

## Meiosis and pollen grain development in *Isolepis cernua* f. *cernua* (Cyperaceae)

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**Abstract** — In this paper, cytogenetic studies and pollen grain development of *Isolepis cernua* (Vahl) Roem. & Schult. f. *cernua* (Cyperaceae) from Argentina are presented. The somatic chromosome number found is  $2n=54$ , being different from previous reports. Primary constrictions have not been observed, suggesting that they are holocentric chromosomes, as in many species of the family. The meiotic behaviour was described showing  $n=27$  and axial orientation of some of the bivalents. Three of four pollen nuclei degenerate while one of them completes the mitosis. These processes originate the pseudomonad, a characteristic feature in the Cyperaceae. This fact does not affect the pollen fertility which is 98%.

**Key words:** Chromosome number, *Cyperaceae*, *Isolepis cernua* f. *cernua*, meiotic behaviour, pseudomonad.

### INTRODUCTION

The genus *Isolepis* (Cyperaceae) comprises about 70 cosmopolitan species, with major concentration in Africa and Australia (WILSON 1981). The species grow in aquatic as well as in terrestrial habits, from the sea level to 3000-4000m of height.

*Isolepis cernua* (Vahl) Roem. & Schult. f. *cernua* (= *Scirpus cernuus* Vahl) is a variable annual species, with filiform culms of 3-20 cm high. It bears 1-3 leaves that are shorter than the culms. The inflorescence is pseudolateral with 1-3 sessile spikelets and a bract that follows the culm's direction.

Regarding cytogenetic features, most Cyperaceae genera show three distinctive characteristics: *i*) chromosomes with diffuse kinetochores, *it*) post-reductional meiosis: the first meiotic division comprises the separation of the sister chromatids using the duplication plane as a separation plane; and the second meiotic division comprises the disjunction of homologous chromosomes (BATTAGLIA and BOYES 1955), and *Hi*) abortion in 3 of the 4 nuclei that result

from meiosis in the microspore mother cell and the origin of the pollen grain from only one of the nucleus (pseudomonad). The last characteristic was found in an other few groups such as the Styphelieae tribe from the Epacridaceae (CHAR *et al.* 1973).

There are different reports on chromosome numbers of *Isolepis cernua* (as *Scirpus cernuus* Vahl). TANAKA (1942, 1948) reported  $2n=48$  for Japanese material, whereas SANYAL and SHARMA (1972) and POJAR (1973) reported  $2n=30$  for Indian and British Columbia populations, respectively.

In the over 5000 species of Cyperaceae (GOETGHEBEUR 1998), the meiotic behaviour and pollen development was described in only 51 of them (KIRPES *et al.* 1996) and no data were reported for *Isolepis cernua*.

The aim of this work is to analyse the meiotic behaviour, gamete number, pollen fertility and the formation of the pseudomonad in *Isolepis cernua*.

### MATERIAL AND METHODS

The material used was collected in the Reserva Natural Otamendi (Provincia de Buenos Aires, Argentina). Voucher specimens (E. R. Guaglianone & L. P. Dopchiz 3080, 3158 y 3112) are deposited in

the Herbarium of Institute Darwinion Argentina (SI).

Flowers and roots tips of twenty individuals from this population were fixed without pretreatment in 3:1 (absolute ethanol: glacial acetic acid). For mitosis studies in roots tips and the staining was done with Feulgen for 90 minutes, after hydrolysing the samples with HCl 5N at room temperature (20°C) for 25 minutes. For meiotic studies the anthers were squashed in 2% acetic haematoxylin (NUNEZ 1968) using ferric citrate as a mordant.

Chromosome measurements were done on photomicrographs using a MiniMop (Kontron) Image Analyser. To estimate the asymmetry of the karyotype the  $A_2$  index was used (Interchromosomal Asymmetry Index) as defined by ROMERO ZARCO (1986) which is independent of chromosome number and size.

Pollen fertility was determined using the stain proposed by ALEXANDER (1969), analysing at least 100 pollen grains in each of ten individuals.

## RESULTS

The chromosome number found in root meristem cells was  $2n=54$  (Fig. 1A) for 5 individuals analysed. The chromosome size varies from 0.23 to 0.58  $\mu$ m. With the average data on chromosome length we calculated the Interchromosomal Asymmetry Index  $A_2=0.227$ .

