

analysis. Bhuyanpirh tea plantations have not been previously surveyed for AM fungi.

Isolation and characterization of AM spores used published methods (Gerdemann and Nicolson, *Trans. Br. Mycol. Soc.* 46(2):235-244, 1963; Kormanic and McGraw, Pp. 34-45 in *Methods and Principles of Mycorrhizal Research*, American Phytopathological Society, 1982; Schenck and Perez, P. 245 in *Manual for the Identification of VA Mycorrhizal Fungi*, INVAM, 1987). 15-day-old maize seedlings were planted in earthen pots after treatment with or without AM fungi. Roots sampled after 75 days of growth had higher mycorrhizal colonization in water-stressed plants compared to well-watered plants (Fig. 1). Biomass and growth was higher in mycorrhizal than nonmycorrhizal plants irrespective of water treatments (Fig. 2). However, the plants irrigated with alternate watering schedules showed higher biomass than those treated with daily watering.

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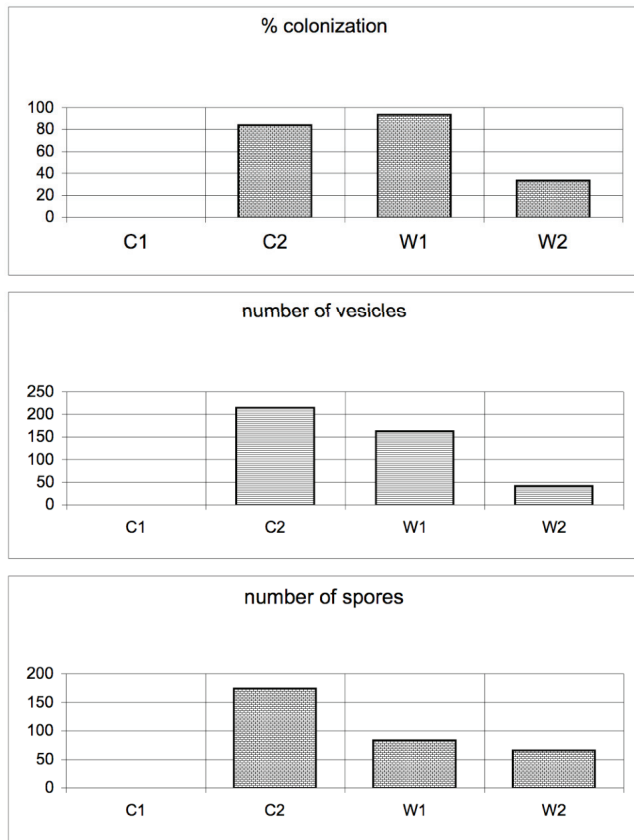


Figure 1. Status of mycorrhization in maize roots inoculated under different treatments. Abbreviations: C1 = daily watering, C2 = AM + daily watering, W1 = AM + alternate day watering, W2 = AM + watering at two day intervals.

