

Karyotype comparison between *Zea luxurians* and maize Amarillo Chico through DAPI-banding and FISH of knob sequence

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Karyotypes of *Zea luxurians* and the maize of race Amarillo Chico from Northwest of Argentina (NOA) were described using DAPI-banding and Fluorescent *In Situ* Hybridization (FISH) techniques.

The variation in DNA content in *Zea* has been proposed that is largely caused by differences in the heterochromatin amounts that form distal blocks named knobs (Laurie and Bennett, *Heredity* 55:307-313, 1985; Poggio *et al.*, *Ann. Bot.* 82:115-117, 1998). These knobs may vary in quantity and size among the different races of maize and its wild relatives (Kato, *Agric. Exp. Stn. Bull.* 635: 1-185, 1976; McClintock *et al.*, Col. Postgrad. Chapingo México, 1981). *Zea luxurians* possesses the highest genome size of the $2n=20$ *Zea* species ($2C= 8.83$ pg), while the DNA content of maize varies between $2C= 4.92$ and 6.79 pg (Tito *et al.*, *Theor. Appl. Genet.* 83:58-64, 1991). The heterochromatic knobs from maize and *Zea luxurians* correspond to C and DAPI-positive bands on mitotic metaphase chromosomes (Tito *et al.*, 1991; Poggio *et al.*, 1998), and there are composed principally by a 180-bp tandem repeat sequence (Peacock *et al.*, *Proc. Natl. Acad. Sci. USA* 78:4490-4494, 1981).

The materials used in this study were the *Zea luxurians* cv. 9478 (Guatemalan) Leg. CIMMYT and the Argentinean race of maize Amarillo Chico (VAV 6451) from NOA Leg. by Vavilov Laboratory, University of Buenos Aires (UBA). The materials were cultivated in the greenhouse of Facultad de Agronomía, UBA.

DAPI banding and FISH performed according to Summer (Chromosome banding. Unwin Hyman, London, 1990) and González *et al.* (*Chrom. Res.* 14:629-635, 2006), respectively, were carried out on mitotic metaphases from *Zea luxurians* and maize. For FISH the knob-180bp sequence was obtained and used as probe. Slides were examined with a Carl Zeiss Axiophot

epifluorescence microscope and the photographs were taken using a Leica CCD digital camera.

Chromosomal parameters were measured in at least 10 metaphases, for each species, using the freeware program MicroMeasure 3.3 (<http://www.colostate.edu/depts/biology/micromeasure>). For karyotyping the relative chromosome length, arm ratio y centromeric index were calculated. Chromosomes were ordered from high to low as usually in maize, and the chromosomal morphology was described according to Levan *et al.* (*Hereditas* 52: 201-220, 1964). Also, intra and inter-chromosomal asymmetry indexes, A1 and A2 described by Romero Zarco (*Taxon* 35: 526-530, 1986), were calculated. These indexes, in addition to the formulae and karyotypic parameters from *Zea luxurians* and the Amarillo Chico maize are shown in Table 1.

Table 1

	Maize Amarillo Chico	<i>Zea luxurians</i>
2n=4x	20	20
DNA content 2C (X +/- Standart Error)*	5.63 pg (+/- 0.05)	8.83 pg (+/- 0.08)
Karyotypic Formula	6 m + 4 sm	5 m + 4 sm + 1 sm-st
TCL	135um	173um
TCL without knobs	124um	168um
% of heterochromatin †	7.65	21.16
Range of knob sizes	1.62um - 0.54um	2.74um - 0.8um
Media of knob sizes	1.04um	1.75um
A1	0.36	0.39
A2	0.27	0.15

Ref.: * From Tito *et al.*, 1991. **TCL**: Total Chromosome Length. †: Calculated as a percent of TCL. **A1**: intra-chromosomal asymmetry index. **A2**: inter-chromosomal asymmetry index. **m**: metacentric. **sm**: sub-metacentric. **st**: sub-telocentric.

The analysis of the karyotype parameters let us to elaborate the idiograms from the taxa analyzed (Fig. 1). It is important to note that the knobs 180pb sequence shown FISH-positive signals on all heterochromatic DAPI-positive bands, as it is indicated in the figure 1.

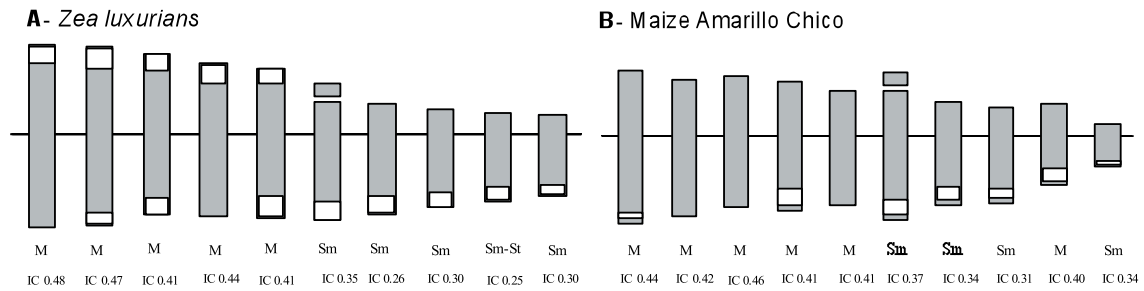


Figure 1: Idiograms of *Zea luxurians* (A) and Maize race Amarillo Chico (B). The white blocks represent the coincident DAPI-positive bands and the 180bp sequence FISH signals. **M**: metacentric. **Sm**: sub-metacentric. **St**: sub-telocentric. **IC**: Centromeric index.

Tito *et al.* (1991) and Poggio *et al.* (1998) found, applying C and DAPI banding to different maize races from NOA, a positive relation between the genome size and the heterochromatin percent corresponding to knobs. We revealed, using DAPI-banding and FISH, that *Zea luxurians* have about 14% more knob-heterochromatin than maize, indeed knobs of *Zea luxurians* are about 40% bigger than the maize ones (Fig. 1). Moreover, we observed that the TCL of *Zea luxurians* is 28.6 % higher than maize, being approximately 26% if knobs are not considered. Also, we have seen that the Total Chromosome Volume of *Zea luxurians* is about 28% higher than maize. Therefore, the differences in DNA content and the chromosome sizes between both species could be explicated for both its dissimilar number and size of knobs, and the different amounts of interspersed DNA.

From the observed in figure 1, we conclude that the difference founded in inter-chromosomal asymmetry in the taxa studied is due to the number, size and distribution of heterochromatic knobs on both chromosomes arms.

Finally, the cytogenetic studies presented here revealed important karyotypic and chromosomal differences between *Zea luxurians* and maize.