4C DNA Variations and Karyotype Diversity in Nine Species of Ferocactus B. & R.

A. B. Das, S. Mohanty and P. Das

Cytogenetics Laboratory, Regional Plant Resource Centre, Bhubaneswar 751 015, Orissa, India

Accepted October 5, 1998

Summary  Cytophotometric estimation of 4C DNA content, karyotype analysis and Interphase Nuclear Volume (INV) were carried out in nine species of Ferocactus of the family Cactaceae. Significant interspecific variations in nuclear DNA amount were noted with a constant somatic chromosome number (2n=22). The 4C DNA content varied from 7.66 pg in E emoryi to 9.74 pg in E gracilis. The INV varied from 390.36 pm3 in E pilosus to 564.32 pm3 in E gracilis. The average chromosome length and volume varied from 2.66 pm in E emoryi to 2.96 pm in E gracilis and 1.92 pm3 in E histrix and 2.77 pm3 in E gracilis. The mean 4C DNA content showed a significant positive correlation with chromosome length, volume and INV. The structural alterations in the chromosomes as well as loss or addition of highly repetitive sequences in the genome suggest variations in the nuclear DNA at interspecific level during macro- and micro-evolution of the species.

Ferocactus of the family Cactaceae is an important genus of horticultural interest. Mostly the plants are perennial, ferociously spined with heavy dark red spines and are commonly known as Barrel Cacti. They grow to 60 cm high in the full sun, both on the rocks and in the grassy pastures (Heywood 1985). Chromosome analysis by earlier worker showed n=11 bivalents (Remski 1954), but there are no report on cytophotometric estimation of nuclear DNA content in Ferocactus. In order to ascertain precisely the importance of DNA in genetic diversity and phylogeny, an understanding of the genetic behaviour at specific level, is necessary. The present study principally deals with somatic chromosome number, karyotype and DNA content in nine species of Ferocactus to find out the extent to which the values have a selective advantage in development of new species.

Material and methods

Healthy and young root tips (2–3 mm) of nine species of Ferocactus (Table 1) were sampled from the living Cactus Collection of the Regional Plant Resource Centre, Bhubaneswar, Orissa. Voucher specimens of each sample were deposited in both the Living Collection Division and the Herbarium at the Centre. Root tips were pretreated in solution of para-dichlorobenzene : aesculine (1 : 1) for 3.5 hr, washed thoroughly and kept at 14°C followed by overnight fixation in 1 : 3 propionic acid : ethanol. Chromosomes were stained using 2% lacto-propionic orcein after cold hydrolysis in 5N HCl for 7 min. Root-tips were then squashed in 45% propionic acid. Ten well scattered metaphates were selected for karyotype analysis of each species, and the genomic chromosome length and volume of a karyotype determined. Form percentage (F%) of individual chromosomes were calculated and total form percentage (TF%) was calculated (Das and Mallick 1993b).

For scoring of INV, 20 root-tips of about 2.5 mm length from each species were fixed in 1 : 3 acetic acid : ethanol for 24 hr at 25°C, and cold hydrolysed in 5N HCl at 20°C for 60 mm. After thorough washing, the root-tips were then put into Schiff’s reagent for 1 hr at 20°C and kept in the dark for staining. Squash preparation was done in 45% acetic acid. Ten randomly selected nuclei were scored from each root-tip. Under oil immersion the mean of two diameters of a nuclei from somatic cells, obtained by measuring at right angles to each other; was used to calculate the volume
Table 1. Somatic chromosome number, chromosome length, chromosome volume, karyotype formula and 4C DNA content in nine species of *Ferocactus*

