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Low chromosome number angiosperms

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Abstract — Plant with very low chromosome number are of interest for the analysis of the structure of chromosome and chromatin organization. Many studies have been carried out on the evolution of the karyotype in the Angiosperms with only two chromosomes in their haploid complement. The results of these analyses in the five Angiosperms with 2n=4 have been reported with the aim to provide further insight into the origin and the organization of their chromosomes.

Key words: *Brachycome*, *Colpodium, Haplopappus*, *Ornitogalum*, plant chromosomes, *Zingeria*.

INTRODUCTION

Five angiosperms with only two chromosomes in their aploid complement (n=2), that is the lowest known chromosome number, are reported. These plants are: *Zingeria biebersteiniana* (Claus) P. Smirnov, (*Pooideae*); *Colpodium versicolor* (Stev.) Schmalh, *(Pooideae*); *Haplopappus gracilis* (Nutt.) Gray, (*Compositae*) and *Brachycome dichromosomatica* C.R. Carter, (*Compositae*) and *Ornitogalum tenuifolium* Delaroche (*Hyacinthaceae*).

Plants with very low chromosome number are of interest as simple system for the examination of the structural organization of their chromosomes. Many studies have been carried out in the seventies and eighties on the karyotype evolution of the plant species with only two chromosomes in their haploid complement. In the present review we refer the most important reports up today regarding the five known angiosperms.

Zingeria biebersteiniana - The wide range in the basic chromosome number (x range from 2 to 18) and prevalence of polyploidy and hybridization are prominent features in chromosomal evolution in *Poaceae* and somatic numbers vary between 2n=4 and 2n=263-265 (DE WET 1987) and the common basic chromosome number in the family are 7, 9, 10 and 12 and x=2 is reported only for *Colpodium* and *Zingeria*. Since *Colpodium* contains species with x=2, 4, 5, 6 and 7 and *Zingeria* contains species with x=2, 4 and 6, both species may represent aneuploid reduction derived from the pleisomorphic number x=7 that is the predominant number in these two tribes and highlight the flexibility in genomic evolution in the *Poaceae*.

BENNETT *et al.* (1986) studied the karyotype of *Z. biebersteiniana* by light and electron microscopy and determined the 4C nuclear DNA content. The two chromosomes were easyly distinguishable at mitotic metaphase in the light microscope after C-banding. Both chromosomes have similar large paracentromeric blocks of heterochromatin as previously described by SEMYONOV and SEMYONOVA (1975) and two characteristics C-bands were present. The former at the telomere of the short arm of the chromosome 1 where NOR is present too and the latter near to the short arm telomere of chromosome 2. By the electron microscope three-dimensional serial thin section reconstruction method each chromosome could be distiguished at each stage of mitosis.

The 4C nuclear DNA amount was estimated as 7.43(0.48 pg, very similar to that of *Haplopappus gracilis* (4C=8.2 pg, BENNET and SMITH 1976). Later BENNET *et al.* (1995) by fluorochromes and fluorescent in situ hybridization studied *Z. biebersteiniana* chromosomes: the fluorochromes banding provided a reliable method of chromosome identification. By two different fluorochromes the large pericentromeric region fluoresced more brightly on chromosome 2 and the size and the distribution of fluorescent segments agree with that found previously with Giemsa banding. The physical location of the 18S-26S and 5S rDNA sequences were reported after fluorescent in situ hybridization. The major site of the 18S-26S rDNA were located at the cytological secondary constric-

tion on chromosome 1 and two additional sites were also detected, the first on chromosome 2 and the second near the major site on chromosome 1. The origin of the minor sites of rDNA might be explained with a tandem fusion of several chromosomes producing two chromosome types only. The pericentric region of chromosome 1 was characterized by a large block of heterochromatin that compresed three segments.

Information is available on DNA composition of centromeric region in *Z. biebersteiniana* (BENNET *et al*. 1995; KOTSERUBA *et al*. 2001) and the sequence of the pericentromeric heterochromatin of *Z. biebersteiniana* were therefore studied by SAUNDERS and HOUBEN (2001), by DNA reassociation experiments and hydroxyapatite chromatography: a centromere-specific tandem repeat and a retrotransposon-like sequences were identified. Similar centromeric specific retro-transposon like elements were detected in the centromeres of many plant species (FRANCKI 2001 and references therein). The pericentromeric heterochromatin of *Z. biebersteiniana* was composed of members of the Zbcen1 tandem repeat family. After fluorescent in situ hybridization, intensely fluorescent signals were detected on the pericentromeric heterochromatin of both metaphase chromosome pairs. These pericentromeric specific Zbcen1 tandem arrays were intermingled with accumulated putative copia-like retrotransposon sequence Zb47A and southern bydridization of the retro-transposon-like sequences with genomic DNA of *Z. biebersteiniana* resulted in a pattern tipical of dispersely organized sequences.