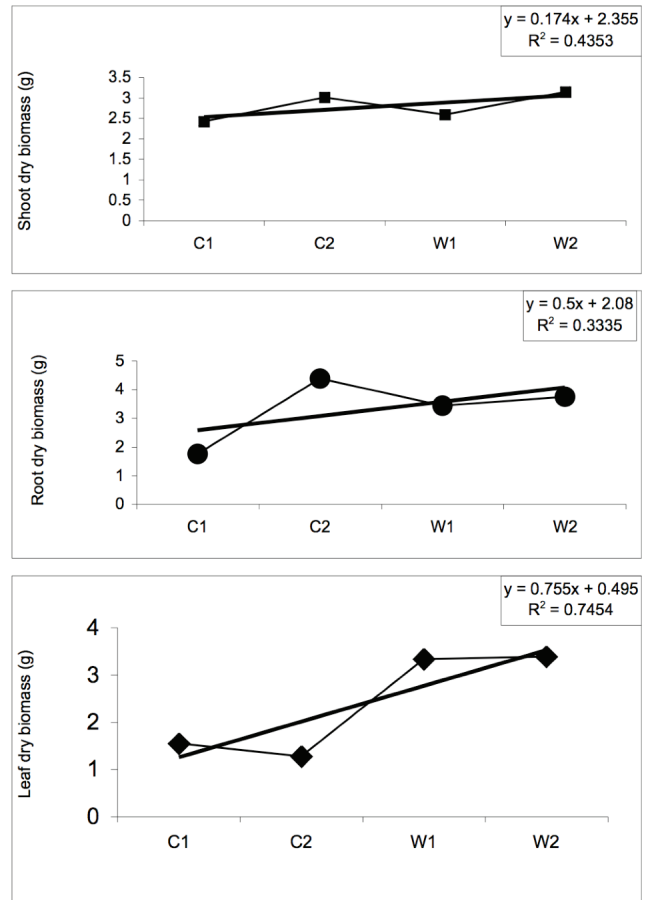


Figure 2. Effect of AM fungi on growth (measured after 75 days of experiment) of maize (host plant) grown under different watering schedules. For abbreviations see Figure 1.

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Analysis of the effect of RAD51 on the spontaneous mutation frequency in maize haploids

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Rad51p plays a central role in homologous recombination and the repair of double-strand breaks in *Saccharomyces cerevisiae*. Double mutants of the two *Zea mays* L. *rad51* homologs (*Zmrad51A1* and *Zmrad51A2*) are viable and develop well under normal conditions in diploids. However, they have meiotic abnormalities, are male sterile and have greatly reduced seed set (Li et al., *Genetics* 176: 1469-1482, 2007). In this article, these alleles will be referred to as *rad51A1* and *rad51A2*.

The purpose of this study was to determine if a higher spontaneous mutation frequency is present in maize plants with both the *rad51A1* and *rad51A2* mutations. For this purpose, the frequencies of mutant sectors on the 5th leaves of haploid plants that were *rad51A1* and *rad51A2* or *Rad51A1* and *rad51A2* were compared. In haploids, all mutant sectors will be detectable because they will not be covered by the non-mutant allele on the normal homolog.

To produce haploids of the two genotypes, a line that produces high frequencies of haploids, RWS, was employed. *Rad51A1/rad51A1*; *rad51A2/rad51A2* female parents were crossed with pollen from RWS male parents. The F1s were field planted, and the haploids selected by their distinctive phenotype. Part of the F1 kernels were pre-selected using the *r1-nj* marker allele. Because RWS is *r1-nj/r1-nj* and the pollen parent is *r1/r1*, kernels with colorless embryos can be selected as maternal haploids.

66 haploids were recovered. Each haploid was genotyped to determine if it was *Rad51A1/rad51A2* or *rad51A1/rad51A2*. The number of mutant sectors on leaf 5 of each of the haploid plants was then determined. The leaf sectors were typically chlorotic or necrotic sectors. 46 of the haploids were *Rad51A1/rad51A2* and 20 were *rad51A1/rad51A2*. The reason for the greater frequency of *Rad51A1/rad51A2* plants is not known; perhaps the double mutant ovules function with a reduced frequency. Unfortunately, 2 of the double mutant plants broke off during development and one had half of each leaf missing, and therefore was not evaluated.

The frequency of mutant leaf sectors on the fifth leaf of the 46 *Rad51A1/rad51A2* plants was 1.41 ± 1.26 and the frequency of mutant leaf sectors on the fifth leaf of the 17 *rad51A1/rad51A2* plants was 2.29 ± 2.37 . Thus, the frequency of leaf sectors on the two plant types appears to be similar, and we can conclude that the spontaneous mutation frequency is not elevated in somatic cells of double-mutant plants. Also, the two plant types appeared to be indistinguishable from each other, so the double mutants do not appear to have an altered leaf morphology.

