SUPPLEMENTAL TEXT S1

MATERIALS AND METHODS

Identification and cloning of teosinte *ZCN7* and *ZCN8* genes

The teosinte *ZCN7* and *ZCN8* genomic sequence was first reconstituted *in silico* using publically available data from genotyping by sequencing studies. Specifically, the data of study SRA051245 of Chia et al., (2012) were downloaded from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra). SRA data of each run were dumped in fastq format using the fastq-dump tool of the NCBI SRA Toolkit v.2.4.2-1 (http://www.ncbi.nlm.nih.gov/Traces/sra/?view=software). Forward and reverse paired reads were split for each sample into two separate files (option - split-files). Reads were quality trimmed by Fastx toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) trimming leading and trailing bases below a quality threshold of 20, removing reads having an average quality below 20 and trimmed reads shorter than 24 bp. For each sample, only reads passing the quality filtering as matching pairs were retained and aligned to the whole B73 reference genome (version AGPv3_22, http://plants.ensembl.org/Zea_mays/Info/Index) using the Bowtie2 aligner (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml; Langmead et al., 2009) with default parameters. The resulting SAM files were converted to sorted BAM using Samtools v.0.1.19 (http://samtools.sourceforge.net/; Li et al., 2009). Sorted BAM files were uploaded on the graphical viewer Tablet v.1.14.04.10 (http://ics.hutton.ac.uk/tablet/; Milne et al., 2013). Polymorphism identification in the two genes between the B73 reference genome and the different *Zea mays* ssp. *parviglumis* inbred lines (SNP and small INDELS) was manually performed using Tablet starting from 1500 bases upstream to 1500 bases downstream the coding region. The artificially reconstructed teosinte *ZCN7* and *ZCN8* genomic sequences were used to design different primer combinations (Supplemental Table S5), allowing to amplify DNA fragments in PCRs with Balsas teosinte genomic DNA as template. All these fragments were overlapping one with each other and were subsequently sequenced in both directions to obtain the real genomic sequence of *ZCN7* and *ZCN8* in Balsas teosinte.

Chromatin preparation and ChIP assays

Chromatin preparation was performed using frozen tissues, according to the protocol described by Luo et al., (2013), which was adapted for maize and teosinte plants. Using frozen tissues we were able to greatly improve the efficiency of fixation of mature leaf tissues compared with vacuum infiltration protocol employed in previous experiments (Mascheretti et al., 2013; see below). B73 and teosinte immature and mature leaf were obtained by manual dissection of V6/V7-stage seedlings, immediately frozen in liquid nitrogen and stored at -80 °C. Before of fixation the samples were thin powdered using mortar and pestle continually cooled in liquid nitrogen. Approximately 1.2 g of the thin powder was stored in a 50-ml Falcon tube containing 25 ml of nuclear isolation buffer (10 mM Hepes pH 7.6, 1 M sucrose, 5 mM KCl, 5 mM MgCl2, 5 mM EDTA) supplemented with 0.6% Triton X-100, 1 mM PMSF and 14 mM β-mercaptoethanol. Subsequently, formaldehyde was added to each sample at 1.5% final concentration and fixation was performed for 15 min at room temperature (RT) and with gentle rotation. Fixation was stopped by adding glycine at 0.17 M final concentration, followed by 5 min of incubation at RT with gentle rotation. The lysate was filtered through a double layer of Miracloth (Calbiochem) into a new 50-ml Falcon tube stored in ice. Nuclei were collected with centrifugation at 4000 x g for 15 min at 4°C and the pellet was washed two times with 1 ml of cold nuclear isolation buffer. The pellet was then suspended with 50/250 µl of nuclear lysis buffer (10 mM Tris-HCl pH 7.6, 1% SDS, 10 mM EDTA pH 8) supplemented with 1 mM PMSF, 1 mg/µl aprotinin, 1 mg/µl pepstatin A, 1 mg/µl leupeptin, and 10 mM Na-butyrate. Sonication was performed with the ultrasonic sonicator Sonoplus HD 2070 (Bandelin) to shear chromatin to an average fragment size of 100 to 800 bp. The sonication round applied was of 75% power and 15 sec, by maintaining the sample in ice for 10 min at least between two rounds. Typically, 5 and 12 rounds were applied for B73 and teosinte samples, respectively to achieve the chromatin 100 to 800 bp average fragment size. Fragmented chromatin samples were cleared by centrifugation at 13000 x g for 10 min at 4°C and aliquots were stored at -80 °C. The efficiency of the fixation procedure was monitored using the strategy described by Haring et al., (2007), showing that this methodology was most effective for fixation of mature leaf with respect that vacuum infiltration of fresh tissues. Subsequently, the chromatin was quantified as previously reported (Locatelli et al., 2009). Approximately, 16 and 5.5 µg of chromatin are obtained from 1 g of immature and mature B73 leaf, respectively, while 8.5 and 3.5 µg of chromatin are obtained from 1 g of immature and mature teosinte leaf, respectively.

For each ChIP assay 5/10 µg of chromatin was employed. Each chromatin aliquot was diluted 10 times with ChIP dilution buffer (1 mM EDTA, 15 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% Triton X-100) supplemented with 1 mM PMSF, 1 mg/µl aprotinin, 1 mg/µl pepstatin A, 1 mg/µl leupeptin and 10 mM Na-butyrate. The immunoprecipitation was performed as described in

Locatelli et al., (2009), with the exception that the ChIP DNA clean and concentrator kit (Zymo Research) was used following the manufacturer's instruction as the final step for DNA purification. Elution for each ChIPed sample was performed to obtain a final volume of 40/80 µl, depending by the amount of the chromatin employed in the assay. One microliter of the ChIPed DNA and a 1:50 dilution of input were used for qPCR analysis.

Supplemental Literature cited

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