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Karyotypical studies of two maize races from Northeast Argentine (NEA): DAPI-banding and Fluorescent In Situ Hybridization (FISH)

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Knobs heterochromatic blocks occur in all *Zea* species with 2n = 20, varying in size and number across maize races and their wild relatives (Kato, *Mass. Agric. Exp. Stn. Bull.* 635: 1-185, 1976; McClintock *et al*., Colegio de Postgraduados, Chapingo, México, 1981; González & Poggio, *Genome* 54: 26–32, 2011; Poggio *et al*., *Cytogenet. Genome Res*. 109: 259–267, 2005). Variation in DNA content has been proposed to be principally due to differences in the amount of heterochromatin, which is mainly located in distal *knobs* (Laurie & Bennett, *Heredity* 55:307-313, 1985; Poggio *et al*., *Ann. Bot*. 82:115-117, 1998). These structures can be observed as subtelomeric bands by DAPI-banding, and using Fluorescent *In Situ* Hybridization (FISH) (González *et al*., *Chrom. Res.* 14: 629-635, 2006). *Knobs* have been described as highly repeated *tandem* arrays of 180-bp and TR-1 (350 bp) sequences, both repeated in different proportions constituting different *knobs* (Dennis & Peacock, *J. Mol. Evol*. 20: 341-350, 1984; Ananiev *et al*., *Proc. Natl. Acad. Sci*. 95: 10785-10790, 1998).

In this study we present the karyotypic formulae, asymmetry indexes and the position and composition of the *knobs* of two races of maize from Argentine Northeast (NEA), Tupi Amarillo (VAV 6563) and Rosado (VAV 6565). DAPI-banding and FISH techniques were applied. The plant material has been provided by the Vavilov Lab. of

Universidad de Buenos Aires (UBA), and cultivated in the greenhouse of the Facultad de Agronomía, UBA.

DAPI-banding was performed according to Summer (Chromosomes banding. Unwin Hyman, London, 1990). DAPI fluorochrome (4'-6-diamidino-2-phenylindole) preferentially stains AT-rich heterochromatin in plants (Guerra, *Genet. Mol. Biol.* 23: 1029-1041, 2000).

The 180-bp and TR-1 *knobs* sequence of maize was obtained from GenBank (http://www.ncbi.nlm.nih.gov/). These sequences were isolated and amplified from total genomic DNA of maize by PCR methods. The sequences obtained were labeled with biotin and digoxigenin by PCR as well by enzymatic methods. For the latter we used enzymatic kits: BioNick Labeling System (Invitrogen) to label with biotin and Dig High Prime (Roche) to label with digoxigenin. FISH was performed according to González *et al.* (*Chrom. Res*. 14: 629-635, 2006). FISH slides were observed with a Zeiss AxioPhot epifluorescence microscope (Carl Zeiss, Germany), and microphotographs were taken with a Leica CCD digital camera.

Chromosomal parameters were measured using the freeware program MicroMeasure 3.3 (http://www.colostate.edu/depts/ biology/micromeasure). The relative chromosome length, arm ratio, and centromeric indexes were calculated to determine the karyotypes. The chromosomes were ordered from the largest to the smallest, as usual for maize, and chromosome morphology was described according to Levan *et al*. (*Hereditas* 52: 201-220, 1964). To estimate the karyotype asymmetry, two numerical parameters were used, according Romero Zarco (*Taxon* 35: 526-530, 1986): A1 (intrachromosomal asymmetry index) and A2 (interchromosomal asymmetry index).

In each race a maximum of 25 individuals were studied (3-5 individuals per maize ear) and at least 10 cell per individual were analyzed.

The karyotype parameters analysis let us to elaborate the idiograms from the two maize races (Figure 1 A and 2A).

DAPI banding allowed identified and locates the *knobs* as DAPI-positive bands on mitotic metaphase chromosomes (Figure 1 B and 2 B). In FISH experiments, simultaneous hybridization with the 180-bp and TR-1 probes showed that these sequences co-localized with all the DAPI-positive bands in both races. Different intensities of hybridization signals

with each probe suggest that the DAPI-positive *knobs* are composed by different proportions of 180-bp and TR-1 sequences.

In table 1 the karyotypic formulae, A1 and A2 indexes, percentage of heterochromatin and number of *knobs* are presented for Tupi Amarillo and Rosado maize races.

We observed that the percentage of heterochromatin of Tupi Amarillo is about 8% higher than Rosado (Table 1), this difference between both maize races is due to the higher number of *knobs* in Tupi Amarillo. This race showed higher intrachromosomal asymmetry (A1) but lower interchromosomal asymmetry (A2) compared with Rosado. This is due to differences in the size and the distribution of the *knobs* on the both chromosomal arms (Figures 1 A and 2 A).

All results obtained in this work allowed us to identify cytogenetically the maize races studied. Then, the variations of the patterns for number, position and sequence composition of the heterochromatic *knobs* are useful markers for a proper cytogenetic characterization of maize races.

The cytogenetic characterization of different Argentinean races of maize will contribute to the knowledge of the genetic variability within native materials, useful for its integration in future breeding plans and biodiversity conservation.

	Tupi Amarillo	**Rosado**
Karyotypic formulae	6m + 4 sm	6m + 1m-sm + 3sm
% of heterochromatin	19,64	11,7
Range of number of *Knobs*	16-22	10-11
A1	0,35	0,27
A2	0,18	0,26

Table 1: Karyotypic parameters of Tupi Amarillo and Rosado maize races. Ref.: A1: intrachromosomal asymmetry index. A2: interchromosomal asymmetry index. m: metacentric. sm: submetacentric.

Figure 1: **A**. Idiogram of maize Rosado. The white blocks represent the coincident DAPI-positive band and 180-bp and TR-1 FISH signals. **B**. DAPI-banding. **C.** FISH using 180-pb as probe on mitotic metaphase chromosomes. Ref.: M: metacentric. SM: submetacentric. IC: centromeric index. Bar 10 μm.

Figure 2: **A**. Idiogram of maize Tupi Amarillo. The white blocks represent the coincident DAPI-positive band and 180-bp and TR-1 FISH signals. **B**. DAPI-banding. **C.** FISH using TR-1 as probe on mitotic metaphase chromosomes. Ref.: M: metacentric. SM: submetacentric. IC: centromeric index. Bar 10 μm.