

Cytogenetic analysis of *Campuloclinium macrocephalum* (Eupatorieae, Asteraceae)

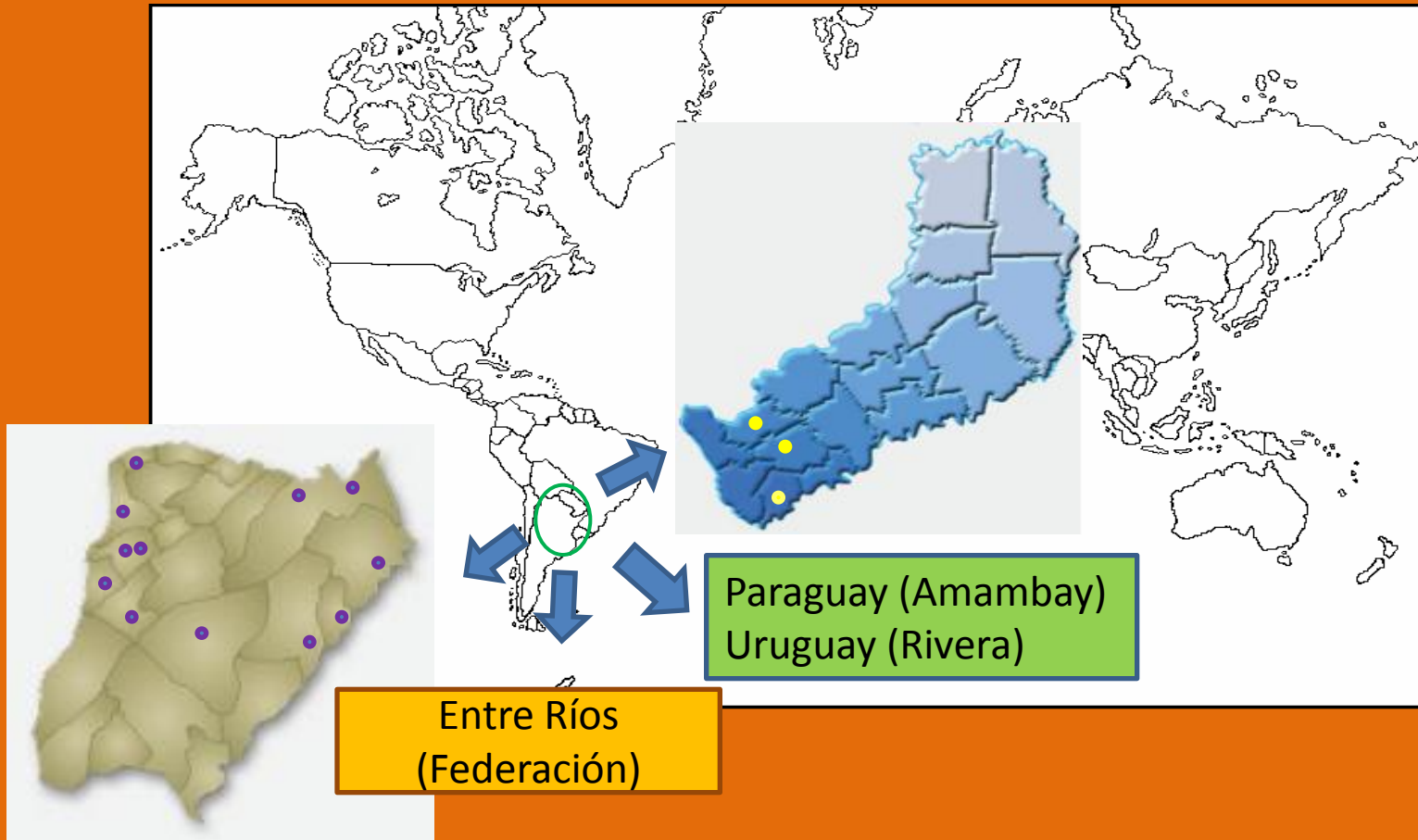


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Campuloclinium macrocephalum (Less) DC.

Distribution



Materials and Methods

MATERIALS AND METHODS

Meiotic analysis

Microsporegenesis

Plants: Argentina : -Corrientes → 12 populations.
-Misiones → 3 populations.
-Entre Ríos → 1 population.
Paraguay → 1 population.
Uruguay → 1 population.

Buds fixed :

-5:1 lactic acid- ethanol solution.
-70% ethanol at 4 °C.

Anthers stained:

- 2% lacto-propionic orcein.

Embryological studies

Plants: Argentina: -Corrientes → 1 population 3x

Buds fixed :

-FAA solution for 48 h.
-70% ethanol at 4 °C.

Mitotic analysis

Plants: Argentina: -Corrientes → 1 population.
-Uruguay → 2 population.

Roots fixed :

-3:1 ethanol- acetic acid solution.
-70% ethanol at 4 °C.

Roots pretreatment:

- Feulgen technique.

