New Methodologies in Plant Breeding for Creating and Perpetuating Major Yield Increases (Plant Breeding)

Increased food production is critical for feeding our growing world population; arable land for food production is decreasing, water is a critical resource and plants need to be resilient to biotic and abiotic stresses. We need to breed improved crops, urgently. Hybrid crop production has been a major plant breeding strategy in a number of different crops for more than a century. Hybrids produce a significantly greater yield than either of the parents used in their formation due to a phenomenon known as heterosis or hybrid vigour. The increased yield provides economic benefit to the farmer but is only evident in the first generation hybrid. Yields decline in subsequent generations if seeds from the hybrid are saved and sown. Parental stocks thus need to be maintained and hybrid seed needs to be produced anew each growing season. Not all crosses give rise to hybrid vigour and hybrid production is expensive due to the technical nature of the process. This project comprised two parallel and fundamental research activities in order to provide new information for the development of methodologies in plant breeding to create and perpetuate major yield increases. The first aimed to understand the molecular and cellular bases of heterosis. The second aimed to understand the molecular basis of asexual seed formation (apomixis) so that it could be used to preserve or capture the benefits of heterosis in seeds saved from hybrids. Improved plant breeding technologies would benefit food production in both developed and developing countries.

Heterosis. In searching for an understanding of the molecular and cellular bases of heterosis, our program has demonstrated that epigenetic controls of gene activity patterns are of central importance in the generation of the increased vegetative and reproductive yields in F1 hybrid plants. Two epigenetic systems which are involved are the production of small ribonucleic acid (RNA) molecules of the 24 nucleotide (nt) residues and DNA methylation patterns dependent upon the association of 24nt sRNAs with DNA. We have identified a novel mechanism of alteration of methylation of the hybrid genome, which we have termed Trans Chromosomal Methylation and Trans Chromosomal deMethylation, which underlie these changes in gene activity levels. In exploring the inheritance of these changes induced in the F1 we have shown that they are heritable and that segregation of genes and their epigenetic controls provide the explanation for the subsequent reduction in the level of hybrid vigour in the F2 and later generations. Changes of the gene activity patterns across the hybrid

genome occur from the earliest stages of seed development through to the final production of seed.

Apomixis. We studied the molecular and cellular basis of apomixis in the daisy-like plant Hieracium because asexual seed formation is not evident in agronomic seed crops. Two dominant genetic loci were known to control asexual seed formation in Hieracium at the start of this project. One known to control the initiation of apomixis and sexual suppression and the other triggering fertilization-independent embryo and endosperm formation in seeds. We have found the locus essential for forming the endosperm of the seed without fertilization is dominant and independent from the two other components of apomixis initiation and fertilization-independent embryogenesis and have begun its mapping and molecular characterisation. Uncovering the molecular basis for apomixis initiation and suppression of the sexual reproductive pathway was a major focus of the project. The gene(s) is located on a single chromosome near the tip of the long arm, surrounded by complex DNA repeat structures. Analyses of locus-linked markers in segregating populations have defined the critical genomic region to target genome walking and gene identification. Analyses have shown that the extensive repeats associated with these critical sequences are not essential for the initiation of apomixis. Genomic sequencing has revealed only 13 intact genes in 2 megabases of sequence. All are expressed in the cell initiating apomixis, 11 have been excluded in analyses and two are under investigation. The analysis of small RNA populations in apomicts, mutants and sexual plants has established that the initiation of apomixis is presaged by specific changes in small RNA molecules, 21 and 22nt in length that impact the function of messenger RNAs required for developmental processes suggesting that as for heterosis, epigenetic processes are involved in apomixis initiation.

Both project activities have generated high quality publications. The heterosis group has attracted additional funding from a multinational agroscience company. The apomixis group has received an invitation from the Bill and Melinda Gates Foundation to submit a proposal for preserving heterosis in crops of importance to Sub-Saharan African farmers. The developed proposal, led by CSIRO involves five additional international research organizations and a multinational private company. This proposal is currently under review.