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Poster Abstract – C.59

COLD STRESS AND CIS-ACTING REGULATORY VARIATION IN MAIZE

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Functional polymorphism in genes can be classified as coding variation, altering the amino-acid sequence of the encoded protein, or regulatory variation affecting the level or the pattern of expression of the gene. While the frequencies and consequences of coding polymorphism can be recognized directly from the DNA sequence, the extent to which variations in non-coding *cis*-regulatory DNA alters gene expression in populations is mostly unknown. However, it has been suggested that regulatory variations are important in modulating gene function since alterations in gene regulation are proposed to influence disease susceptibility, and to be the primary substrate for the evolution of the species. If this hypothesis is correct, it implies that *cis*-acting regulatory variation is a common phenomenon.

Our recent studies of allele-specific expression among non-imprinted genes coming from two maize inbred lines (B73 and H99) in the F1 hybrid lines (B73XH99 and H99XB73) confirmed the postulated presumption, since more than 58% of the genes tested showed greater than 1.5 fold differences in expression among the alleles, with no difference among reciprocal hybrids. In addition, we observed significant variations in allelic expression ratios across different tissues.

We now examined the influence of abiotic stress on allele-specific expression in the two reciprocal hybrids, B73XH99 and H99XB73, using Single nucleotide polymorphism (SNP) assay developed for maize to measure the relative expression of each allele of a gene in a heterozygous individual. The method utilizes SNP markers in the transcript itself to distinguish between the transcripts derived from each of the two parental alleles.

RNA samples were isolated separately from the aerial part of seedlings and their roots after 24 and 72 hours cold treatment, as well as from their respective controls. Preliminary results reveal that cold induces significant variations in expression ratios for the alleles that initially showed no disparity in expression, and it enhanced alteration in the relative expression ratios for those alleles that already showed differential expression patterns. In addition, transcriptional changes induced by cold varied between the aerial parts of the seedlings and their roots. We also analyzed the nucleotide sequence polymorphisms in the 5' upstream regulatory region of some of the genes and tried to identify any association between polymorphisms and differences in allelic expression.

Our findings suggest that cis-acting regulatory variation in addition to being a widespread phenomenon in maize, is also relevant to stress response, with different alleles/haplotypes responding differentially to stress in different parts of the plant. The heterozygous state found in hybrids for many genes could

therefore represent a buffering mechanism to improve stress tolerance.

Results also support the use of SNP assay as a valid quantitative method that indirectly scans for the influence of cis-acting effects on gene expression which helps us not only to appreciate the extent of functionally important regulatory variations but also to focus on candidate haplotypes that have differences in expression for detailed molecular characterization of specific polymorphisms.