Meiotic configurations

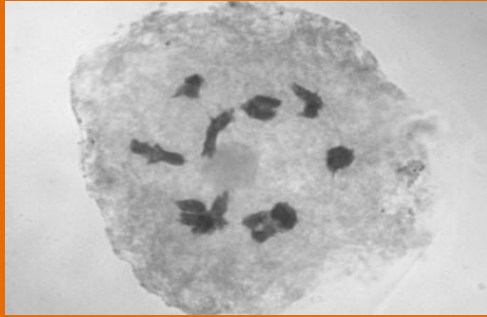
| Populations | 2n/Ploidy Level | Meiotic Configurations | Percentage | Number of cells |
|-------------|-----------------|--|---|-----------------|
| A | 20/2x | 10II | 100 | 18 |
| B | 30/3x | 2I + 2II + 8III 20I + 2II + 1IV | 50 50 | 2 |
| C | 30/3x | 4I + 8II + 1IV + 1VI 11I + 5II + 3III 6I + 6II + 4III 3I + 3II + 7III 8I + 5II + 4III | 20 20 20 20 20 | 5 |
| D | 30/3x | 3II + 8III 1I + 7II + 5III 2I + 8 II + 4III 9II + 4III | 25 25 25 25 | 4 |
| E | 30/3x | 10I + 10II 3I + 9II + 3III 7I + 7II + 3III 11I + 8II + 1III 7I + 10II + 1III 8I + 8II + 2III 7I + 9II + 3III 4I + 10II + 2III 5I + 8II + 3III 5I + 11II + 1III 7I + 8II + 2III 6I + 6II + 4III 6I + 9II + 2III | 5.55 5.55 16.66 5.55 5.55 5.55 5.55 5.55 16.66 5.55 5.55 5.55 5.55 | 18 |
| F | 30/3x | 4I + 13II 6I + 6II + 4III 10I + 7II + 2III 4I + 10II + 2III 4I + 3II + 6III 3I + 5II + 5III 8I + 8II + 2III 6I + 8II + 2III 2I + 5II + 6III 1I + 10II + 3III 3I + 6II + 5III 5I + 8II + 3III 5I + 5II + 5III 6I + 5II + 4III 4I + 4II + 6III | 5.55 11.11 5.55 5.55 5.55 5.55 5.55 11.11 5.55 5.55 11.11 5.55 5.55 5.55 5.55 | 15 |
| G | 30/3x | 8I + 10II + 1III 6I + 2II + 2 III + 2IV 7I + 10II + 1III 10I + 7II + 2III 4I + 7II + 4III 15I + 6II + 1III 8I + 8II + 2III 12I + 9II | 12.50 12.50 12.50 12.50 12.50 12.50 12.50 12.50 | 8 |

| Populations | 2n/Ploidy Level | Meiotic Configurations | Percentage | Number of cells |
|-------------|-----------------|--|---|-----------------|
| H | 30/3x | 9I + 9II + 1III 8I + 8II + 2III 7I + 10II + 1III | 50 25 25 | 4 |
| I | 40/4x | 1I + 8II + 3III + 4IV (42) 2I + 12II + 2III + 2IV 1I + 14II + 1III + 2IV 12II + 4IV 2I + 19II 10II + 5IV | 16.66 16.66 16.66 16.66 16.66 16.66 | 6 |
| J | 40/4x | 17II + 2III 15II + 3IV (42) 2I + 15II + 2III | 33.33 33.33 33.33 | 3 |
| K | 40/4x | 18II + 1IV 11II + 2III + 3IV 20 II | 60 20 20 | 5 |
| L | 40/4x | 4I + 4III + 8II + 2IV 4II + 6III + 4IV 1I + 13II + 3III + 1IV | 33.33 33.33 33.33 | 3 |
| M | 40/4x | 2I + 11II + 4III + 1IV 18II + 1IV 2I + 14II + 2III | 33.33 33.33 33.33 | 3 |
| N | 40/4x | 18II + 1IV 7I + 10II + 3III + 1IV 3I + 12 II + 3III + 1IV 1I + 7 II + 3III + 4IV 2I + 14II + 2III + 1IV 1I + 11II + 3III + 2IV 2I + 7II + 7III + 3IV | 14.28 14.28 14.28 14.28 14.28 14.28 14.28 | 7 |
| Ñ | 40/4x | 20II 18II + 1IV 1I + 16II + 1III + 1IV 1I + 12II + 1III + 3IV 2I + 16II + 2III 14II + 3IV 16II + 2IV | 14.28 14.28 14.28 14.28 14.28 14.28 14.28 | 7 |
| O | 40/4x | 20II 2I + 17II + 1IV 2I + 19II 1I + 15II + 3III 16II + 2IV | 42.85 14.28 14.28 14.28 14.28 | 7 |
| P | 40/4x | 10I + 9II + 4III 4I + 16II + 1IV 8I + 13II + 2III | 33.33 33.33 33.33 | 3 |
| Q | 40/4x | 6I + 17II 3I + 17II + 1III 1I + 18II + 1III 16II + 2IV 5I + 16II + 1III 2I + 15II + 2IV | 28.57 14.28 14.28 14.28 14.28 14.28 | 7 |

