Nuclear DNA content and chiasma behaviour in six species of Gymnocalycium Pfeiff. of the family Cactaceae

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SUMMARY - Meiotic studies including chiasma frequency determination of Pollen Mother Cells (PMCS) and 4C DNA content from root tip cells of 6 species of Gymnocalycium of the family Cactaceae revealed significant interspecific variation in the genome. The haploid chromosome number $n = 11$ was recorded in G. bruchii, G. denudatum, G. borridispinum, G. mihanovicbii, G. quehlumum and G. saglionis. The chiasma frequency significantly varied from 30.45 to 34.28 per nucleus. The formation of univalent in some of the cells, spindle anomalies i.e. late or early separation leads to the formation of pentads, sexads or octads instead of tetrads formation in the meiotic telophase II through differential pollen sterility from 2.83 to 7.31% in G. mihanovicbii and G. saglionis respectively. The 4C DNA amount of root tip cells varied significantly from 12.021 to 14.661 pg in G. saglionis and G. bruchii respectively. Significant variation in DNA amount with gross or minor alteration of chiasma frequency leads to genetic drift among the species.

Key words: chiasma, cytophotometry, haploid chromosome number, 4C DNA, meiosis.

INTRODUCTION

Gymnocalycium was one of the first globular cactus genera to be established in 1845 with an approximate 90 species occurred in Bolivia, Paraguay, Uruguay and far south into Brazil towards Argentina (CULLMANN et al. 1986). The Gymnocalyciums are among the most rewarding of cacti; with their beautiful flowers and their defiant wild spine formations they are very popular among cactus growers. Most species are self-sterile, but develop fruit and seed reliably and rapidly after artificial fertilization with the pollen, of another plant (HEYWOOD 1985). The genus is easily recognized by its usual sealy pink, white or yellow flowers, whose ovary and tube bear very large, blunt, membranous scales with completely loare axils. The body is generally squat globular to compressed disc-shaped, and almost always has round ribs, virtually all species feature lateral furrows between the areoles. The chromo
some analysis reported earlier deals with mainly of mitotic studies in different species of Gymnocalycium that showed $2n = 22$ in *G. anisitsii*, *G. gibbosum* and *G. platense* (Katagiri 1953). *In situ* nuclear DNA content was estimated in a number of taxa at intergeneric, interspecific and intraspecific levels (Nagato et al. 1981; Dhillon and Mikesch 1982; Watson 1987; Das and Das 1994; Das et al. 1995). Interspecific nuclear DNA content depends largely on repetitive and nonrepetitive sequences of the genome (Bennet et al. 1977; Ress and Dale 1974; Ress and Narayan 1977). Various facets of researches on chromosome and DNA is gaining importance. To ascertain the diversity in DNA and meiotic chromosomes among the species of Gymnocalycium, understanding of the interspecific variations, if any, is necessary. The meiotic behaviour and chiasma frequency are the major basic information in the conventional breeding programme for creation of new hybrids of horticultural interest. Interspecific chromosome pairing behaviour, genomic compatibility and nuclear DNA content has not been studied in the genus Gymnocalycium earlier. The present study principally deals with the 4C DNA estimation in relation to genomic behaviour in 6 species of Gymnocalycium.

**Materials and Methods**


For Feulgen cytophotometric estimation of 4C DNA, ten fixed root tips of each species were hydrolysed in 1N HCl for 12 min at 60°C, washed in distilled water and stained in Schiff's reagent for 2 h at 140°C; each root tip squash was prepared in 45% acetic acid. Ten scorings were made from each slide and 4C DNA content was estimated in metaphase chromosomes using Nikon Optiphot microscope with microspectrophotometer following the method of Sharma and Sharma (1980) and applying monochromatic light at 550 nm. *In situ* DNA values obtained on the basis of optical density were converted to picograms (pg) using Van't Hof's (1965) 4C nuclear DNA values 67.1 pg for *Allium cepa* as standard. To find out the significant differences of 4C DNA content among 6 species of Gymnocalycium, if any, Analysis of variance (ANOVA) test was performed (Sokal and Rohlf 1973).

