tion of novel mechanisms determining nutrient partitioning, and generate new unbiased hypotheses.

Microarray slides were assembled using clones obtained from 20-part-normalized cDNA libraries representing the major events in endosperm development. Approximately 22,300 ESTs were sequenced, aligned, assembled into contigs using a similarity score of 80%, and annotated using TBLASTN software. It is notable that the distribution of ESTs across the original cDNA libraries was not uniform. The highest proportion of the sequences could be associated with endosperm tissue, the lowest with 8-day-old embryos. Of the 8,950 ESTs identified, 6,719 were singletons and 2,231 formed contigs. EST sequences were analyzed with the BLAST2GO software (http://www.blast2go.de). In the first phase, homology searches using public domain non-redundant databases identified significantly homologous sequences for 48.4% of the ESTs considered. These ESTs represented 3,090 single hit and 1,240 multiple hit sequences.

In the second phase, an attempt was made to associate biological processes to each of the ESTs showing sequence homology using the gene ontology (G.O.; http://www.geneontology .org) and KEGG databases (http://www.genome.jp/kegg). Approximately 85% of these unigenes could be assigned a functional annotation, with the remainder (ca. 15%) having an obscure or unknown function. Twenty-four distinct patterns of expression were resolved to establish the complex regulatory hierarchies that exist to orchestrate the dynamic metabolic, transport, and control processes occurring in developing endosperm. This classification is consistent with the many functions of maize endosperm and is comparable with that reported by other workers (Verza et al., Plant Mol. Biol. 59:363-374, 2005). It appears that our maize endosperm gene set is rather comprehensive and provides a good representation of the entire transcriptome including genes linked to accumulation of storage products and energy supply. More specifically, most of the transcripts appeared to be involved in carbohydrate metabolism (12.0%), followed by those involved in storage protein synthesis (7.9%), translation (11.2%) and transcription (5.3%), nucleotide metabolism (2.5%), and RNA processing (2.1%). Among physiological processes, those transcripts implicated in protein turnover (5.6%), energy metabolism (3.1%), electron transport (1.2%), amino acid metabolism (4.4%), amino acid and sugar transport (7.8%), the latter being intrinsically linked to the accumulation of storage protein and starch, nucleic acid metabolism (2.5%), lipid (2.1%) and fatty acid metabolism (1.6%), and secondary metabolites (2.0%) were represented in our EST collection. Moreover, genes encoding for protein involved in cell wall (2.8%), cytoskeleton (2.8%), and stress and defense (5.1%) appear to be related to relevant cellular processes assigned in the functional classification. Finally, the assignment of other important classes of transcripts, such as DNA (1.2%) and protein folding (0.5%), transcription regulators (5.3%; mostly representing transcription factors) and signal transducers (13.3%) provides new perspectives for data mining and for studies of coordinated gene regulation in developing maize endosperm. Thus, ESTs corresponding to the majority of genes (or their alleles) are represented in the maize endosperm cDNA libraries constructed, and the use of the maize Zeastar Unigene chip to examine endosperm gene expression appeared feasible.

Microarray slides containing the entire Zeastar unigene set, spotted in duplicate, were hybridized with probes derived from endosperm tissue harvested at 14 DAP - a developmental stage in which synthesis of starch and storage protein is known to begin- of normal, *o2*, *o7*, and *o2o7* A69Y inbreds. To reduce hybridization artifacts, all probes were labelled both with Cy3 and with Cy5 and used in dye-swapping experiments on a series of three independent slides. The expression data obtained were assayed for consistency by performing F-tests at 95% confidence levels. Replicates appeared to be in general agreement; thus, we are confident that the alterations of the transcriptomes described here are consistent with the biology of endosperm development. Moreover, we selected a series of thirty clones, believed to be of particular interest and exhibiting distinct patterns of expression, for detailed analysis, using qRT-PCR to confirm the changes in expression levels determined using the arrays. RNAs isolated from the four genotypes were used as templates for amplification. The relative expression levels determined by qRT-PCR showed good agreement with those determined using arrays with high correlation coefficients.