Meiotic behavior in Populations 2x, 3x y 4x

| Populations | A | B | C | D | E | F | G | H | I | J | K | L | M | N | Ñ | O | P | Q |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Metaphase I | | | | | | | | | | | | | | | | | | |
| Regular (%) | 100 | 0 | 0 | 0 | 10.52 | 15.15 | 0 | 14 | 74.30 | 89.30 | 93.08 | 87.80 | 75.51 | 75.37 | 87.30 | 70 | 57.89 | 79.56 |
| Chrom- outside plate(%) | 0 | 100 | 100 | 100 | 89.44 | 84.85 | 100 | 86 | 25.70 | 10.67 | 6.91 | 12.19 | 24.48 | 24.62 | 12.69 | 30 | 42.10 | 20.43 |
| Number of cell analyzed | 50 | 36 | 28 | 40 | 57 | 66 | 25 | 50 | 175 | 159 | 217 | 123 | 49 | 134 | 63 | 100 | 57 | 93 |
| Anaphase I | | | | | | | | | | | | | | | | | | |
| Regular I(%) | 100 | 42.59 | 52.98 | 63.70 | 69.17 | 51.28 | 81.90 | 76.05 | 62.41 | 84.48 | 58.82 | 72.77 | 94.28 | 79.77 | 90.06 | 78.60 | 85.32 | 89.44 |
| Laggards (%) | 0 | 47.59 | 42.55 | 34.69 | 25.70 | 34.64 | 17.14 | 24.28 | 37.11 | 14.94 | 21.76 | 26.17 | 5.71 | 20.22 | 9.27 | 19.53 | 14.22 | 9.31 |
| Bridges with fragment. (%) | 0 | 1.63 | 0 | 0 | 2.80 | 7.68 | 0 | 0 | 0.23 | 0 | 4.70 | 0 | 0 | 0 | 0 | 0 | 0 | 0.62 |
| Bridges without fragment (%) | 0 | 8.19 | 3.73 | 1.61 | 2.33 | 3.84 | 0.95 | 0 | 0 | 0 | 0 | 0.52 | 0 | 0 | 0.66 | 0.46 | 0.45 | 0 |
| Bridges + laggards(%) | 0 | 75 | 134 | 124 | 386 | 78 | 0 | 0 | 0 | 0 | 5.88 | 0 | 0 | 0 | 1.39 | 0 | 0 | 0.62 |
| Laggards+bridge+ fragment(%) | 0 | 0 | 0.74 | 0 | 0 | 2.56 | 0 | 0 | 0.23 | 0.57 | 0.58 | 0.52 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of cell analyzed | 50 | 75 | 134 | 124 | 386 | 78 | 210 | 71 | 423 | 174 | 170 | 191 | 105 | 89 | 151 | 215 | 218 | 161 |
| Tetrads | | | | | | | | | | | | | | | | | | |
| Regular (%) | 100 | - | 24.27 | 43.01 | 76.30 | 46.54 | 80.26 | 91.42 | 80.61 | 74 | 87.95 | 59.83 | 93.54 | 78.63 | 94.64 | 88.93 | 81.67 | 98.25 |
| Irregular (%) | 0 | - | 75.72 | 56.98 | 23.70 | 53.46 | 19.73 | 8.57 | 19.38 | 18 | 12.04 | 40.16 | 6.45 | 21.36 | 5.35 | 11.06 | 18.32 | 1.74 |
| Number of cell analyzed | 50 | - | 103 | 93 | 249 | 318 | 451 | 175 | 227 | 511 | 498 | 244 | 279 | 117 | 616 | 262 | 562 | 343 |
| Pollen Fertility | | | | | | | | | | | | | | | | | | |
| Fertile (%) | 99.35 | 25.25 | 32.04 | 51.67 | 58.42 | 57.40 | 52.69 | 44.74 | 81.76 | 83.49 | 54.69 | 90.40 | 87.67 | 91.03 | 88.20 | 83.86 | 86.38 | 95.76 |
| Esterile (%) | 1.36 | 74.74 | 67.95 | 43.32 | 41.57 | 42.60 | 46.64 | 54.83 | 16.85 | 15.40 | 45.30 | 9.20 | 9.93 | 8.45 | 11.04 | 15.64 | 13.18 | 3.54 |
| Unreduced (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0.66 | 0.41 | 1.38 | 1.10 | 0 | 0.38 | 2.38 | 0.51 | 30 | 0.49 | 0.43 | 0.68 |
| Number of cell analyzed | 514 | 891 | 1.554 | 538 | 1.027 | 1.242 | 3.469 | 3.585 | 1.442 | 2.635 | 2.002 | 2.313 | 2.930 | 2.744 | 3.994 | 2.442 | 2.761 | 2.907 |

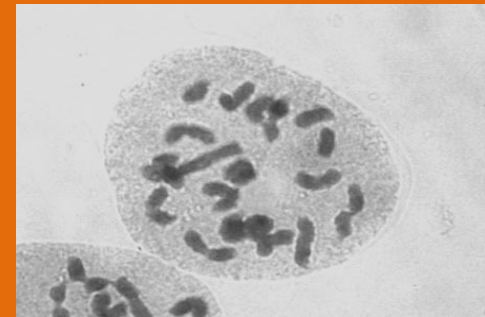
Diakinesis, Metaphase, Anaphase, Telophase and Polyads



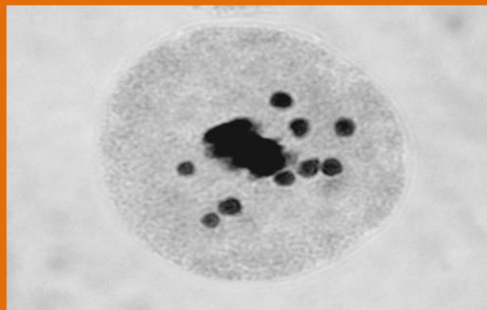
Population A: 10 II



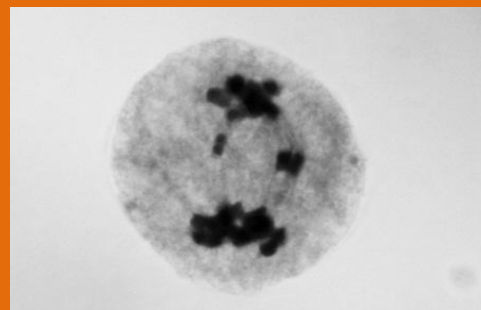
Population G: 4I+7 II+4III



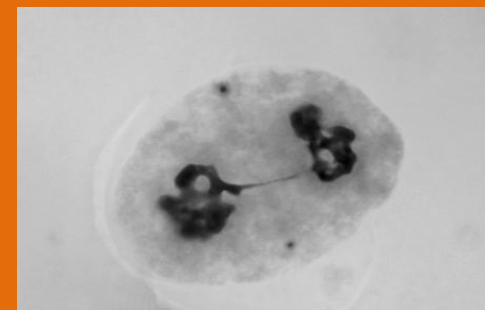
Population O: 20 II



Metaphase I with 9 off-plate chrom.