All the individuals from the population studied showed regular meiosis (Fig. 1C, D, E) and high fertility (98%). In diplotene and diakinesis stages (Fig. 1E) two bivalents were found associated to the nucleole. At least five bivalents with two chiasmata were observed (Fig. 1D). From this phase up to prometaphase I (Fig. 1F) the bivalents gradually condensate. In diplotene and diakinesis (Fig. 1C, D, E) 27 bivalents are perfectly seen, 12 of which are larger. To analyse the bivalents in metaphase I is difficult due to their proximity to the equatorial plate and the high condensation of the chromatin (Fig. 1G). However, we observed that some of the bivalents were arranged perpendicular to the equatorial plane as occurs with holocentric chromosomes (Fig. 1G). This arrangement would correspond to the axial orientation (Fig. 1F, G) according to WHITE (1973). Telophase I is normal (Fig. 1H). The stage previous to telophase II could not be found suggesting that meiosis II is a very short stage. The four nuclei resulting immediately after telophase II showed the same size (Fig. 1I).

In this study, we observed the primary division of the pollen grain (Fig. 2A-G). One of the four nuclei increased its size and underwent mitosis, while the other three remain smaller (Fig. 2A).

In the viable nucleus, 27 chromosomes were counted in late prometaphase (Fig. 2B, C), supporting the somatic chromosome number that we found ( $2n=54$ ). The viable nucleus separates from the sterile nuclei by a thin wall (Fig. 2C). The former and the sterile nuclei start to divide simultaneously, but these last stop the process in prophase (Fig. 2A, B, C) and have less dense cytoplasm. In anaphase II (Fig. 2E, F) some of the chromosomes of the viable nucleus orientate their major axis perpendicular to the fuse fibers, and migrate parallelly to the equator plane. No defined centromere has been observed for the chromosome in any stage (somatic mitosis, primary pseudomonadite mitosis, or meiosis). The viable nucleus ends its division originating the vegetative and generative nuclei (Fig. 2HI). The other three sterile nuclei are situated in the opposite pole and gradually decrease in size and become necrotic.

## DISCUSSION

The finding of  $2n=54$  for *Isolepis cernua* is a new report for the species. The discrepancy with the chromosome count of  $2n=30$  found by SANYAL and SHARMA (1972) and POJAR (1973) could be due to differences in the taxonomic criterion or the existence of intraspecific variation in the chromosome number. The chromosomes are very small and the karyotype symmetrical. The chromosomes do not show localized centromeres (monocentric) and, despite their small size, in some cases we could see their migration parallel to the equator. Thus, as in many Cyperaceae (HEILBORN 1924; GREILHUBER 1995; EUCENO and CASTROVIEJO 1991; PAZY and PLITMANN 1994; LUCENO *et al.* 1998), *Isolepis cernua* seems to have holocentric chromosomes.

In most of the cells studied, the bivalents in metaphase I tend to be clumped so that only in some of them we observed the axial orientation. All these evidences suggest that the meiotic process is prereductional. However, to confirm

