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Oral Communication Abstract – S.01

THE MAGIC OF HETEROSIS IN TOMATO

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Agricultural heterosis was observed nearly 100 years ago when hybrid plants out yielded their inbred parents and today this “hybrid vigor” is a major provider for global food production. The genetic basis of heterosis has been debated with respect to the relative importance of dominant, overdominance and epistasis where one of the problems has been the use of whole genome segregating populations where interactions often mask the effects of individual quantitative trait loci (QTL). To partition heterosis into its mode of inheritance components we employed a population of tomato (*Solanum lycopersicum*) introgression lines (ILs), which carry single marker-defined chromosome segments from the distantly related wild species *S. pennellii*. Combined quantitative genetic and phenomic analysis of ILs, which is largely devoid of epistasis, revealed 841 QTL for 35 diverse traits that were measured in the field on homozygous and heterozygous plants. The mode of inheritance of genomic regions associated with greater reproductive fitness was characterized by the prevalence of overdominance, which was virtually absent for the non-reproductive traits. We show that the alliance of overdominance with improved reproductive fitness is a general attribute of sexually reproducing organisms and propose that this naturally selected association is the ancestral basis for heterosis for improved agricultural yields.

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Oral Communication Abstract – S.02

HETEROSIS IN MAIZE: FROM QTL ANALYSIS TO DEVELOPMENT AND EVALUATION OF NEAR ISOGENIC LINES FOR HETEROTIC QTL

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Heterosis, i.e., the superiority of hybrids over parental lines, is sizable for allogamous species, especially maize (*Zea mays* L.). Heterosis has been extensively exploited but, despite a century of investigations, its genetic basis is not completely understood, yet. To gain information on its genetic control, we undertook a long term research in maize, aimed at providing a framework of comprehensive quantitative trait locus (QTL) phenotyping, to be integrated with map based cloning.

As a first step, we jointly applied classical genetic and QTL analysis in a set of recombinant inbred lines (RILs), derived from the heterotic single cross B73 x H99. RILs were crossed to the three testers B73, H99 and B73 x H99, following a North Carolina III (NCIII) mating design and testcrosses were evaluated, together with the RILs, in three environments. Level of heterosis for several agronomic traits and underlying genetic effects (allelic and non-allelic interactions) were estimated. Several QTL with heterotic effects on agronomic traits were detected and most of them were characterized by dominant or overdominant gene action, whereas non-allelic interaction proved to be of minor importance.

We then developed genetic materials suitable for validation and precise estimate of the effects of six heterotic QTL chosen for their appreciable additive and dominance effects. For this purpose, we adopted a residual heterozygous lines (RHL)-based introgression program to produce pairs of near-isogenic lines (NILs) homozygous either for one or the other parental inbred allele (i.e. B73 or H99) at the selected heterotic QTL regions. In addition, during the process of NILs production, we were able to preliminarily validate the phenotypic effects of two major QTL for heterosis, mapped on chromosome 3 (bin 05) and 4 (bin 10).

Once NILs were obtained, we then approached a study aimed at verifying and characterizing QTL heterotic effects. The six pairs of NILs were crossed with the two parental inbred lines B73 and H99. The 24 testcrosses are now being evaluated in a multi-year research conducted over several environments at low and high plant densities (4.5 and 9.0 plants m^{-2}, respectively). This investigation is warranted because, in several studies, the level of heterosis has proven to be particularly important in coping with environmental stress.

Results obtained in the first year of testing allowed us to confirm additive and dominance effects of heterotic QTL for traits that showed strong heterosis in our previous studies. From non-stress to stress condition, for yield per plant and other agronomic traits the contribution of additive effects declined, while that of dominance increased. These preliminary findings thus confirm the importance of heterosis in coping with stress and its possible role in enhancing crop sustainability.

The ultimate objective of QTL mapping is to identify the causal genes that underlie these QTL. Starting from F_1 hybrids obtained by crossing contrasting NILs, we produced large F_2

populations, each segregating only for one QTL region. We limited our attention to the introgressed QTL mapped on chromosome 4 (bin 10) and 10 (bin 03) because of their sizable effects on yield and other traits. F_2 populations were genotyped at markers flanking the segregating QTL, and F_3 families are now being produced by selfing informative recombinant F_2 individuals. The so obtained F_3 families will be evaluated for agronomic traits in order to fine map the heterotic QTL.

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HETEROSIS IN MAIZE: NEW TOOLS AND COMPLEXITIES

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Heterosis is the phenomenon whereby the progeny of particular inbred lines have enhanced agronomic performance relative to both parents. Although several hypotheses have been proposed to explain this fundamental biological phenomenon, the responsible molecular mechanisms have not been determined. The maize inbred lines B73 and Mo17 produce a heterotic F_1 hybrid that is being used as a model to study heterosis.