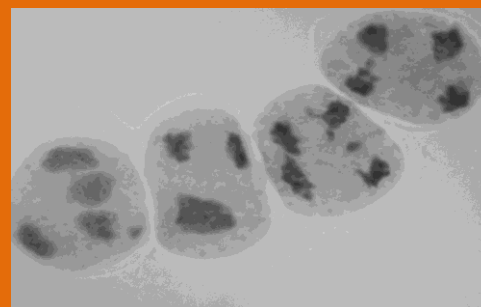
Anaphase I with 3 laggards



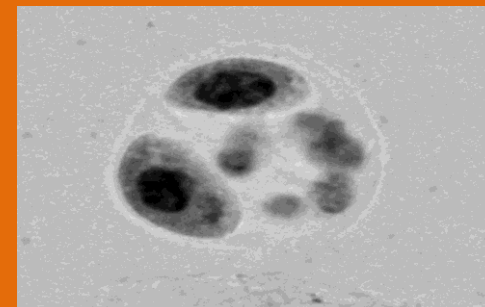
Anaphase I with bridge



Telophase II formation of nuclei of different sizes

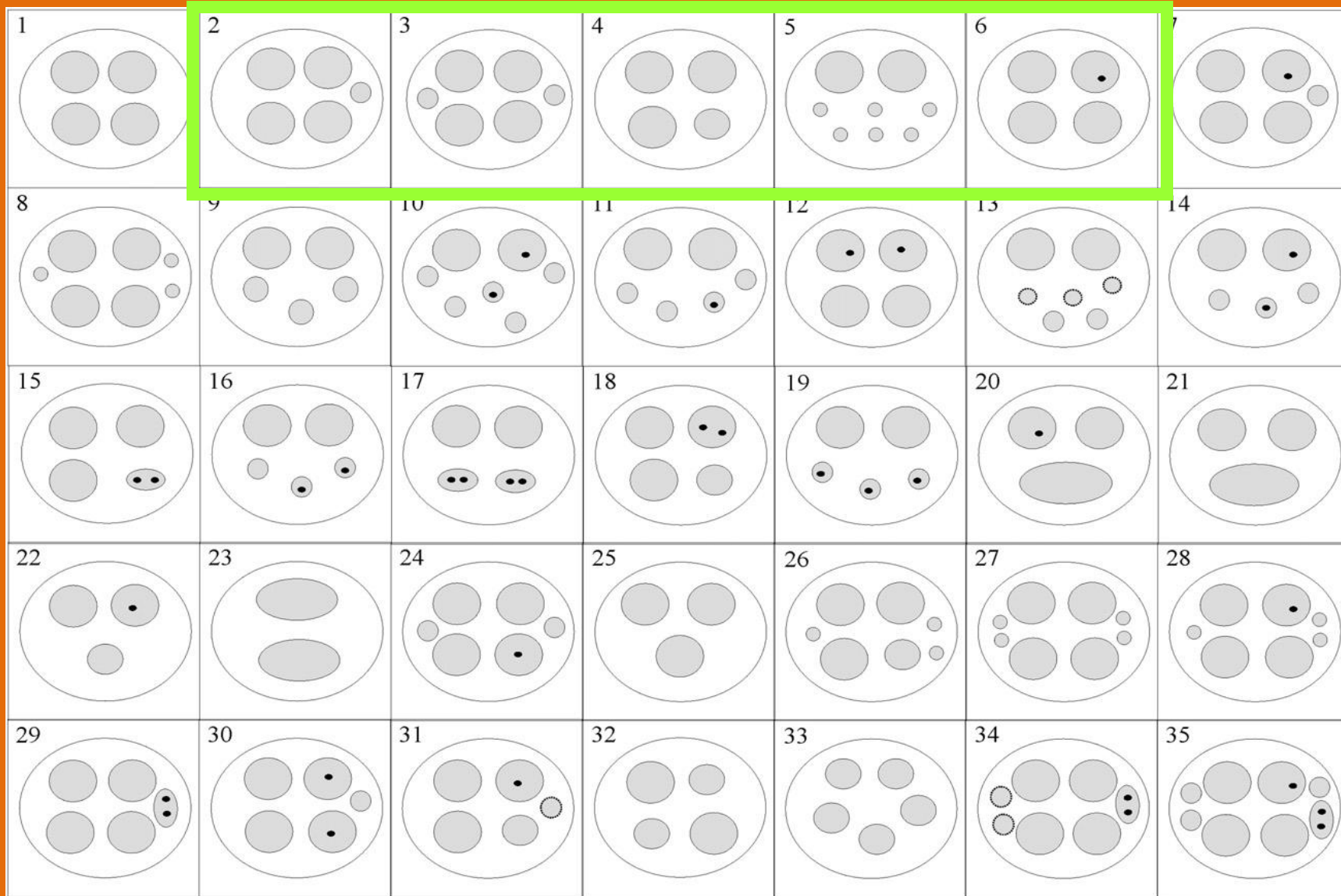


Telophase II with formación de micronuclei



Polyad

Microspore types present in the triploid populations of *C. macrocephalum*

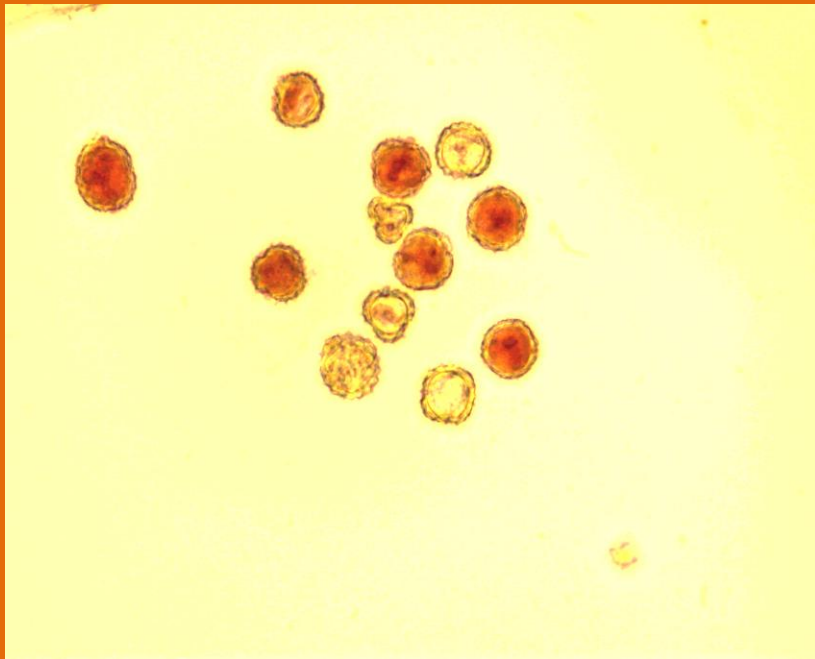


Different types and percentages of **meiotic** products in four triploid populations

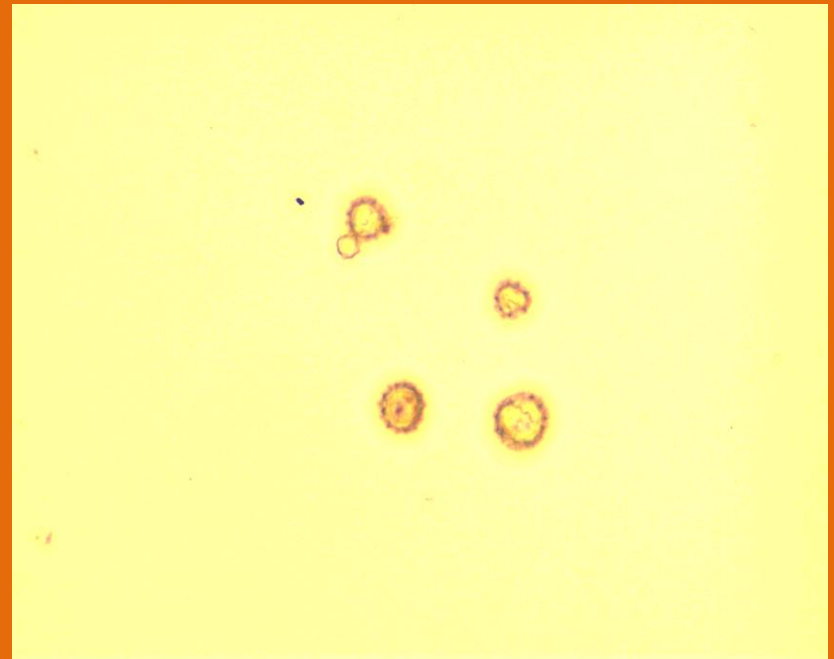
| Types | Description | B | C | D | E |
|----------------|---|-------|-------|------|-------|
| 1 | Normal | 25.00 | 43.01 | 76.3 | 46.54 |
| 2 | 1 micro-microspore | 35.00 | 23.65 | 9.63 | 23.58 |
| 3 | 2 micro-microspores | 11.00 | 3.22 | 0.40 | 7.23 |
| 4 | 3 normal microspores y 1 wich core reduced | 0.00 | 3.22 | 0.00 | 0.00 |
| 5 | 3 microspores normals y 1 wich core reduced | 0.00 | 1.07 | 0.00 | 0.00 |
| 6 | 1 micronucleus | 11.00 | 10.75 | 8.43 | 9.43 |
| 7 | 3 normal microspores. 1 microspore with one micronucleus and 1 micro-microspore | 5.00 | 4.3 | 0.00 | 4.71 |
| 8 | 3 micro-microspores | 2.00 | 2.15 | 0.00 | 0.00 |
| 9 | 2 normal microspores and 3 micro-microspores | 0.00 | 1.07 | 0.00 | 0.31 |
| 10 | 1 normal. 1 microspore with a micronucleus | 0.00 | 1.07 | 0.00 | 0.00 |
| 11 | 2 normal microsperes. 3 normal microspores and 1 micro-microspore with micronucleus | 0.00 | 1.07 | 0.00 | 0.00 |
| 12 | 2 microspores with 1 each | 3.00 | 3.22 | 0.00 | 1.57 |
| 13 | 2 normal microspores. 2 micro-microspores and 3 microspores without cytoplasm | 0.00 | 1.07 | 0.00 | 0.00 |
| 14 | 1 normal microspore. 1 microspore with a micronucleus. 2 normal micro-microspores and 1 micro-microspores with one micronucleus | 0.00 | 1.07 | 0.00 | 0.00 |
| 15 | 1 micro-microspore with 2 micronucleus | 3.00 | 0.00 | 0.00 | 0.00 |