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>NSC</th>
<th>Karyotype formula</th>
<th>TCL</th>
<th>TCV</th>
<th>4C DNA</th>
<th>INV</th>
<th>TF%</th>
<th>ACL</th>
<th>ACV</th>
<th>ADNA</th>
<th>AINV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. echidne</em></td>
<td>22</td>
<td>2</td>
<td>2B+4C+12D+4E</td>
<td>56.74±0.21</td>
<td>52.30±0.47</td>
<td>8.23±0.002</td>
<td>410.58±1.45</td>
<td>40.29±0.68</td>
<td>2.58</td>
<td>2.37</td>
<td>0.37</td>
<td>18.66</td>
</tr>
<tr>
<td><em>F. emoryi</em></td>
<td>22</td>
<td>4</td>
<td>2A+2B+10D+6E</td>
<td>58.65±0.28</td>
<td>48.22±0.39</td>
<td>7.66±0.006</td>
<td>425.40±1.24</td>
<td>43.16±0.48</td>
<td>2.66</td>
<td>2.19</td>
<td>0.34</td>
<td>19.33</td>
</tr>
<tr>
<td><em>F. fordii</em></td>
<td>22</td>
<td>6</td>
<td>4A+2C+14D+2E</td>
<td>60.21±0.35</td>
<td>53.86±0.46</td>
<td>8.44±0.004</td>
<td>480.00±3.50</td>
<td>39.39±0.45</td>
<td>2.73</td>
<td>2.44</td>
<td>0.38</td>
<td>21.81</td>
</tr>
<tr>
<td><em>F. glaucescens</em></td>
<td>22</td>
<td>4</td>
<td>2A+2B+14D+4E</td>
<td>62.87±0.23</td>
<td>56.37±0.38</td>
<td>8.97±0.005</td>
<td>540.22±1.98</td>
<td>38.65±0.56</td>
<td>2.85</td>
<td>2.56</td>
<td>0.41</td>
<td>24.55</td>
</tr>
<tr>
<td><em>F. grucilos</em></td>
<td>22</td>
<td>4</td>
<td>2A+2C+14D+4E</td>
<td>65.22±0.27</td>
<td>61.02±0.24</td>
<td>9.74±0.004</td>
<td>564.32±2.35</td>
<td>42.69±0.35</td>
<td>2.96</td>
<td>2.77</td>
<td>0.44</td>
<td>25.65</td>
</tr>
<tr>
<td><em>F. histrix</em></td>
<td>22</td>
<td>2</td>
<td>2B+14D+6E</td>
<td>54.23±0.37</td>
<td>42.41±0.37</td>
<td>6.68±0.007</td>
<td>390.75±1.67</td>
<td>41.12±0.47</td>
<td>2.46</td>
<td>1.92</td>
<td>0.30</td>
<td>17.76</td>
</tr>
<tr>
<td><em>F. macrodiscus</em></td>
<td>22</td>
<td>4</td>
<td>2A+2C+12D+6E</td>
<td>59.74±1.25</td>
<td>52.13±0.68</td>
<td>8.28±0.002</td>
<td>450.23±1.30</td>
<td>40.47±0.42</td>
<td>2.71</td>
<td>2.36</td>
<td>0.38</td>
<td>20.46</td>
</tr>
<tr>
<td><em>F. potsii var. alamosanus</em></td>
<td>22</td>
<td>6</td>
<td>2A+2B+2C+14D+2E</td>
<td>63.28±0.27</td>
<td>57.45±0.24</td>
<td>9.35±0.007</td>
<td>457.90±1.21</td>
<td>35.23±0.35</td>
<td>2.87</td>
<td>2.61</td>
<td>0.43</td>
<td>20.81</td>
</tr>
<tr>
<td><em>F. pilosus</em></td>
<td>22</td>
<td>4</td>
<td>2B+2C+14D+4E</td>
<td>54.55±0.23</td>
<td>43.67±0.17</td>
<td>6.70±0.006</td>
<td>390.36±0.98</td>
<td>38.12±0.41</td>
<td>2.47</td>
<td>1.98</td>
<td>0.30</td>
<td>17.74</td>
</tr>
</tbody>
</table>

2n = Somatic chromosome number, NSC = Number of secondary constricted chromosomes, TCL = Total chromosome length, TCV = Total chromosome volume, INV = Interphase nuclear volume, ACL = Average chromosome length, ACV = Average chromosome volume, ADNA = Average DNA, AINV = Average interphase nuclear volume.
(Das and Mallick 1993b).

For Feulgen cytophotometric estimation of 4C DNA, ten fixed root-tips from each species were hydrolysed in 5N HCl for 60 min at 20°C, washed in distilled water and rinsed in SO₂ (Fox 1969). Root tips were stained in Schiff’s reagent for 2 hr at 14°C; each root-tip squash was prepared in 45% acetic acid. Ten scorings were made from each slide and 4C DNA was estimated from metaphase chromosomes under monochromatic light at 550 nm using a Nikon Optiphot microscope attached with micro-spectrophotometer (Sharma and Sharma 1980). In situ DNA values were obtained on the basis of optical density which were converted to picograms (pg) using Bennett and Smith’s (1990), 4C nuclear DNA value (79.46 pg) for Pisum sativum vs. Minerva Maple as a standard.

