P299

A transgenic cereal crop with enhanced folate: rice expressing wheat HPPK/DHPS

Gillies SA, McIntosh SR, Henry RJ
Grain Foods CRC, Centre for Plant Conservation Genetics, Southern Cross University, PO Box 157, Lismore NSW Australia.

Folate is a B-group vitamin critical for normal cellular function and division. It acts in one-carbon transfer systems essential in nucleotide synthesis, methylation and gene expression. Insufficient intake causes megaloblastic anaemia and there are strong linkages to cardiovascular disease, various cancers and cognitive decline. Low levels prenatally can lead to low birth weight and premature infants and catastrophic neural tube defects including spina bifida and anencephaly. Vertebrates are unable to synthesize folate *de novo*, accordingly plant foods are the primary source. Cereals unfortunately, which provide over half the worlds population with 80% of their diets are particularly poor in folate. Consequently the majority of developed nations have fortification programmes. In the developing world however, such programmes are logistically far more difficult. A practicable alternative is metabolic engineering, to create a cereal crop plant producing high levels of folate. Folate is produced in a multi-step process from a pterin ring, p-aminobenzoate (pABA) and glutamate residues. Here we show rice plants transgenic for wheat 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase/7,8-dihydropteroate synthase (HPPK/DHPS) which operates at a central point in the production pathway, gives elevated folate levels. Consequently we have a cereal crop transgenic for a single cereal gene expressing enhanced folate levels.

P300

Transposon-mediated gene search: finding a needle in a haystack

Singh J[1], Lam A[1], Bloss P[1], Bregitzer P[2], Lemaux P[1]
[1] Department of Plant and Microbial Biology University of California, Berkeley CA, 94720 USA, [2] National Small Grains Germplasm Research Facility, USDA ARS, Aberdeen, ID, USA, 83210

The maize *Ac/Ds* transposon system is an effective approach for gene identification and cloning in heterologous species. Using this system, single-copy *Ds* insertion lines (TNPs) were generated in barley to identify and tag genes and determine gene function. Coupled with availability of extensive genomic resources, a robust platform is available for effective use of transposon approaches to isolating, characterizing and mapping genes in barley. From 200 independent TNPs, *Ds* flanking sequences were determined in >100 lines. Results from BLAST searches indicate that ~86% of these sequences are from known or putative genes, encoding, for example, MLA1, wall-associated kinases, ubiquitin-conjugating enzyme, ATP-binding transporter, terpene synthase, ankyrin1-like protein and cytochrome P450. As mapping information on insertion sites becomes available, it will be used to tag agronomically important genes by identifying *Ds* elements in close proximity to traits/genes of interest, followed by *Ds* reactivation to achieve saturation mutagenesis. For maximal utility of this approach, which depends on the marked tendency of *Ds* to transpose to linked locations, we studied characteristics important to TNP reactivation, *i.e.*, status of terminal inverted repeats and 8 bp duplications and the nature of insertion sites. Frequency of TNP reactivation was also determined over 3-4 generations of transposition and the observed rates provide the foundation for tagging and “transposon-walking” (repeated localized transposition) strategies. Remobilization frequencies of primary, secondary, tertiary and quaternary TNPs, coupled with the tendency for localized *Ds* transposition, validates a *Ds*-mediated saturation mutagenesis approach to tagging and characterizing linked genes in large genome cereals like barley and wheat. Recent studies also show that a high frequency (83%) of *Ds* flanking sequences in barley map in high to moderate recombination regions of wheat. Such information and resources will be utilized for wheat and barley improvement.

P301

Towards the understanding of genetic and molecular basis of heterosis in wheat (*Triticum aestivum* L.)

Sun Q, Ni Z, Peng H, Yao Y, Du J
Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, China

Hybrid cultivars have been used in many crop plants as well as wheat, however, the genetic and molecular basis of heterosis remains to be revealed. By using an 'immortalized F_2'' population, the genetic basis of wheat heterosis was dissected. A total of 42 heterosis loci were mapped for the 7 agronomic traits, with one showing dominance effect and 41 showing overdominance effects, suggesting that overdominance plays an important roles in wheat heterosis. Large numbers of digenic interactions were resolved by using two-way ANOVA. All kinds of genetic effects, including partial-, full-, and overdominance at single-locus level and all three forms of digenic interactions (additive by additive, additive by dominance, and dominance by dominance), contributed to heterosis in the immortalized F_2 population, indicating that these genetic components were not mutually exclusive in the genetic basis of heterosis. Differential gene expression patterns were observed between hybrids and their parental inbreds, and by using dialell crosses, our studies indicated that the differential gene expression patterns in leaves were correlated with heterosis for agronomic traits, which suggested that these differentially expressed genes could play important roles in heterosis. Large scale identification and characterization indicated the differentially expressed genes are involved in diverse biological processes. By using the internode length heterosis as a model trait, the gibberellin (GA) related gene regulatory pathways were found to be responsible for the heterosis in plant height. Our results indicated that among the 18 genes analyzed, genes encoding enzymes that promote synthesis of bioactive GAs, and genes that act as positive components in the GA response pathways were up-regulated in hybrid, whereas genes encoding enzymes that deactivate bioactive GAs, and genes that act as negative components of GA response pathways were down-regulated in hybrid. A model for GA and heterosis in wheat plant height was proposed.

P302

Understanding of molecular mechanisms of the water soluble carbohydrate trait in *Triticum aestivum*

Xue GP[1], McIntyre CL[1], Tabe L[2], Jenkins CLD[2], Shorter R[1]
[1]CSIRO Plant Industry, 306 Carmody Rd., St Lucia, Qld 4067, [2]CSIRO Plant Industry, GPO Box, Canberra ACT 2601, Australia

Water soluble carbohydrates (WSC) in wheat stems at anthesis are composed of mainly fructans, sucrose, glucose and fructose, and are important carbon sources for grain filling. Variation in stem WSC concentrations among wheat genotypes is one of the genetic factors thought to influence grain weight and yield under water-limited environments. Here, we describe the molecular dissection of carbohydrate metabolism in the stems of *Triticum aestivum* at the WSC accumulation phase. Gene expression profiling analysis of carbohydrate metabolic enzymes revealed that the mRNA levels of two fructan synthetic enzyme families (1-SST and 6-SFT) in the stem were positively correlated with stem WSC and fructan concentrations, while the mRNA levels of enzyme families involved in sucrose hydrolysis were inversely correlated with WSC concentrations. Transcripts for 6-SFT and 1-SST as well as a protein kinase (WPK4) were induced by sucrose, and appeared to be co-regulated in wheat stems. The data suggest that differential carbon partitioning in the wheat stem is one mechanism that contributes to variation in WSC accumulation.