| 16 | 1 micro-microspore and 2 microspores with 1 micronucleus each | 2.00 | 0.00 | 0.00 | 0.00 |
| 17 | 2 micro-microspores with 2 micronucleus | 1.00 | 0.00 | 0.00 | 0.00 |
| 18 | Without micro-microspores and 1 microspore with 2 micronucleus | 1.00 | 0.00 | 0.00 | 0.62 |
| 19 | Without micro-microspores and 3 microspores with 1 micronucleus each | 1.00 | 0.00 | 0.00 | 0.00 |
| 20 | 2 normal microspores. 1 microspore with 1micronucleus and large size microspore | 0.00 | 0.00 | 0.88 | 0.00 |
| 21 | 2 normal microspores. 1 large size microspore | 0.00 | 0.00 | 1.60 | 0.00 |
| 22 | 2 large size microspores. 1 with micronucleus and 1 small microspore | 0.00 | 0.00 | 0.40 | 0.00 |
| 23 | Dyad | 0.00 | 0.00 | 0.40 | 0.00 |
| 24 | 4 normal microspores. 1 withmicronucleus and 2 micro-microspores | 0.00 | 0.00 | 0.40 | 0.31 |
| 25 | Triad | 0.00 | 0.00 | 1.20 | 0.00 |
| 26 | 3 normal microspores. 1 small microspore and 3 micro-microspores | 0.00 | 0.00 | 0.00 | 1.88 |
| 27 | 4 normal microspores and 4 micro-microspores | 0.00 | 0.00 | 0.00 | 0.62 |
| 28 | 3 normal microspores. 1 microspore with one micronucleus and 3 micro-microspores | 0.00 | 0.00 | 0.00 | 0.31 |
| 30 | 2 normal microspores. 2 microspores with a micronucleus each and 1 micro-microspore | 0.00 | 0.00 | 0.00 | 0.31 |
| 31 | 3 normal microspores. 1 small microspore and 1 nucleus without cytoplasm | 0.00 | 0.00 | 0.00 | 0.62 |
| 32 | 2 normal microspores and 2 small microspores | 0.00 | 0.00 | 0.00 | 0.31 |
| 33 | 5 medium size microspores | 0.00 | 0.00 | 0.00 | 0.31 |
| 34 | 4 normal microspores. 1 micro-microspore with two nucleus and 2 micro-microspores | 0.00 | 0.00 | 0.00 | 0.31 |
| 35 | 4 medium size microspores. 1 micro-microspore with two nucleus and 2 micro-microspores with cytoplasm | 0.00 | 0.00 | 0.00 | 0.31 |
| Total of cells | | 100 | 93 | 249 | 318 |