Correlation coefficients were calculated between different cytological characters to determine genomic characteristics. ANOVA were performed among the nuclear DNA values following Duncan’s multiple range test (Harter 1960).

Observations

Chromosome characteristics

Somatic chromosome number in root-tip cells of nine species of Ferocactus was 2n=22. On the basis of the size of the chromosome and the position of the constrictions, a number of chromosome types were found to be common within the species studied though there were minute differences of the karyotype. A general description of the representative types of chromosomes is given in Fig. 1.

Type A: Large to medium sized chromosome with primary and secondary constrictions at sub-median and terminal in position respectively.

Type B: Large to medium sized chromosomes with two constrictions one in the sub-median position and other in the sub-terminal position.

Type C: Small sized chromosomes having median primary constriction with satelite bodies on the long arm.

Type D: Medium to small chromosome with nearly sub-median primary constriction.

Type E: Medium sized chromosomes with sub-median primary constriction.

The karyotype formula of all the species revealed differences in the chromosome structure (Table 1, Figs. 2–10). All the types of chromosomes (Type A, B, C, D, E) were found in the karyotype of F. potsii var. alamosanus. However, Type A chromosomes were absent in three (F. echidne, F. histrix, F. piosus) out of nine species studied (F. echidne, F. emoryi, F. fordii, F. glaucescens, F. gracilis, F. histrix, F. macrodiscus, F. pilosus, F. potsii var. alamosanus). Type C chromosomes were common in all the species, except in F. emoryi and F. histrix. Numerical differences in Type D and E chromosomes were found in all species (Table 1). The total chromosome length and volume ranged from 54.23 μm and 42.41 μm³ in F. histrix to 65.22 μm and 61.02 μm³ in F. gracilis (Table 1). The total form percentage (TF%) ranged from 35.22 in F. potsii var. alamosanus to 43.16 in F. emoryi.

4C nuclear DNA amount and INV

In situ 4C DNA content varied significantly among the different species of Ferocactus from 7.66 pg in F. emoryi to 9.74 pg in F. gracilis; the average chromosome length and volume ranged from 2.46–2.96 μm and 1.92–2.77 μm³ respectively (Table 1). INV also varied with species, the minimum (390.36 μm³) was found in F. pilosus and the maximum (564.32 μm³) in F. gracilis. The frequency polygon of INV in the different species showed variations in the distribution around the mean keeping a constant sharp peak at its mean value (Fig. 11). ANOVA showed significant variations in the nuclear DNA content among the species of Ferocactus at (F=0.5%). Critical differences showed marked differences among the mean values of nuclear DNA (Table 2). The nuclear DNA
content was directly proportional to the total chromosome length, volume and INV in studies of *Ferocactus* (Table 3).

**Discussion**

*Karyotype genome length and nuclear DNA amount*

Investigation on somatic chromosome of nine *Ferocactus* species revealed that the metaphase chromosome number (2n=22) was common in all the species, but the combination of the type of chromosomes and the number of secondary constricted chromosomes varied among the species. Type A chromosomes were present in all species except *F. echidne, F. histrix* and *F. pilosus*. Only Type B chromosomes were present among the three types of secondary constricted chromosomes found, in *F. histrix*. Combination of Type A and B were noted in *F. emoryi, F. glaucescens, F. potsii var. alamosanus*. However Type A and C were common in *F. fordii, F. gracilis, F. macrodiscus*. Karyotype of *F. echidne,* and *F. pilosus* showed Type B and C chromosomes. A gradual numeric alteration of D and E Type chromosomes was evident in all the species. Significantly more number of D type chromosomes compared to Type E were found in all the species. The secondary constricted chromosomes varied from 2–6 among the species; the lowest number was obtained in *F. histrix*. Evidently, structural changes as well as changes in parts of heterochromatins might have played a vital role (Das et al. 1995, 1996, Rai et al. 1997) in inducing differences at species level.