The regulation of gene expression levels in hybrid combinations can be studied via eQTL mapping, a combination of traditional QTL mapping and global expression profiling. The maize IBM population of recombinant inbred lines (RILs) was developed from a cross between the inbred lines B73 and Mo17. Each RIL is mosaic and homozygous for either the B73 or the Mo17 allele at each locus. A genetic map based on the IBM RILs containing over 9,000 markers (ISU_IBM Map7) was used in conjunction with eQTL analyses to gain insight into the regulation and mechanisms related to heterosis. As a first step, 30 IBM RILs were crossed onto both B73 and Mo17. In combination with the RILs *per se*, the resulting cross-types provide a contrast of gene expression for the heterozygous genotype and both homozygous genotypes across all loci polymorphic between B73 and Mo17.

Four replications of each RIL, B73xRIL, and Mo17xRIL genotype were hybridized to a custom cDNA microarray using a loop design that included all pair-wise comparisons between each RIL and its crosses with B73 and Mo17. In each cross-type hundreds of significant associations were identified between genetic markers and gene expression levels. Although many of these eQTLs exhibit additive gene action, large numbers exhibit dominant gene action. Substantial numbers of the eQTLs act *in trans*.

Natural Antisense Transcripts (NATs) can regulate gene expression by virtue of their ability to form double-stranded RNA duplexes. B*oth* sense and antisense transcripts accumulate to detectable levels for over 70% of a random set of maize genes. Significantly, these sense and antisense transcripts exhibit significantly different expression patterns between the B73 and Mo17 inbreds. To investigate the genetic mechanisms that regulate the accumulation of antisense transcripts, two replications of each of the 90 genotypes (30 RILs, 30 B73xRIL, and 30 Mo17xRIL) described above were hybridized to a custom, strand-specific, oligonucleotide microarray. Many eQTLs that regulate both the absolute levels of sense and antisense transcripts as well as those that regulate the *ratios* of complementary sense and antisense transcripts were identified. We hypothesize that the complex genetic interactions identified in this study contribute to heterosis.

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Oral Communication Abstract – S.04

UNDERSTANDING ALLELIC VARIATION IN MAIZE: TOWARDS DISCOVERING OF THE MOLECULAR BASES OF HETEROSIS

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Heterosis, or hybrid vigor, is the increased performance of hybrid relative to the parents and is a result of the variation that is present within a species. Maize hybrids exhibit high levels of heterosis and as such provide an excellent system for the study and understanding of the phenomenon. Intraspecific comparison of sequences and expression levels in maize have documented a surprisingly high level of alleic variation, which includes variation in the content of genic fragments, variation in repetitive elements surrounding genes, and variation in gene expression levels. A major unresolved question is how the combined allelic variation and interactions in a hybrid give rise to heterotic phenotypes.

Our research is focused on the analysis of gene expression in the hybrid and inbred parents at the allele specific-level, which, as compared to solely measuring total transcript amount without allelic differentiation, may provide a different perspective and understanding of gene regulation and the molecular basis of heterosis. By quantifying allele-specific transcript levels we estimated that 70% of maize genes show differences in expression of at least 1.5 fold due to *cis*-regulatory variation. Allelic expression varied spatially in different tissue types and temporally as a consequence of environmental changes. Some of the allelic imbalances revealed expression overdominance or bidirectional expression, meaning that either allele was overexpressed depending on tissue, or growth phase or/and environmental conditions. In an attempt of identifying a source of *cis*-acting variation we assessed the effects of the maize intraspecific structural genome diversity on gene expression. Transposable elements located in or near genes could affect expression through the donation of transcriptional regulatory signals as well as through epigenetic silencing. To test the latter hypothesis we searched for the presence of both sense and antisense co-transcripts and examined the methylation state of the analysed genes and their promoters.

Our results show that *cis*-regulatory variation is very common in maize and that the repetitive sequences can influence gene transcription. It has been proposed that allele expression variability may explain phenotypic variability within species, and that the relatively modest changes in gene activity are a key feature of many speciation events. Our findings may thus uncover a possible mechanism that connects genomic plasticity to the evolution of whole organisms. In addition, *cis*-regulatory variation and observed expression overdominance may provide a possible molecular explanation of the heterosis phenomenon in maize. Expression overdominance could provide heterozygotes with a wider range of transcription modulation than the corresponding homozygotes, which in turn could determine both flexibility and stability.