Fertility of populations poliploid

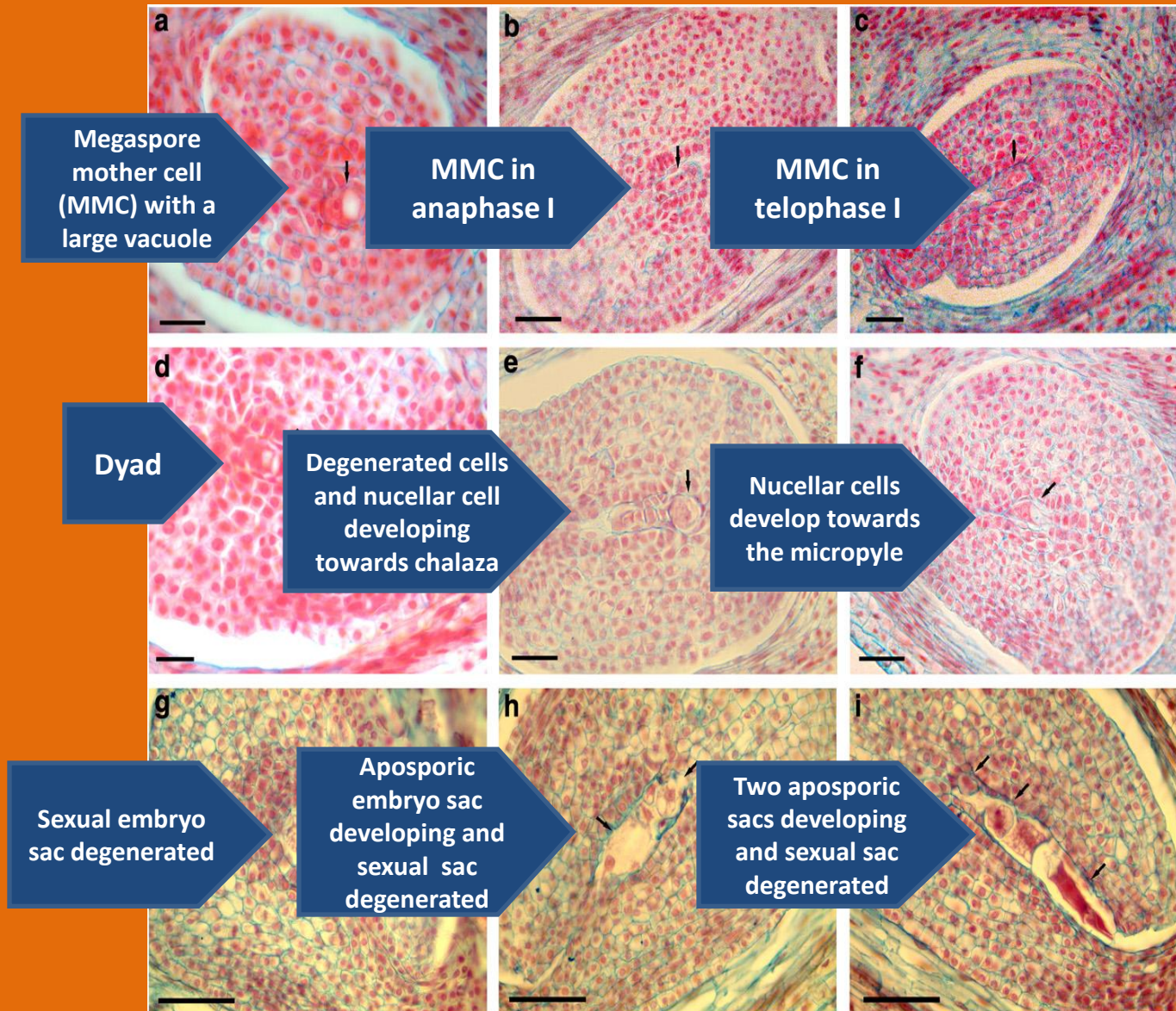
Grains pollen viable and non-viable



Grains pollen non-viable



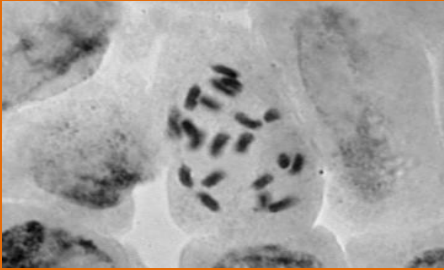
Megasporogenesis and megagametogenesis in a triploid population of *C. macrocephalum*.



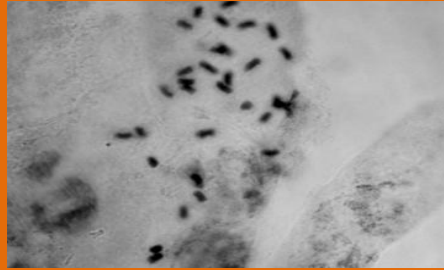
Chromosome count in three populations

| Population | Ploidy | Individuals | B chromosomes or Supernumerary | Total cell | Total individuals | |
|------------------|--------|-------------|--------------------------------|------------|-------------------|------------|
| 3739 | 2x | A | 0 | 1 | 1 (14.28%) | |
| | 3x | B | 1 | 1 | 1 (14.28%) | |
| | 4x | C | 1 | 1 | 1 | 5 (71.42%) |
| | | D | 1 | 1 | 1 | |
| | | E | 0 | 1 | 1 | |
| F | | 0 | 2 | 2 | | |
| G | 0 | 3 | 3 | | | |
| Total | | | | | 7 (100%) | |
| 3722 | 2x | D | 0 | 5 | 1(20%) | |
| | | | 1 | 1 | | |
| | 4x | E | 0 | 1 | 4(80%) | |
| | | | F | 0 | | 1 |
