Parents for Zhuo-Zi No.1 are ZP 99-01 (Female) and ZP 99-02 (male). ZP 99-01 was developed by continued inbreeding and selection from a local variety with purple plants and grain, selecting for ears of superior plants from large populations. ZP 99-02 was developed by continued inbreeding and selection from the improvement population No. 02. Both parents have good or high general combining ability; high specific combining ability; high vigor; normal to high production; resistance to biological stress; late maturity; medium tall plant and ear height; fair stalks; vigorous roots; semi-erect and mid-sized leaves; 1~2 ears per plant and purple-black grain on a deep-purple cob. The main agronomic traits of Zhuo-Zi No.1 are summarized in Tables 1 and 2, and Figure 1. It produces a purple color in the seedling, leaf tip, leaf periphery, leaf ear, leaf sheath, stalk, tassel and its branches, anther surface, silk, husk leaf, ear handle, cob, pericarp, aleurone, and leaf blade of the main plant and in tillers at maturity. It has normal color (pale-yellow or nearly white) endosperm, embryonic bud, shield blade, shoot sheath and root system. The biomass yield and grain yield can reach 75000~7950 kg/ha and 7500~8025 kg/ha respectively. The anthocyanidin-3-monoglucoside (maize morado color) content of purple maize Zhou-Zi No.1 is estimated to be 225~300 kg/ha. Anthocyanin content varies in different tissues and organs. It is 0.023% in the stalk and leaf blade, 1.007% in the tassel, 2.228% in the husk leaf, 0.268% in the silk, 0.728% in the cob, 0.106% in the grain, and 0.869% in the stalk coat (Table 3.). Thus, the husk leaf and tassel have a higher anthocyanin content.

Figure 1. Ear of Zhuo-Zi No. 1.

Table 1. The agronomic traits of Zhuo-Zi No. 1.

No.	Trait name	Average value, character and resistance
1	Plant height cm	313.5
2	Ear height cm	124.7
3	Tassel length cm	42.6
4	Tassel branch number	14.0
5	Leaf number	23.5
6	Ear length cm	18.3
7	Ear diameter cm	4.5
8	Row number per ear	15.7
9	Grain numer per row	37.8
10	Weight of 100 grain g	398.6
11	Ear number each plant	1.6
12	Husk number each ear	14.8
13	Grain type	Semi-dent
14	Ear form	Cylinder
15	Resistance to	
	E. turcicum	HR
	B. maydis	HR
	C. lunada	HR
	C. zeae-maydis	MR
	U. zeae	MR
	F. moniliforme and *P. inflatum*	MR
	R. solani and *R. cerealis, zeae*	R
	S. holci-sorghi	HR
	MRDV	MR
	SCMV-MDR	MR
16	Tolerance lodging	
	drought	

Table 2. The biochemical composition of grain of Zhuo-Zi No. 1.

Component	% (g/100g grain), g/L
Protein	10.86
Lipid	5.02
Starch	74.23
Lysine	0.36
Water	10.7
Unit Weight (g/L)	792

Table 3. The anthocyanin content of different tissues and organs of Zhuo-Zi No. 1.

Tissue name	Anthocyanin % (g/100g)
Stalk and leaf blade*	0.023
Tassel	1.007
Husk leaf	2.228
Silk	0.268
Ear cob	0.728
Grain	0.106
Stalk sheath	0.869

*Sampled at maturity

BERGAMO, ITALY
CRA – MAC

The *opaque2* and *opaque7* mutants reveal extensive changes in endosperm metabolism as revealed by transcriptome-wide analyses

--Hartings, H; Lauria, M; Lazzaroni, N; Pirona, R; Rossi, V; Motto, M

The changes in storage reserve accumulation during maize (*Zea mays*) grain maturation are well established; however, the key molecular determinants controlling carbon flux to the grain and the partitioning of carbon to starch and protein are more elusive (Motto et al., Cellular and Molecular Biology of Plant Seed Development, Larkins and Vasil, eds., 1997). The *Opaque-2* (*O2*) gene, one of the best-characterized plant transcription factors, is a good example of the integration of carbohydrate amino acids and storage protein metabolism in maize endosperm development. Evidence also indicates that the *Opaque-7* (*O7*) gene plays a role in affecting endosperm metabolism.

To advance our understanding of the nature of the mutations associated with an opaque phenotype, we used nearly isogenic inbreds for *o2* and *o7* mutants, and for the double mutant combination *o2o7*, to provide genome-scale information about gene expression patterns by cDNA microarray. Classifying genes based on similarities or differences in transcript profile with phenotype can confirm existing knowledge, lead to the dissection and revela-

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.

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

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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,