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Characterization of maize and teosinte using the variation in their knob sequences

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In maize, the wide variation in nuclear DNA content is mainly caused by differences in heterochromatin amounts (Tito et al., Theor. App. Genet. 83:58-64, 1991; Poggio et al., Ann. J. Bot. 82:115-117, 1998). Knob heterochromatin of maize and teosinte differs from all other classes of heterochromatin due to its cytological appearance and DNA sequence composition (Peacock et al., J. Mol. Evol. 20:341-350, 1981). It is a useful cytological marker because it is polymorphic and was found at 22 different positions within the maize karyotype (Kato, Mass. Agric. Exp. Stn. Bull. 635:1-185, 1976). Knobs consist of thousands to millions of tandem 180- and 350-bp (TR-1) repeats which are present in cytologically detectable knobs in different proportions relative to one another (Ananiev et al., Proc. Natl. Acad. Sci. 95:10785-10790, 1998). Maize knob heterochromatin is also visible in interphase or nondividing somatic cells using a simple DAPI-banding method.

The aim of this work is to analyze the variation in the number of knobs and its sequence composition from different Northwest Argentinean strains of maize and some Mexican teosintes. DAPI banding and fluorescent in situ hybridization (FISH) on interphase cells, using TR-1 and 180-bp repeats as labelled probes, were performed.

Maize materials were kindly provided by Ing. Cámara Hernández from the Vavilov Laboratory of the Facultad de Agronomía (FA) of the Universidad de Buenos Aires (UBA). Mexican teosintes were obtained from the following locations: *Zea diploperennis* from Las Joyas, Jalisco; *Zea luxurians* from Guadalajara; *Zea mays* ssp. *parviglumis* from Mesa Central and *Zea mays* ssp. *mexicana* from Balsas River valley. Plants were cultivated in the greenhouse of FA-UBA. DAPI banding and FISH techniques were done according to the methods of González et al., 2006 (Chrom. Res. 14:629-635).

The experiments showed variations in number and sequence composition of DAPI+ knobs in five maize strains. However, the pattern was recurrent for each race (Table 1).

Table 1. Number and sequence composition of maize knobs by DAPI banding and FISH experiments. Ref: VAV: accession; a.s.l.: about sea level; n/d: no data available.

Maize strain	Cultivation altitude	DAPI+ knobs	180 pb knobs	TR-1 knobs	180 pb + TR-1 knobs
Race Amarillo Chico (VAV6451)	2000 mt a.s.l.	9	5	2	2
Race Orgullo Cuarentón (VAV6482)	910 mt a.s.l.	18	16	n/d	n/d
Race Amarillo Chico (VAV6476)	1690 mt a.s.l.	10	4	0	6
Race Blanco y ocho rayas (VAV6483)	1250 mt a.s.l.	13	9	0	4
Imbreed Line IFSC 13043	00 mt a.s.l.	10	6	0	4

Actually, we are studying the knob sequence composition of different teosintes. We found that the FISH experiments, using the 180 bp repeat as probe, show strong hybridization signals on almost all the DAPI + knobs of *Z. m. ssp. parviglumis* and *Z. luxurians*, but on *Z. m. ssp. mexicana* knobs these signals are weaker. FISH experiments on *Z. diploperennis* chromosomes using 180-pb and TR-1 probes simultaneously revealed that the two sequences were localized on all DAPI + knobs. These results need to be confirmed for other teosinte accessions.

These experiments demonstrate that the variants of the patterns for number and sequence composition of the heterochromatic knobs, along with their subsequent localization within chromosomes, are useful markers for a proper cytogenetic characterization of maize races and teosintes. The knowledge of these variations will allow further research on the correlation described previously for the presence of knobs and crop altitude (Rosato et al., Am. J. Bot. 85:168-174, 1998). This methodology could then be extrapolated to the cytogenetic characterization of commercial inbreds and maize hybrids.

On the other hand, the cytogenetic characterization of different Argentinean races of maize will contribute to the information about the availability of genetic variability within native materials, useful for its integration in future breeding plans and biodiversity conservation.