| | | | G | 0 | | 3 |
| H | 0 | 3 | | | | |
| Total | | | | | 5(100%) | |
| San Roque | 3x | I | 1 | 9 | 6 (75%) | |
| | | | 2 | 1 | | |
| | | J | 0 | 2 | | |
| | | | 1 | 8 | | |
| | | | 3 | 1 | | |
| | | K | 0 | 2 | | |
| | 1 | | 1 | | | |
| | L | 1 | 1 | | | |
| | M | 1 | 3 | | | |
| | N | 0 | 1 | | | |
| 1 | | 4 | | | | |
| 4x | Ñ | 0 | 2 | 1 (12.50%) | | |
| 5x | O | 2 | 1 | 1 (12.50%) | | |
| Total | | | | | 8 (100%) | |

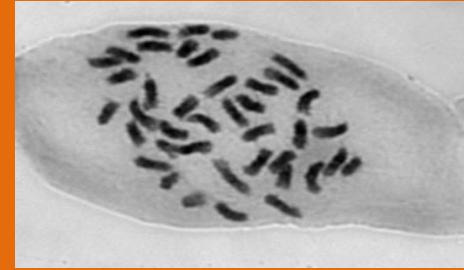
Populations with different chromosome numbers



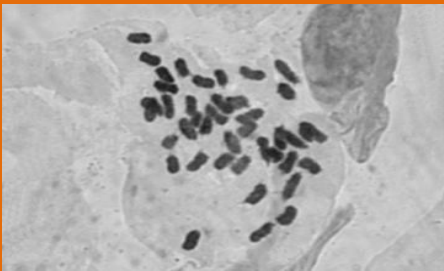
3739: 20 chromosomes (A)



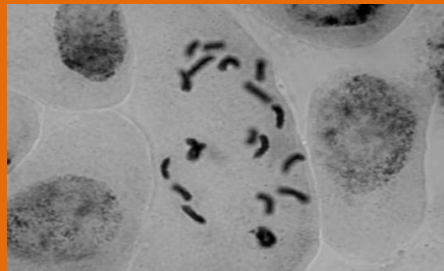
3739: 31 chromosomes (B)



3739: 40 chromosomes (E)



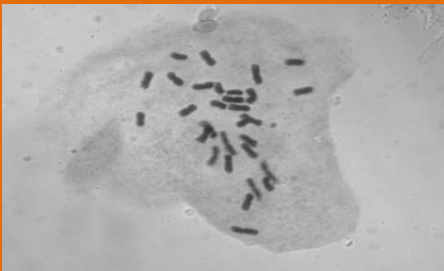
3739: 41 chromosomes (C)



3722: 20 chromosomes (D)



3722: 40 chromosomes (E)



S. Roque: 30 chromosomes (k)



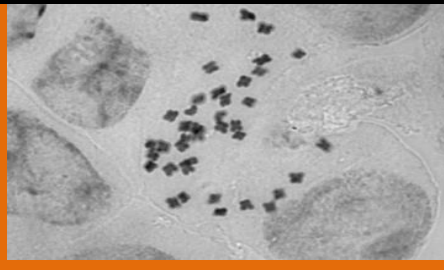
S. Roque: 31 chromosomes (J)



