Cytogenetic Study of Paracentric Inversions in

Tridax procumbens (Compositae)

Herbán Cequea1,* Damelis Díaz de Cequea1, José Imery1 and Mauro Nirchio2

1 Departamento de Biología, Escuela de Ciencias, Universidad de Oriente, Núcleo de Sucre, Apartado 245, Cumaná 6101, Venezuela
2 Departamento de Acuicultura, Escuela de Ciencias del Mar, Universidad de Oriente, Núcleo de Nueva Esparta, Margarita, Venezuela

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Summary

Meiotic analyses at pachytene, anaphase I and II, metaphase II and the tetrad stage were carried out on several Tridax procumbens L. plants. Aberrations such as reverse inversion loops in pachytene, dicentric bridges and (or) fragments in pollen mother cells at anaphase I and II, dicentric bridges at metaphase II and 4–7 microspores at the tetrad stage, indicated that these plants were heterozygous for a paracentric inversion, which may involve one or more homologous chromosomes. The 75% fertility showed that a simple or double crossing-over took place within inversion reverse loops between inverted and normal chromosome segments at pachytene, which reduced fertility by the formation of genetically abnormal gametes.

Key words

Paracentric inversions, Fertility, Meiosis, Chromosomes, Crossing-over.

Tridax procumbens L. is a perennial herb, widely distributed in Mexico, The Antilles and South America. In Venezuela, it is common on roadides and in vacant lots in towns (Aristeguieta 1964). Cytological studies have shown that 2n = 36 (Raghavan and Ventakasubban 1941). In a paracentric inversion, if simple or double crossing-over takes place within inversion reverse loops between inverted and normal chromosome segments at pachytene, dicentric bridges and acentric fragments in some pollen mother cells (PMCs) at meiotic anaphase I and II, are observed (McClintock 1938), which reduces fertility by the formation of genetically abnormal gametes. On the other hand, if no crossovers take place, then only normal PMCs are visible at anaphase. The meiotic configurations expected at anaphase following crossovers within the inversion loop and proximal to it, have been reported by McClintock (1938), Brandham (1969), Ekberg (1974), Sybenga (1975) and Pickering (1991).

This study reports different meiotic configurations at pachytene, anaphase I and II, telophase I and II, suggesting that crossing-over occurs within inversion reverse loops in a paracentric inversion of a T. procumbens population.

Materials and methods

Tridax procumbens seeds used in this study were collected from a naturalized population in Cumaná, Sucre State, Venezuela during the year 2000. Selected seeds from 5 plants were germinated in 50% Hoagland’s solution. Seedlings approximately 7 cm long were planted in water expanded Jiffy-7 peat pellets until numerous roots were observed. Seven week-old seedlings were transferred to 5 kg polyethylene sacks containing sand, peat and soil in a 1:1:1 ratio and maintained in a greenhouse.

Flower buds were harvested between 13:00 and 13:30 and fixed in ethanol: propionic acid © 2003 The Japan Mendel Society


* Corresponding author, e-mail: hcequea@cumana.sucre.udo.edu.ve
(3 : 1) for 48 h. Anthers were removed from individual florets, squashed and stained in orcein FLP 1.5% (Cequea and Nirchio 1998). Meiotic configurations at pachytene, anaphase I and II, telophase I and II and the tetrad stage were scored for presence of reverse inversion loops, bridges, fragments and the number of microspores in PMCs.

Five hundred pollen grains per floret were stained in Buffalo Black NBR (1 g naphthol Buffalo Black in 100 ml warm FLP solvent, cooled to room temperature and filtered). Pollen grains having uniformly and darkly stained cytoplasm were considered normal, while those incompletely or lightly stained blue was considered abnormal.

The classification of anaphase configurations into categories was adapted from the system used by Brandham (1969) and Ekberg (1974). The categories are described as follows:

- **Anaphase I**: Normal, Bridge + fragment, 2 bridges + 2 fragments, single fragment, 2 fragments.
- **Anaphase II**: Normal, bridge, bridge + 2 fragments, single fragment, 2 fragments.

*T. procumbens* plants were kept at the botanical herbarium at the Universidad de Oriente under the number 3002.

