustments were made. Therefore, maintaining genetic diversity among crop genotypes offers a measure of protection against potential losses from crop pests and facilitates the creation of segregating populations from which plants with superior gene combinations can be selected.

At present Egyptian cotton genetic resources are very limited meaning that cotton breeders should focus their exertion to maintain and expand genetic diversity. Patterns of genetic and geographical variation in the cotton collections need to be analyzed in order to better screen for desirable genes. It may be possible to both increase diversity within regions and achieve greater genetic gains in yield and fiber quality by tapping genetic resources outside one's geographic area. Introgression of genes from feral/exotic cotton and through inter-intra-specific hybridization introgress desirable heritable traits (insect, pathogen, and environmental stress resistance; unique fiber properties; biochemical properties; etc.) from related races into acceptable agronomic genotypes.

Recognition and description of genetic variation is accomplished at both the phenotypic and the genotypic levels. Pedigree information and traditional breeding studies provide useful information on genetic diversity. The ability to utilize DNA technology, such as molecular markers, provides a more accurate and reliable glimpse into these genetic relationships.

Although molecular markers are not a direct product of quantitative genetics, the explosion of interests in their use in plant breeding is large part because of the implementations they have for helping solve problems that are common to quantitative genetics and plant breeding (Dudley, 1997). The availability of molecular markers provides an additional dimension to the use of quantitative genetics in plant breeding. Potential applications of molecular markers include marker-assisted selection, identification of the number of genes controlling quantitative traits, grouping germplasm into related groups, selection of parents and marker-assisted backcrossing.

Increasing the scope of this work will assist in a better understanding of the diversity existing in *Gossypium*, of the heritable systems of cotton plant, and of the systematics of the genus. The DNA-based studies also have afforded the opportunity to examine genetic relationships across different pools of

cultivated germplasm. More information will be available on why plant resistance genes are clustered together, or what candidate genes should be considered when manipulating quantitative traits loci for crop improvement (Paterson, 1996).

Clustering of various line types is apparent, indicating genetic distance estimates among cotton genotypes and will reveal useful information for future breeding efforts. This information can in turn be used to plan crosses and, perhaps, maximize genetic diversity and heterosis.

The uses of molecular markers in assessing genetic relationships among genotypes have many advantages. These usefulnesses are promoted by their: (1) large numbers; (2) lack of environmental interaction; (3) ability to be organized into linkage groups and (4) in a number of cases, estimates of genetic relationships determined through marker-based distance calculations have correlated well with plant performance and pedigree. This makes them useful tools for discriminating among genetic relationships in cotton. At present, little information regarding genetic distance among cultivated cotton genotypes has been conducted.

Molecular markers have been used to assess the relationship among species in the genus *Gossypium*, providing a clearer phylogenetic picture of this group of plants. The visualization of DNA polymorphisms within any germplasm is known as "DNA fingerprinting". The fingerprints may be used as a tool for determining the identity of a specific DNA sample or to assess the relatedness between samples. Fingerprints are also used as a source for genetic markers to generate linkage maps or to identify molecular markers linked to phenotypic trait and/or genetic loci. Many DNA fingerprinting techniques have been developed in the past few years and are generally based on one of two strategies:

I- classical, hybridization-based, fingerprinting

It involves the cutting of genomic DNA with restriction Endonuclease followed by electrophoretic separation of the DNA fragments. Restriction fragment length polymorphism (RFLPs) is an example for this technique.

II- PCR- based fingerprinting

It involves the *in vitro* amplification of particular sequence of DNA using specific or arbitrary primers and a thermostable Polymerase. Amplification products are separated by electrophoresis and detected by staining or use of labeled primers. Techniques in this category include Random Amplified Polymorphic DNA (RAPD), DNA amplification fingerprinting (DAF) and Arbitrarily primed- Polymerase chain reaction, PCR, (AP-PCR).

Amplified Fragment Length Polymorphism (AFLP) technology is a DNA fingerprinting technique that combines both of these strategies. It is based on the selective amplification of subset of genomic restriction fragments using PCR. DNA is digested with restriction Endonuclease, and double-stranded DNA adapters are ligated to the ends of the DNA fragments to generate template DNA for amplification (Lin and Kuo, 1995). The DNA polymorphism identified using AFLP are typically inherited in Mendelian fashion and may therefore be used for typing, identification of molecular markers, produce a marker-assisted breeding programs, and mapping of genetic loci.

The current study was planned through the eyes and the practical view of conventional plant breeders, who have the desire to learn and accept innovative methods that enhance the available crop improvement techniques. Genetic improvement through biotechnology needs conventional breeding for many reasons. Of them, the elite cultivars will be the parents of the next generation of improved genotypes. Second, field testing across locations or cropping systems and over years will be needed to determine the best selections due to the GXE interaction (Kang and Gauch, 1996). Third, the concept of gene pools has been enlarged to include Transgenes and native exotic gene pools that are becoming available through comparative analysis of plant biological repertoires (Lee, 1998).

Based on the previously stated concepts this study was initiated for the identification of cotton genetic materials, blurring the line between genetic diversity and improvement efforts. To date only a few studies have been conducted, touching molecular characterization of cotton genetic reticulation and diversity. Only a few studies using AFLP markers, aimed at characterization of cotton species reticulation and genetic diversification, have been conducted. This study showing the robustness and power of AFLP

technique for cotton nuclear genome analysis and discriminating among different cotton species.

The main objectives of the current study are:

- 1- Employing AFLP markers for studying intra- and inter-specific genetic diversity and phylogenetic relationships among a diverse collection of cotton taxa.
- 2- Utilizing traditional and molecular breeding methodologies for the identification of diverse genotypes and, consequently, the accurate choice of the genetically unique parents as potentially important new sources of favorable alleles for Egyptian cotton improvement.