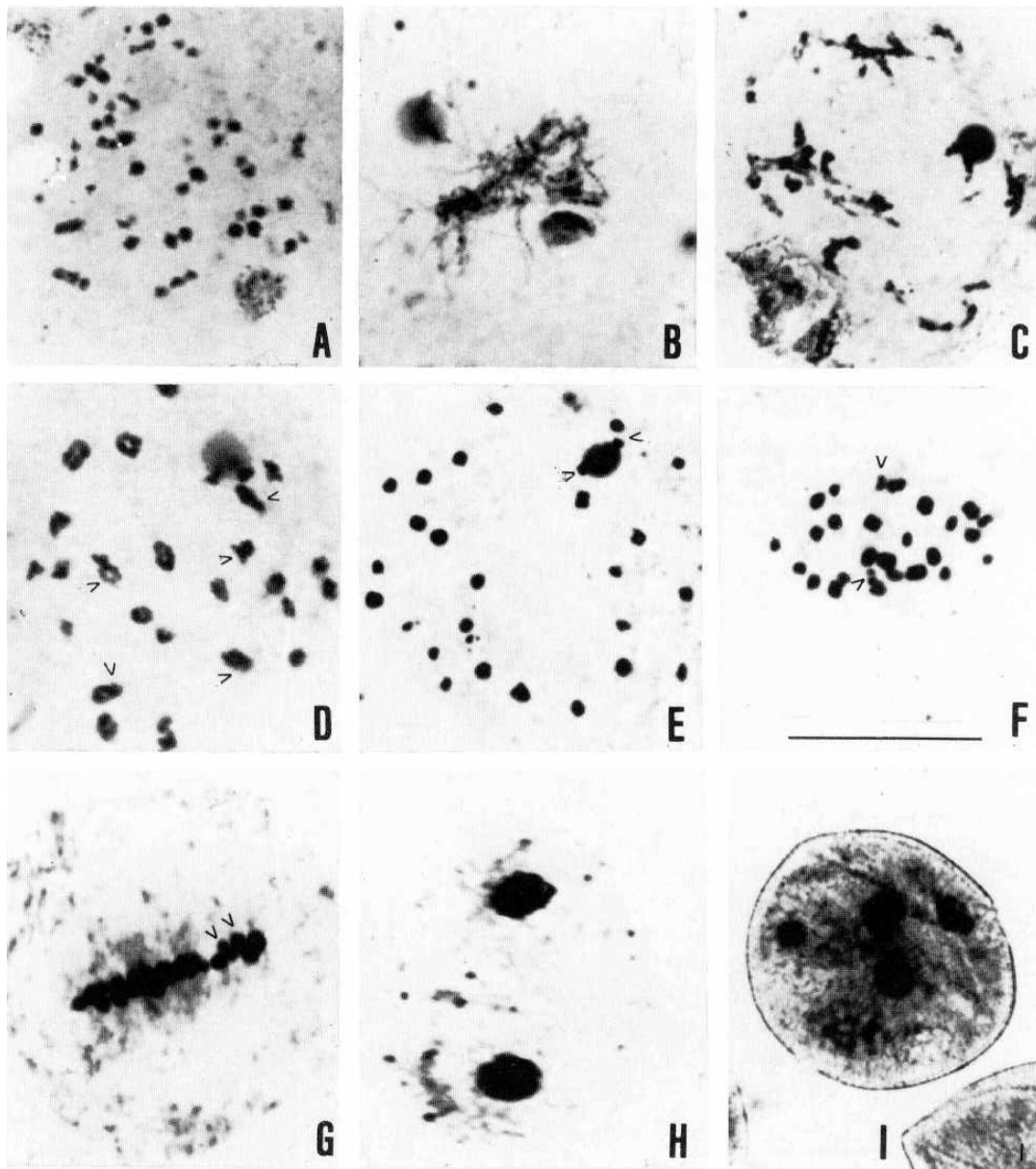


Fig. 1 — A: *Mitotic metaphase*. B-I: *Meiosis*. B: Paquitene. C: Early Diplotene. D: Diplotene. In D the arrows show bivalents with two chiasmata. E: Diakynesis. The arrows show two bivalents associated to the nucleole. F: Prometaphase I. G: Metaphase I. The arrows shows bivalent with axial orientation. H: Telofase I.1: Telofase II. Bar= 10  $\mu$ m.

this, more detailed analysis of the other meiotic stages, such as anaphase I, are required. The three inviable nuclei degenerate while the remaining one develops into a pollen grain. The three non-functional nuclei fuse originating an amorphous chromatin mass. The pollen grain is a pseudomonad showing three degenerated microspores. This phenomena was documented in another Cyperaceae (KIRPES *et al.* 1996). This fact could be due either to the particular shape of the pollen grain, which forces

this arrangement or to the nutritional condition caused by the anther periplasmodium (TANAKA 1940a, b). Any of these two hypothesis could be responsible for the pseudomonad formation. This degeneration does not affect the pollen fertility.