Gene expression profiling, based on a unigene set composed of 7,250 ESTs, allowed us to identify a series of mutant related up-regulated (17.1%) and down-regulated (3.2%) transcripts. Several differentially expressed ESTs homologous to gene encoding enzymes involved in amino acid synthesis, carbon metabolism (TCA cycle and glycolysis), storage protein and starch metabolism, gene transcription and translation processes, signal transduction, and protein, fatty acid, and lipid synthesis were identified. Our analyses demonstrate that the mutants investigated are pleiotropic and play a critical role in several endosperm metabolic processes. Pleiotropic effects were less evident in the *o7* mutant, but severe in the *o2* and *o2o7* backgrounds, with large changes in gene expression patterns, affecting a broad range of endosperm-expressed genes involved in several metabolic pathways. Although more work is required to define gene functions and dissect the complex regulation of gene expression, the genes isolated and characterized to date give us an intriguing insight into the mechanisms underlying endosperm metabolism.

Research in this laboratory was supported by the European Communities BIOTECH Programme, as part of the Zeastar project (2001-2005) and by Ministero per le Politiche Agricole, Alimentari e Forestali, Roma: special grant “Zeagen”.

BERGAMO, ITALY
CRA-MAC
COLLERETTO GIACOSA, ITALY
ISPA-CNR

The *Zea mays* (L.) b-32 ribosome-inactivating protein efficiently inhibits growth of *Fusarium verticillioides*

--Balconi, C; Lanzanova, C; Giuffrida, MG; Baro, C; Hartings, H; Lupotto, E; Motto, M

Fungi of the genus *Fusarium* are widely distributed pathogens of maize, causing diseases for seedlings, roots, stalks and kernels (Bottalico, J. Plant Pathol. 80(2):85-103, 1998; Reid et al., Phytopathol. 89:1028-1037, 1999). In addition to their effects on yield,

Fusarium species can affect grain quality, producing a number of toxic compounds, including fumonisins (Munkvold, Ann. Rev. Phytopathol. 41:99-116, 2003), involved in human and animal health (CAST, Task force rep. 38. Ames, IA: CAST, 2003). Therefore, the development of maize plants carrying resistance to *Fusarium* ssp. (Lew et al., Cereal Res. Commun. 25:467-470, 1997) as well as resistance to mycotoxin production is highly desired.

In maize endosperm, a cytosolic albumin with a molecular weight of 32 kDa, termed b-32, is synthesized in temporal and quantitative coordination with the deposition of storage proteins (Soave et al., Cell 27:403-410, 1981). It was shown that the *b-32* genes form a small gene family (Hartings et al., Genet. Res. Camb. 65:11-19, 1995).

Endosperm-derived native b-32 was shown i) to enzymatically inactivate ribosomes, through its capacity to specifically modify rRNA, inhibiting protein synthesis in vitro (Maddaloni et al., J. Genet. Breed. 45:377-380, 1991; Bass et al., Plant Cell 4:225-234, 1992), and ii) to inhibit the growth of *Rhizoctonia solani* mycelia in in vitro bioassays (Maddaloni et al., Transgenic Res. 6:393-402, 1997). Similarly, Balconi et al. (European J. Plant Pathol. 117:129-140, 2007) found that maize RIP-b-32 protein was effective in wheat transgenic lines as an anti-fungal protein by reducing *Fusarium* head blight (FHB) symptoms.

To verify if maize plants expressing b-32 in various tissues have an increased tolerance to fungal pathogens, transgenic plants were obtained through genetic transformation using a chimeric gene containing the *b-32* coding sequence downstream of a constitutive *35SCaMV* promoter. A set of four independent homozygous progenies expressing b-32 were selected for a detailed analysis of b-32 expression in leaves and for pathogenicity tests.