Flower buds were fixed overnight in 1:3 propionic acid: ethanol at room temperature and kept in 70% ethanol for meiotic studies. Random scorings of chiasma frequencies were done in at least very clear five Pollen Mother Cells (PMCs) showings well spread bivalents at diakinesis of meiotic prophase I stage. For staining, 2% acetocarmine was used for meiotic chromosome study. For the statistical analysis of the variance usual t-tests were followed.
OBSERVATIONS

Meiotic studies.

The scoring and statistical analysis of chiasma frequency of different species of *Gymnocalycium* showed significant interspecific variation. The haploid chromosome number $n = 11$ was found in all the 6 species studied (Figs.1-6). Cell division was synchronous in all the species (Fig.7). The mean chiasma per cell varied significantly from 30.45 in *G. saglionis* to 34.28 in *G. bruchii* (Table 1). The highest chiasmata per bivalent (3.116) was also observed in *G. bruchii*. The formation of univalents, late or early separation of the bivalents were noted in *G. saglionis* and *G. quehlianum* and about 2-7% of pollen sterility was recorded (Figs. 89). The lowest pollen sterility was noted in *G. mihanovichii* (2.83%) and the highest pollen sterility was noted in *G. saglionis* (7.3 1 %). The mean chiasma per cell showed characteristics chiasma number in all the species.

Nuclear DNA amount.

Nuclear DNA content in the root-tip cells of the six species of *Gymnocalycium* showed significant differences that varied from 12.021 pg in *G. saglionis* to 14.661 pg in *G. bruchii*. The average 4C DNA content per chromosome varied among the species. The correlation values between the mean chiasma per bivalent and mean DNA content per chromosome were highly significant. The nuclear DNA content differed significantly (Tables 1 and 2) among the studied species. The 4C DNA amount was found to be directly correlated with chiasma frequency.

DISCUSSION

A careful investigation on chiasma behaviour at diakinesis and metaphase I in 6*Gymnocalycium* species confirmed presence of high number of rod type bivalents in the pollen mother cells (PMCs) of all the species. The lowest chiasma per bivalent in *G. saglionis* (2.768) as compared to other species suggest a consequent increase of heterochromatin region leading to chiasma terminalization (TORREZAN and PAGLIAIUNI 1995). Spindle abnormality was found in *G. saglionis* and *G. quehlianum* which was evident by the formation of univalents, late separation in the PMCs. The pollen sterility was also higher in these two species (67%). Moderate rate of pollen sterility was noted in rest of the species studied (25%). The species of five to eight microspores during microsporogenesis in telophase II, evidently, showed high percentage of sterility. All these facts suggest the genetic control of chiasma frequency (GALE and
TABLE 1 - Meiotic chromosome number, chiasma frequency, pollen sterility and 4C DNA content in 6 species of *Gymnocalycium*.

<table>
<thead>
<tr>
<th>Haploid Species</th>
<th>Mean number of chromosome number (n)</th>
<th>Mean number of chiasma per cell ± SE</th>
<th>Mean number of chiasma per bivalent ± SE</th>
<th>Pollen sterility in percentage ± SE</th>
<th>4C DNA in pg ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. bruchii</em></td>
<td>11</td>
<td>34.28 ± 0.66</td>
<td>3.116 ± 0.05</td>
<td>5.14 ± 0.53</td>
<td>14.661 ± 0.13</td>
</tr>
<tr>
<td><em>G. denudatum</em></td>
<td>11</td>
<td>32.98 ± 0.53</td>
<td>2.998 ± 0.02</td>
<td>4.23 ± 0.56</td>
<td>13.782 ± 0.09</td>
</tr>
<tr>
<td><em>G. quebianum</em></td>
<td>11</td>
<td>33.06 ± 0.44</td>
<td>3.005 ± 0.02</td>
<td>6.49 ± 0.88</td>
<td>12.920 ± 0.12</td>
</tr>
<tr>
<td><em>G. borridispinum</em></td>
<td>11</td>
<td>31.16 ± 0.35</td>
<td>2.832 ± 0.01</td>
<td>3.20 ± 1.23</td>
<td>14.134 ± 0.14</td>
</tr>
<tr>
<td><em>G. mibanovichii</em></td>
<td>11</td>
<td>31.80 ± 0.34</td>
<td>2.890 ± 0.02</td>
<td>2.83 ± 0.85</td>
<td>13.122 ± 0.15</td>
</tr>
<tr>
<td><em>G. saglionis</em></td>
<td>11</td>
<td>30.45 ± 0.52</td>
<td>2.768 ± 0.03</td>
<td>7.31 ± 1.29</td>
<td>12.021 ± 0.11</td>
</tr>
</tbody>
</table>