### Results

Meiosis was abnormal in the majority of stages. In pachytene an inversion loop was observed (Fig. 1). The designations of the anaphase I configurations used by Brandham (1969) were adopted, thus: N stands for a normal configuration, B, L and F stand for one dicentric bridge, one loop and one acentric fragment, respectively. A single crossover within the loop gives rise to the BF (bridge fragment) type (Fig. 2). Moreover, we expected that the anaphase stages would appear normal following 2-strand doubles within the loop. Three-strand doubles give rise to BF and 4-strand doubles produce BBFF (Fig. 3). In the case of single crossovers within the loop these result in the formation of BF or LF depending on which chromatids are involved in the crossovers. Following double crossovers within the loop, the configurations N, BF, LF, BBFF, as well as LLFF are expected. It should be emphasized that LLFF is only formed as a result of a 4-strand double crossover within the loop and a simultaneous crossover in the proximal region. In LF and LLFF the dicentric sister-chromatids form loops at anaphase I. The existence of such loops in *T. procumbens* was difficult to prove due to the condensed state of the chromosomes at anaphase I. Acentric fragments rounded like dots with similar or different sizes were also clearly observed (Figs. 3, 4). Moreover, the fragments usually remained on the equatorial plane of the spindle (Figs. 4, 5). The meiotic stages at interphase and prophase II were normal in appearance. The chromatid bridges, as a rule, were broken at the end of the second division of meiosis. Thus, a persisting bridge from anaphase I was usually observed at metaphase II or at subsequent meiotic stages (Fig. 6). The tetrad stage appeared abnormal with 5–7 micronuclei (Figs. 7, 8). Chromosome aberrations observed in an inversion heterozyg-
Figs. 1–8. Aberrations in PMCs in *Tridax procumbens* heterozygous for paracentric inversions. 1) Pachytene with an inversion loop (arrow), 2) Anaphase I with a dicentric bridge connecting bivalent at 2 poles, 3) Anaphase I with 2 bridges + 2 fragments, 4) Telophase I with 2 fragments at the equatorial plate, 5) Telophase II with 3 fragments. 6) Anaphase II with a bridge connecting 2 nuclei (arrow), 7) Tetrad stage with 5 microspores, 8) Tetrad stage with 5 microspores. One microspore with 2 small nuclei. Bar represents 10 μm.
gous at anaphase I and II are shown in Table 1. The observed pollen fertility average was 75%.

**Discussion**

A paracentric inversion heterozygote was revealed in *Tridax procumbens* by the occurrence of a reverse-loop pairing at the pachytene stage in the pollen mother cells, the presence of cells with single bridges, double bridges +2 fragments, 2 and 3 fragments only at anaphase I and II, telophase I and II, and a tetrad stage with 5–7 micronuclei following the frequency and type of crossover taking place within the inversion loop and in the proximal segment between the inversion and centromere (Brandham 1969, Ekberg 1974, Sall et al. 1990).

In *Tridax procumbens* the pachytene stage was not suitable for cytological analyses due to the dense appearance of this stage, it was not possible to follow a bivalent throughout its entire length, although in good slides it was possible to distinguish part of it (Fig. 1). The fragment had different sizes (Figs. 4, 5), which may indicate that proximal and distal inversions took place.

Bridges without fragments were observed in 23% of anaphase I configurations. The low frequency of bridges and fragments (0.07%) was probably due to the high frequency of 4 strand double crossovers within the reverse loop and a simultaneous crossover in the proximal region, rather than to single and 3 strand double crossovers within the loops of one or more large inversions. Bridges without fragments in inversion heterozygotes may be due to: 1) late segregation or stickiness, 2) fragments hidden by other chromosomes, 3) association of the fragment with one of the normal chromatids as a result of distal crossovers, 4) the incorporation of fragments into the 2 chromosome groups resulting in temporal disappearance. Similar results have been observed in other plant species (McClintock 1938, Das 1955, Kreft 1969, Pickering 1991).

Predicted fertility from first and second anaphase configurations in *T. procumbens* was 72% which corresponds well with observed values 75%. Furthermore, crossover values in PMCs may not correspond exactly and could result in discrepancy between pollen grains (based on male fertility) and the proportion of aberrations in PMCs (Sall et al. 1990).

Crossing over must occur at a specific site on the reverse loop in *T. procumbens*, because the fragments observed were of a consistent size (Figs. 4, 5). Similar results have been found in *Agave stricta* (Brandham 1969). As bridges with and without small fragments occurred in this species, background levels of bridge formation not attributable to inversions may exist. In addition to fragments of similar size, fragments of different sizes were observed (Fig. 3), which indicates that crossing over may occur randomly on the reverse loop where more than one homologous chromosome may be involved in the paracentric inversion heterozygote. Walters (1950) observed a high frequency of bridges and fragments of variable sizes at anaphase I (1.1/cell) in an interspecific *Brachytyrum* hybrid (2n=49) having low pairing at meiosis (37–49 univalents in metaphase I PMCs) and concluded that the bridges and fragments resulted from chromosome breakage and reunion rather than inversion heterozygosity.

Rees and Thompson (1955) also observed bridges and small fragments in inbred lines of rye resulting from breakage and reunion of chromosomes. As these breaks appeared to correspond to the sites of chiasmata, the authors proposed that chiasmata formation and breakages might be influenced by similar factors.

Jones (1968, 1969) also observed that a change in the distribution of chiasmata in inbred rye was accompanied by a change in the distribution pattern of fragment sizes caused by breakage and fusion, or U-type exchange. Thus, distal chiasmata were always associated with smaller fragments than proximal chiasmata. In barley and interspecific hybrids between barley (*H. vulgare*) and other *Hordeum* species, most of the chiasmata were terminal (Linde-Laursen 1982, Bothmer et al. 1983) and any fragments resulting from breakage and fusion tended to be small.

The tetrad stage with 5–7 microspores instead of the expected 4, could be the result of acentric
fragments produced by crossing over in the reverse inversion loop that produces genetically abnormal gametes.

In conclusion, anaphase bridges and fragments observed in *T. procumbens* confirm that these configurations could be the result of paracentric inversions. However, additional cytogenetic studies must be done in order to identify which chromosomes are involved in these inversions heterozygous.

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**References**


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