The integration patterns of the *b-32* transgene were determined by genomic Southern-blots using *Eco*RI-*Hin*dIII double digests as appropriate enzymes to estimate the transgene copy number. Four Basta resistant progenies (SM 3.4; SM 16.1; SM 19.4; SM 20.2), one Basta sensitive progeny (SM 20.4) and the B73 inbred line were analyzed using a *nos-bar* (resistance gene) and *b-32* probe. The *b-32* probe detected the presence of the *b-32* endogenous gene in the control B73 inbred line and in the negative control progeny SM 20.4. A band at the same position is present in transgenic progenies SM 3.4, SM 16.1, SM 19.4, and at a slightly different position in the SM 20.2 progeny, and is most likely due to a recombination event involving the endogenous gene and transgene, indicating that several insertion events have occurred. In addition to the endogenous *b-32* band (native gene), all transgenic progenies contained a few additional bands corresponding to insertions of the transgene. The unique hybridization patterns observed indicated that each progeny resulted from independent transformation events.

Comparison of b-32 expression among various individuals was performed, after immuno-blot imagine scanner acquisition, using IMAGINE MASTER 1D Elite Version 3.01 (NonLinear Dynamyc Ltd) software. A differential b-32 content in leaf protein extracts was recorded in the transgenic progenies. As expected, SM 20.4, i.e. the negative control, showed non-detectable b-32 content (n.d.) in leaf tissues. Proteomic experiments were performed on protein leaf extracts of one of the transgenic lines expressing a high b-32 level (SM 20.2) and were compared to the negative control progeny (SM 20.4). The overlapping of the two-dimensional electrophoresis maps clearly showed the presence of additional spots in SM 20.2 progeny in comparison to SM 20.4 progeny, which was Basta-sensitive and b-32 western negative. These spots were cut from gels and digested with trypsin to allow protein identification by the "peptide mass fingerprinting" (PMF) strategy (Pappin et al., Curr. Biol. 3:327-332, 1993). Both induced b-32 spots and herbicide resistance spots were successfully identified.

Transgenic progenies were tested in bioassays to evaluate the response to *Fusarium* attack in leaf tissues. Preliminary experiments supported the choice of bioassay parameters for a reliable evaluation of transgenic progenies. Results indicated that fungal colony diameters measured on the inoculated leaves of SM 20.4 (the negative control) were, at all detection times, significantly (Student's t test = $P \leq 0.05$) larger than those observed in all four progenies expressing b-32. A good correlation between the b-32 content in the leaves and the level of resistance to *Fusarium* attack was observed. In the case of progenies with high b-32 content in the leaves, in addition to reduced mycelial growth around the cut edges of the leaves, very weak growth on leaf surfaces was observed in comparison with progeny exhibiting the lowest b-32 content in leaves.

The data obtained indicate that maize b-32 is an effective antifungal protein in reducing progression of *Fusarium* infection. Additionally, the reduction in *Fusarium* induced symptoms was related to b-32 concentration in leaf tissues. The expression of antifungal proteins in plants or plant tissues in which they are not normally expressed may be very useful in reducing pathogen colonization and growth; from this perspective, a reduction of *F. verticillioides* infection in maize leaves and stalk could be very useful in limiting the spread of fungal infection to the exposed silks, and consequently, in the reduction of grain fumonisin contamination.

Research developed through the FP5 project, SAFEMAIZE (ICA4-CT2000-30033), and the MIPAAF-funded project AFLARID

BHUBANESWAR, INDIA
Regional Plant Resource Centre

Effect of water stress on performance of maize inoculated with *Glomus* sp. isolated from a tea plantation of Keonjhar, Orissa

--Gupta, N; Routaray, S

Arbuscular mycorrhizal (AM) fungi have been reported to help maize grow under drought and other stresses (Gupta and Routaray, Acta Agric. Scandinavica 55(2):151-157, 2005; Subramanian et al., New Phytol. 129:643-650, 1995; Subramanian and Charest, Mycorrhiza, 7:25-32, 1997). We have expanded this work to study the effects of tea plantation arbuscular mycorrhizal (AM) fungi on maize grown under well-watered and water-stressed pot culture conditions. Fungi were isolated from the drought-prone tea (*Camellia sinensis* L.) plantations of the Bhuyanpirh tea estate of M/S Orissa Tea Plantation Limited, which is situated in Tarmakanta about 48 km away from Keonjhar, Orissa, India. The plantation area has an elevation of more than 600 m and was once covered by dry and mixed deciduous sal forests. The soil is red clay-loam and poor in nutrient content. The rhizosphere soil of different tea plants was collected 10 inches below ground in polythene bags for