TABLE 2 - ANOVA of 4C DNA content, chiasma frequency and pollen sterility in 6 species of *Gymnocalycium*.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>4C DNA content.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between the species</td>
<td>5</td>
<td>141.40</td>
<td>28.28</td>
<td>23.96**</td>
</tr>
<tr>
<td>Within the species</td>
<td>54</td>
<td>64.23</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD = 0.12 in 5% level; CD 0.35 in 1% level.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chiasma frequency.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between the species</td>
<td>5</td>
<td>4.25</td>
<td>0.85</td>
<td>21.25**</td>
</tr>
<tr>
<td>Within the species</td>
<td>54</td>
<td>1.89</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD = 0.17 in 5% level; CD 0.32 in 1% level.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pollen sterility.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between the species</td>
<td>5</td>
<td>120.24</td>
<td>24.04</td>
<td>36.98**</td>
</tr>
<tr>
<td>Within the species</td>
<td>54</td>
<td>35.22</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD = 0.4% in 5% level; CD = 0.70 in 1% level.</td>
<td></td>
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</tbody>
</table>

** = Highly significant at 1% level.

Figs. 1-6. - Meiotic prophase I showing diakinesis stage in different species of *Gymnocalycium* (x 3015); Fig. 1. - *G. bruchii*; Fig. 2. - *G. denudatum*; Fig. 3. - *G. borridispinum*; Fig. 4. - *G. mibanovichii*; Fig. 5. - *G. quebianum*; Fig. 6. - *G. saglionis*.

Fig. 7. - Synchronous meiotic metaphase I showing 11 bivalents in each cells in *G. mibanovichii* (x 1224).

Fig. 8. - Early separation in *G. quebianum* (x 3015).

Fig. 9. - Abnormal microspore formation in *G. saglionis* (x 1224).
However, the formation of chiasma is controlled polygenetically by major genes which operate on a hierarchial system (PARKAR 1975). The distribution of histogram of the mean chiasma frequency per nucleus and nuclear DNA value of each species showed species specific characteristics. 4C DNA content of the somatic cells showed significant interspecific variation. The highest mean 4C DNA per nucleus was 14.661 pg in G. bruchii whereas, 12.021 pg, the lowest DNA amount was found in G. saglionis. The high pollen sterility in the species might be due to heterogenous pairing during bivalent formation in the process of spontaneous mutation irrespective of genome size (DAS and MALLICK 1992). The wide range of microspore formation in G. queebianum with a constant nuclear DNA value around the mean of somatic cells are due to the elimination of abnormal nonfunctional pollen grains during microsporogenesis (HE et al. 1996).

Critical investigations of the 4C DNA amount showed significant variations between the different species of Gymnocalycium (Tables 1 and 2). The maximum 14.661 pg 4C DNA content was noted in G. bruchii and the minimum 12.021 pg in G. saglionis. The average DNA amount per chromosome also varied markedly. The chiasma frequency, however, showed a high correlation with 4C DNA amount (0.623). The higher amount of DNA in G. bruchii might be due to high repetitive DNA sequences in the genome rather than AT or GC-rich sequences of the gene (MARTÉL et al. 1997). DNA values and chiasma frequency in these species of Gymnocalycium are reported for the first time, although such interspecific variations were noticed in several other species (DAS and MALLICK 1989a, b 1991; DAS 1991; DAS and DAS 1994; DAS et al 1995; BRAUTIGAM and BRAUTIGAM 1996; ROSER et al. 1997; BUITENDIJK et al. 1997). The variability of the stable DNA amount might be attributed to the loss or addition of many repeats in the micro-and macro-environment during evolution of new species (PRICE 1976).

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