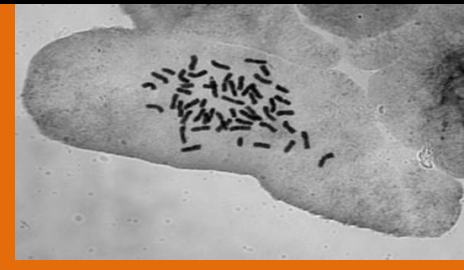
S. Roque: 32 chromosomes (I)



S. Roque: 33 chromosomes (J)



S. Roque: 40 chromosomes (Ñ)



S. Roque: 52 chromosomes (O)

karyotype of cytotypes 2x y 4x of population 3722

| 3722 2x | | | | | | | | | | | |
|-------------------------|------|------|------|------|------|------|-------|------|------------|------|---------------------|
| Pair | SA | | LA | | TL | | CI | | Morphology | RA | LR (%) |
| 1 | 2.03 | 0.31 | 2.60 | 0.17 | 4.64 | 0.45 | 43.80 | 3.41 | m | 0.77 | 10.99 |
| 2 | 2.07 | 0.23 | 2.56 | 0.18 | 4.63 | 0.39 | 44.70 | 1.81 | m sat | 0.80 | 10.96 |
| 3 | 2.16 | 0.15 | 2.46 | 0.20 | 4.63 | 0.35 | 46.82 | 0.52 | m | 0.88 | 10.96 |
| 4 | 1.92 | 0.15 | 2.51 | 0.27 | 4.43 | 0.32 | 43.37 | 3.29 | m | 0.76 | 10.50 |
| 5 | 1.81 | 0.11 | 2.32 | 0.18 | 4.14 | 0.26 | 43.77 | 1.96 | m | 0.77 | 9.80 |
| 6 | 1.54 | 0.19 | 2.10 | 0.10 | 3.65 | 0.28 | 42.27 | 2.05 | m | 0.73 | 8.64 |
| 7 | 1.37 | 0.14 | 2.07 | 0.18 | 3.44 | 0.30 | 39.86 | 1.56 | m | 0.66 | 8.15 |
| 8 | 1.80 | 0.14 | 3.09 | 0.16 | 4.90 | 0.29 | 36.79 | 0.97 | sm | 0.58 | 11.6 |
| 9 | 1.57 | 0.13 | 2.92 | 0.22 | 4.50 | 0.36 | 35.01 | 0.55 | sm sat | 0.53 | 10.66 |
| 10 | 1.20 | 0.14 | 2.04 | 0.24 | 3.25 | 0.34 | 37.09 | 2.80 | sm | 0.58 | 7.70 |
| Total karyotype length: | | | | | | | | | | | 42.23 μm |

| 3722 4x | | | | | | | | | | | |
|----------------------------|------|------|------|------|------|------|-------|------|------------|------|---------------------|
| Pair | SA | | LA | | TL | | CI | | Morphology | RA | LR (%) |
| 1 | 2.05 | 0.12 | 2.55 | 0.16 | 4.61 | 0.55 | 44.61 | 0.46 | m | 0.77 | 12.25 |
| 2 | 1.86 | 0.05 | 2.45 | 0.10 | 4.31 | 0.14 | 43.25 | 1.77 | m sat | 0.80 | 12.22 |
| 3 | 2.02 | 0.13 | 2.16 | 0.16 | 4.18 | 0.34 | 48.29 | 1.17 | m | 0.88 | 12.22 |
| 4 | 1.83 | 0.12 | 2.18 | 0.19 | 4.02 | 0.26 | 45.68 | 2.11 | m | 0.76 | 11.7 |
| 5 | 1.67 | 0.06 | 2.11 | 0.14 | 3.79 | 0.19 | 44.10 | 1.12 | m | 0.77 | 10.92 |
| 6 | 1.48 | 0.12 | 1.83 | 0.08 | 3.31 | 0.20 | 44.67 | 1.04 | m | 0.73 | 9.63 |
| 7 | 1.27 | 0.05 | 2.00 | 0.18 | 3.28 | 0.16 | 38.93 | 2.73 | m | 0.66 | 9.08 |
| 8 | 1.02 | 0.09 | 1.78 | 0.15 | 2.81 | 0.24 | 36.37 | 0.35 | sm | 0.58 | 12.93 |
| 9 | 1.61 | 0.27 | 2.84 | 0.18 | 4.45 | 0.22 | 36.25 | 2.20 | sm sat | 0.53 | 11.89 |
| 10 | 1.10 | 0.15 | 1.97 | 0.10 | 3.08 | 0.18 | 35.91 | 2.37 | sm | 0.58 | 8.58 |
| Lenght Total of cariotype: | | | | | | | | | | | 37.89 μm |

Idiogram of cytotype 2x of population 3722



A1: 0.92

Chromosome B or Supernumerary:

2x → 5.29 μm (3.52-6.27 μm)
4x → 6.47 μm (2.35-5.78 μm)
3.42 μm (3.52-7.35 μm)

Conclusions

The different configurations observed at diakinesis in all populations analyzed suggest that the species is autopolyploid.

Meiosis in diploid populations are very stable with respect to the polyploid, where resulted in a high frequency of irregular meiotic products such as dyads, triads and tetrads having a variable number of microcytes, occasionally with micronuclei resulting in unbalanced pollen grains , non-viable and of different sizes.

The presence of a xylopodium, combined with aposporous apomixis, are two important factors that contribute to the invasiveness of *C. macrocephalum*.

The mitotic analysis of populations showed the existence of different cytotypes within a population (Mixed populations). Besides of found individuals with different numbers of B or supernumerary chromosomes.

Thank you!!!