MOLECULAR TECHNIQUES FOR THE GENETIC IDENTIFICATION OF COTTON PLANTS AND ASSOCIATED SOIL MICROORGANISMS.

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Introduction:

The genetic material (DNA) of living organisms is structurally the same whether it is found in humans, plants, fungi or bacteria. This fact enables the genetic engineer to use molecular genetic techniques to transfer genes from one species to another and so produce, for example, transgenic cotton plants expressing bacterial genes for novel characteristics such as herbicide and insect resistance. This fact also makes it possible to universally employ related techniques to help in the genetic identification of individual people, varieties or strains in human, plant or microbial populations. Thus, the methods used in the compilation of human genetic fingerprints for the identification of people in immigration, forensic and criminal cases could equally be used to differentiate between plant species, cultivars and progeny in plant breeding programs, to identify the particular pathogen strain in an outbreak of plant disease, or to characterise the varieties of mycorrhizal fungi in an agricultural field. The sensitivity of such techniques rests in their ability to detect the rare or subtle differences that exist between the genes of one individual and another.

Molecular genetic techniques for fingerprinting different organisms:

The genetic information with which an organism is born essentially determines what that individual will develop into. Thus, assortments of genes and the "sequences" of genetic information that make-up these genes form the basis for the differences between organisms at the species, population or individual level. Modern molecular genetic techniques are now sensitive enough to detect the small variations (polymorphisms) in gene sequence that are specific to a particular individual. These methods, known collectively as DNA fingerprinting techniques, use specific DNA sequences (called probes or primers) to pinpoint regions of genes that are known to be highly variable amongst different organisms or individuals. This variation can be analysed by radioactively labelling specific DNA probes

that then bind to the variable gene regions (microsatellite or restriction fragment length polymorphism [RFLP] analysis), or by using specific DNA primers to multiply (amplify) the variable gene regions using a new technique called the polymerase chain reaction (PCR). In both cases, potential differences between organisms can be detected by separating the DNA pieces (fragments) according to their size and then looking for the different patterns of DNA fragments that result.

Fingerprinting of cotton cultivars as an aid to plant breeding:
Molecular genetic techniques are increasingly being employed in attempts to identify and track the genes for valuable agronomic characteristics, such as host plant resistance to pests and diseases and improved product yield and quality, during the breeding of crop plants. Such characteristics normally result from the interaction of a number of genes, called Quantitative Trait Loci (QTL), which can affect the final attributes of a plant in an additive manner. These genes are therefore most effective when transferred to new cultivars together, however, this is often a very difficult thing to achieve in plant breeding because the genes are distributed throughout the plant genome rather than in a localised "block". By detecting most or all of the QTL responsible for a useful agronomic characteristic, molecular genetic techniques could play a important role in confirming that progeny plants in a plant breeding program receive all of the desired genes from the selected parental cultivars. In a possible scenario, methods such as PCR and RFLP analysis could be employed to create genetic "fingerprints" for a range of *Gossypium* species and varieties, thereby providing genetic "markers" for the potentially valuable traits that they possess, and helping in the identification and characterisation of parental varieties and their progeny. Molecular techniques would assist in the addition (introgression) of valuable QTL, such as those for disease resistance and fibre quality, into elite varieties during classical cotton plant breeding programs, and of novel transgenes, such as those for insect and herbicide resistance, during back-crossing programs. To date, we have employed PCR analysis to identify differences between the three *Gossypium* species *G. hirsutum*, *G. barbadense* and *G. arboreum*, and between the four current *G. hirsutum* cultivars Deltapine 90, Siokra 1-4, Sicala 33 and Sicala VI. This work, although at an early stage, shows promise in achieving the above objectives.

An epidemiological survey of *Verticillium dahliae* isolates:
Verticillium wilt disease in cotton, caused by strains of *Verticillium dahliae*, is estimated to cost the Australian industry at least $15 million annually. Until recently it has not been possible to differentiate between isolates of these fungi in order to study outbreaks of the disease. However, molecular genetic techniques now offer the potential for the rapid identification of individual fungal strains in environmental samples such as soil or plant tissue. For example, we have successfully employed PCR technology to identify strains of *V. dahliae* isolated from various cotton growing areas in N.S.W. and Queensland as part of an epidemiological survey conducted by Dr. Stephen Allen. In these experiments, we were able to demonstrate distinct genetic differences between fungal strains isolated from widely separated regions including Croppa Creek, Bourke, Breeza and Dalby. These preliminary results suggest that the population of *V. dahliae* in Australia is composed of numerous genetic types, a fact that was not previously recognised and one that could have important implications in terms of disease epidemiology. The sensitivity of this technique is such that an individual strain could be traced throughout the growing season and also between seasons, so that the virulence, spread and persistence of the organism could be determined. If incorporated into a service to growers and plant pathologists, such techniques could play an important role in the control of Verticillium wilt in cotton by providing valuable information necessary for the design of effective disease control strategies.

Identification and characterisation of VAM fungi in cotton fields:
Numerous studies have demonstrated the benefits of natural or artificial infection of crop plants with VA mycorrhizal (VAM) fungi, the most notable of which is the stimulation of growth rate due to increased accumulation of nutrients, particularly phosphorus. Mycorrhizae are also implicated in the increased resistance of host plants to root pathogens, and the vast networks of hyphae are believed to add to the overall structure and stability of soils. One factor limiting the potential benefits of these fungi in agriculture, however, is the lack of persistence of natural fungal inoculum in soils that must be fallowed or rotated to decrease weed and disease loads. The resulting "long-fallow disorder" is characterised by stunted plants that are slow to recover, and can be traced to a reduction of mycorrhizae formation in the young crop. A major problem facing the study of mycorrhizae is the inability

of researchers to confidently identify fungal strains, a requirement that is crucial for any comprehensive analysis of the infectivity and persistence of these organisms in agricultural soils. While the identity of some fungal isolates can be determined from spore morphology or the morphology of mycorrhizae in non-sporing forms, the characterisation of morphologically similar fungi has been a near impossible task. We have recently begun to employ molecular genetic techniques, such as PCR amplification, in an effort to determine the identity and genetic diversity of the fungi responsible for VA mycorrhizae in Australian cotton crops. Preliminary experiments have suceeded in distinguishing between different "species" of VAM fungi, and we hope to develop a rapid procedure for the identification of strains directly from small soil or root samples. This procedure should then assist researchers to determine the relative impact of particular strains of VAM fungi on the growth and performance of different cotton crops, and to survey existing and new fields for the presence of the most effective mycorrhizal strains.

Conclusion:

The utility and future potential of molecular techniques for the genetic identification and characterisation of different organisms is clearly demonstrated in the examples presented in this paper. The power of these methods springs from the same advances in molecular genetic technology that has led to the ability to genetically engineer plants, and like the latter, genetic fingerprinting promises to have a major impact on cotton research and the cotton industry in Australia.

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