The gradual shifting and alteration of TF% values might be due to the structural alterations in the genome. The structural alteration in the morphology as well as variation in the secondary constricted chromosomes in different species might be due to partial duplication of chromosomes or translocation between the chromosomes with or without secondary constrictions during speciation (Das 1991, Das and Mallick 1993a, b, Das and Das 1997). The total chromosome length varied from 54.23 μm in *F. histrix* to 65.22 μm in *F. gracilis*. The average nuclear DNA content significantly varied from 0.30 pg. in *F. histrix* to 0.44 pg. in *F. gracilis*. Correlation coefficient studies revealed significant relationship between nuclear DNA and chromosome length (r=0.961), volume (r=0.996) and INV (r=0.846). The differences in the chromosome volumes or length may be attributed to the differential degree of condensation and spiralization of chromosome arm.

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**Table 2. ANOVA of 4C DNA content in different species of *Ferocactus***

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between species</td>
<td>8</td>
<td>214.125</td>
<td>26.765</td>
<td>76.910**</td>
</tr>
<tr>
<td>Within species</td>
<td>81</td>
<td>28.256</td>
<td>0.348</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**=Significant at P<0.01, DF=degrees of freedom, SS=sum of squares, MS=mean squares, F=variance ratio.
<table>
<thead>
<tr>
<th>Species</th>
<th>F. echidne</th>
<th>F. emoryi</th>
<th>F. fordii</th>
<th>F. glaucescens</th>
<th>F. gracilis</th>
<th>F. histrix</th>
<th>F. macrodiscus</th>
<th>F. potsii var. alamosanus</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. emoryi</td>
<td>0.57ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. fordii</td>
<td>0.21ns</td>
<td>0.78*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. glaucescens</td>
<td>0.74*</td>
<td>1.31**</td>
<td>0.53ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. gracilis</td>
<td>1.51**</td>
<td>2.08**</td>
<td>1.30*</td>
<td>0.77*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. histrix</td>
<td>1.55**</td>
<td>0.98*</td>
<td>1.76**</td>
<td>2.26**</td>
<td>3.06**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. macrodiscus</td>
<td>0.05ns</td>
<td>0.62ns</td>
<td>0.16ns</td>
<td>0.69*</td>
<td>1.46**</td>
<td>1.60**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. potsii var. alamosanus</td>
<td>1.12*</td>
<td>1.69**</td>
<td>0.91*</td>
<td>0.38ns</td>
<td>0.39ns</td>
<td>2.68**</td>
<td>1.07*</td>
<td></td>
</tr>
<tr>
<td>F. pilosus</td>
<td>1.53**</td>
<td>0.96*</td>
<td>1.74**</td>
<td>2.27**</td>
<td>3.04**</td>
<td>0.02ns</td>
<td>1.58**</td>
<td>2.65**</td>
</tr>
</tbody>
</table>

**= Significant at 1% level, *= significant at 5% level, C.D. values at 5% level 0.621, C.D. at 1% level 1.402.
Nuclear DNA amount in relation to genomic chromosome volume

The average DNA content showed a high degree of correlation with the average chromosome length \((r=0.959)\), volume \((r=0.994)\) and average INV \((r=0.973)\). However, in eukaryotic system, chromosome volume and INV is determined not only by DNA but by the basic and non-basic proteins as well.

Diversification in DNA amount

4C DNA values were reported for the first time in these nine species of *Ferocactus*. Significant differences of 4C DNA were recorded among the species; such variations are in agreement with the findings of other workers (Price et al. 1980, Resslar et al. 1981, Das and Mallick 1989a, b, c, Mohanty et al. 1986, 1997a, b). The maximum (9.35 pg) nuclear DNA content was noted in *F. potsii* var. *alamosanus* with all types of chromosomes and the minimum (6.68 pg) in *F. histrix* without Type A, and C chromosomes. The variability of DNA amount has often been attributed to loss or addition of highly repetitive DNA sequences rather than the AT- or GC-rich sequences in a genome (Martel et al. 1997) which reached a certain level and became stabilized during micro-evolution and gradual selection. The diversity of DNA amount has often been attributed to loss or addition of highly repetitive DNA sequences in a genome which reached a certain level and got stabilised during micro-evolution and gradual selection (Price et al. 1980, Das and Das 1997, Das et al. 1995, 1996).

Acknowledgements

The authors wish to thank the Department of Forest and Environment, Government of Orissa, for providing necessary facilities.

References


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