Studies on Nuclear DNA and Meiotic Chromosomes in 8 Species on *Mammillaria*

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In situ nuclear DNA content was estimated in a number of taxa at intergeneric, interspecific and intervarietal levels (Nagato et al. 1981, Dhillon and Mikesch 1982, Sharma and Mukhopadhyay 1984, Mukherjee and Sharma 1986, 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 Narayan 1977). The genus *Mammillaria* of the family Cactaceae is widely distributed in Mexico, South of USA, Greater and Lesser Antilles and Coast of Venezuela (Cullmann et al. 1986). It has 62 species in India with varieties, cultivars and forms of horticultural importance (Das and Panda 1995, Panda and Das 1995). The *Mammillarias* are fairly small cacti, globular or elongated with small flowers arranged usually in a ring around the crown. Fruits are smooth, juicy, club shaped, with berries mostly of brilliant red colour (Heywood 1985). The chromosome analysis reported earlier deals with mainly of mitotic studies in different species of *Mammillaria* that showed 2n=22 in *M. bravoae* and *M. elongata* (Remski 1954). Various facets of researches on chromosome and DNA is gaining importance. To ascertain the diversity in DNA and meiotic chromosomes among the species of *Mammillaria*, 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 have not been studied in the genus *Mammillaria* earlier. The present study principally deals with the 4C DNA estimation in relation to genomic behaviour in 8 species of *Mammillaria*.

Materials and methods

Fresh and young flower buds of 8 species of *Mammillaria* namely *M. asteriflora* Cels, *M. bella* Backbg., *M. bravoae* Craig, *M. brevispina* Boed., *M. collinsii* (B. & R.) Orcutt, *M. elongata* DC., *M. klissingiana* Boed. and *M. matudae* H. Bravo were collected from the experimental cactus garden of Regional Plant Resource Centre, Bhubaneswar having more than 550 species of cacti.

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 hr at 14°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 8 species of *Mammillaria*, 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) showing well spread bivalents at diakinesis of meiotic...
prophase I stage. For staining, 2% aceto-carmine 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 *Mammillaria* showed significant interspecific variation. The haploid chromosome number n = 11 was found in all the 8 species studied (Figs. 1–8). Cell division was synchronous in all the species. The mean chiasma per cell varied significantly from 22.25 in *M. klissingiana* to 24.28 in *M. asteriflora* (Table 1). The highest chiasmata per bivalent (2.207) was observed in *M. asteriflora* too. The formation of univalents, late or early separation of the bivalents and presence of more than four microspores in telophase II were noted in *M. collinsii* and *M. klissingiana* and about 20–22% of pollen sterility was recorded (Figs. 9, 10). The highest pollen sterility was noted in *M. collinsii* (24.20%) and the lowest pollen sterility was noted in *M. elongata* (10.31%). The mean chiasma per cell showed characteristic chiasma number.

**Nuclear DNA amount**

Nuclear DNA content in the somatic cells of the 8 studied species showed significant differences that varied from 18.262 pg in *M. klissingiana* to 24.124 pg in *M. brevispina*. 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, 2) among the studied species which was less than 20 pg in *M. klissingiana*. The 4C DNA amount was found to be directly correlated with chiasma frequency.

**Discussion**

Investigation on chiasma behaviour at diakinesis and metaphase I in 8 *Mammillaria* 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 *M. klissingiana* (2.02) as compared to other species suggest a consequent increase of heterochromatin region leading to chiasma terminalization (Torrezan and Pagliarini 1995). Spindle abnormality was found in *M. collinsii* and *M. klissingiana* leading to the formation of univalents, late separation in the PMCs. The pollen sterility was the minimum in *M. elongata* (10.31%) and the maximum in *M. collinsii* (22.83%). Moderate rate of pollen sterility was noted in *M. klissingiana* (20.83%). The species with 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 Ress 1970, Ress and Dale 1974). However, the formation of chiasma is controlled polygenetically by major genes which operate on a hierarchical 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 24.124 pg in *M. brevispina* whereas, 18.261 pg, the lowest DNA amount was found in *M. klissingiana*. High pollen sterility in the species having comparatively large genome size might be due to heterogenous pairing during bivalent formation in the process of spontaneous mutation (Das and Mallick 1992). The wide range of microspore formation in *M. collinsii* with a constant nuclear DNA value around the mean of somatic cells are due to the elimination of abnormal microspore in form of nonfunctional spores during microsporogenesis. *Arabidopsis* mutant, however, showed variation in the DNA amount in the microspore (He et al. 1996).
Critical investigations of the 4C DNA amount showed significant variations between the different species of *Mammillaria* (Tables 1, 2). The maximum 24.124 pg 4C DNA content was noted in *M. brevispina* and the minimum 18.264 pg in *M. klissingiana*. The average DNA amount per chromosome also varied markedly. The chiasma frequency, however, showed a high correlation with 4C DNA amount (0.765). The higher amount of DNA in *M. brevispina* might be due to high repetitive DNA sequences in the genome. DNA values and chiasma frequency in these species of *Mammillaria* are reported for the first time, although such interspecific variations were noticed in several other species (Price 1976, Mukherjee and Sharma 1986, Chattopadhyay and Sharma 1990, Das and Mallick 1989a, b, 1991, Das and Das 1994, Das et al. 1995). 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).

**Summary**

Meiotic studies of Pollen Mother Cell (PMCs) and 4C DNA content from root tip cells of 8 species of *Mammillaria* of the family Cactaceae revealed significant interspecific variations in the
genome. The haploid chromosome number \( n=11 \) was recorded in *M. asteriflora*, *M. bella*, *M. bravoae*, *M. brevispina*, *M. elongata*, *M. klissingiana* and *M. matudae*. The chiasma frequency significantly varied from 22.25 to 24.28 per nucleus. The formation of univalent in some of the cells, spindle anomalies i.e. early or late separation leads to the formation of pentads, sexads or octads instead of tetrad formation in the meiotic telophase II through differential pollen sterility from 10.31 to 22.83\% in *M. elongata* and *M. collinsii* respectively. The 4C DNA amount of root tip cells varied significantly from 18.262 to 24.124 pg in *M. klissingiana* and *M. brevispina* respectively. Significant variation in DNA amount with gross or minor alteration of chiasma frequency leads to genetic drift among the species.

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References


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