KIRPES *et al.* (1996) analysed the systematic significance of pollen arrangement in Cyperaceae in relation with other families. They consider that the presence of pseudomonad is synapomorphic for the family. The finding that

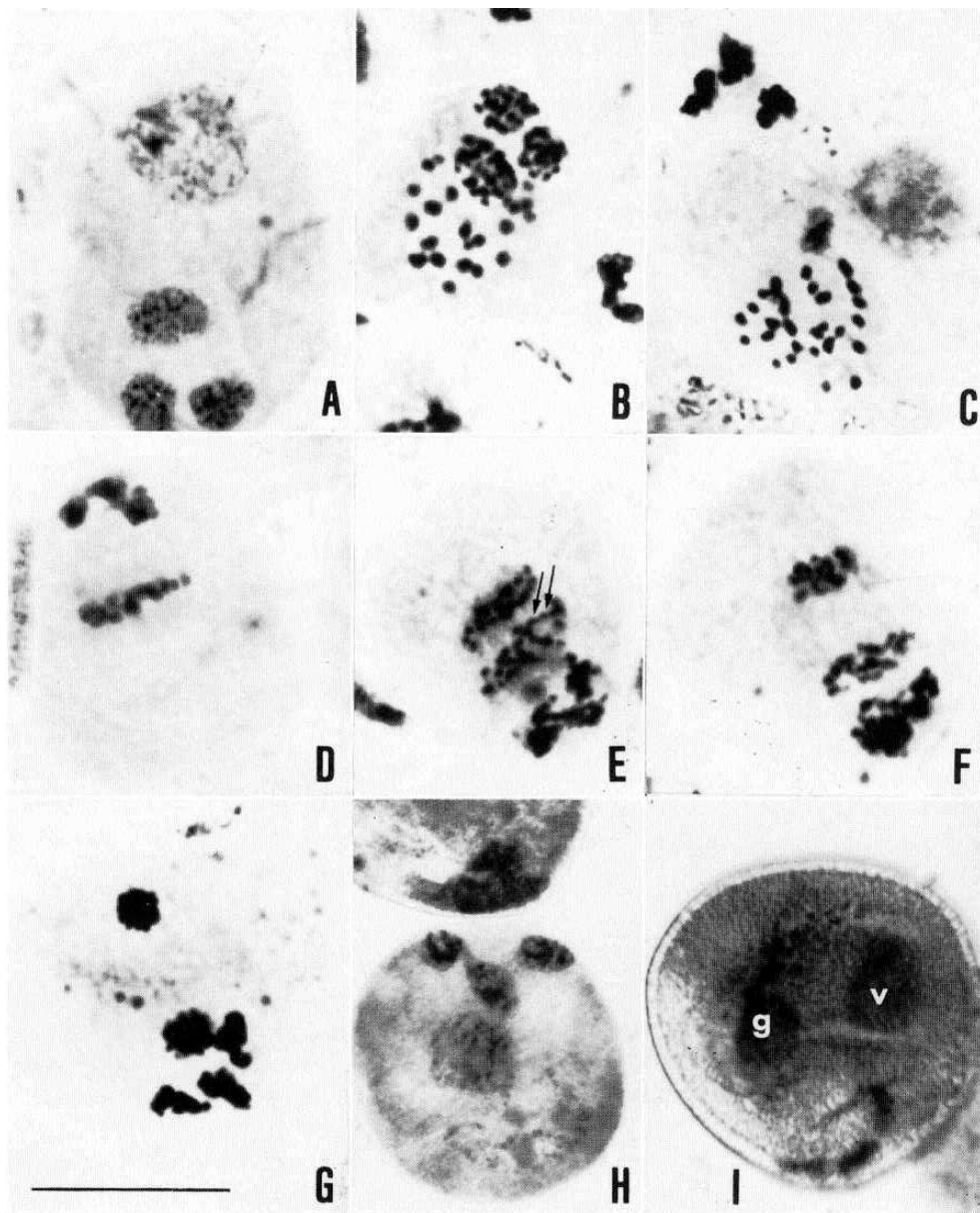


Fig. 2 — *Pollen mitosis* A: Early prophase. B-C: advanced Prometaphase. D: Metaphase. E-F: anaphase. In E the arrows show chromosome arrangement parallel to the equatorial plate. G: Telofase. H: The vegetative and generative nuclei are formed. I: The generative cell is included inside the vegetative cell. Bar= 10  $\mu$ m

*Isolepis cernua* f. *cernua* has a pseudomonad confirms many reports of this kind in supporting the phylogenetic significance of pollen development in Cyperaceae family.

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