Chromosome structure and chromatin organization of *Z. biebersteiniana* were studied by CREMONINI *et al*. (2003): not only nuclear DNA content after Feulgen staining was determined by microdensitometric analysis but the determination of Feulgen absorption at different thresholds of optical density allowed the quantitative estimation of condensed chromatin in interphase nucleus. Chromosome banding patterns by fluorochromes confirmed the previously observed large bands in the centromeric region of both metaphase chromosome pairs and, after the cold and 9-aminoacridine pretreatments, the metaphase chromosomes stained with acetocarmine and aceto orceine were analysed by image analysis system revealing a segmentation that resembled Giemsa/Reverse banding in animal chromosomes. By a specific monoclonal antibody the DNA methylation pattern of metaphase chromosomes were analysed. 5-methyl cytosine residues were present in several chromosome sites and differences might be present between corresponding regions of homologues.

Each chromosome of *Z. biebersteiniana* would be composed of two or more ancestral chromosomes, however the large monocentric heterochromatic block indicate that, after a fusion event, further structural changes occurred in the genome. KOTSERUBA *et al*. (2003) analysed the allotetraploid *Zingeria trichopoda* (2n=8) and its relationships with *Z. biebersteiniana*. The copy number of tandem repeat family Zbcen1 was reduced in *Z. trichopoda* and genomic in situ hybridization demonstrated that *Z. tricopoda* evolved fron an interspecific hybrid between *Z. biebersteiniana* and a second species with a similar number of chromosomes: a candidate could be *Colpodium versicolor*.

Haplopappus gracilis - The first reports on *Haplopappus gracilis* with 2n=4 which is the lower number known in higher plants are from 1957 (JACKSON 1957; 1959).

H. gracilis belongs to the *Haplopappus spinulosus* complex where *H. ravenii* Jackson and *H. wogginsii* Blake are present too. *H. gracilis* has several chromosome racse and ecotype with two metacentric chromosomes and two shorter NOR chromosomes and the Mexican race presents a centric transposition in the metacentric chromosomes (JACKSON 1973). Its various ecotypes extend from 600 m in Arizona to 2400m in New Mexico, Arizona and Colorado and no correlation between geographical position and DNA content is present (JACKSON et al. 1993).

The four chromosomes of this species can be divided in two pairs, the former shows V-shaped chromosomes and the latter J-shaped chromosomes (JACKSON 1959; 1962; TANAKA 1981). TANAKA (1967) evidenced an aceto-orcein banding pattern in *H. Gracilis* metaphase chromosomes.

By interspecific hybrid between *H. gracilis* and *H. ravenii* (2n=8), JACKSON (1962) and TANAKA (1967) showed that the two chromosomes of *H.gracilis* are a product of a fusion occurred in the four chromosomes of *H. ravenii*. Chromosome 1 (the V-shaped) possessed three heterochromatic regions due to the fusion of three chromosomes of *H. ravenii* with elimination of centromeres and loss of an euchromatic region, chromosome 2 (the satellited J-shaped) was presumed to be composed of both chromosomes 4 and an euchromatic segment translocated from chromosome 2. Sometimes light variation in the short arm of chromosome 2 are observed in strains collected in

New Mexico (JACKSON 1963) and the relationships between centromeric regions of chromosomes and nuclear envelop was analysed by TANAKA (1981).

SPARVOLI *et al.* (1966) determined the duration of the mitotic cyle and its phases in the meristematic root cells by patterns of incorporation of ^{3}H-thymidine and the duration of perinuclear DNA replication was analysed too (SPARVOLI *et al.* 1977). By ^{3}H-thymidine autoradiographic studies, the distribution of DNA replicating sites was elucidated by TANAKA (1968) attesting a complex organization of the chromosomes of this species.

Later YONEZAWA and TANAKA (1973) confirmed the results of SPARVOLI *et al.* (1965) and, moreover determined the patterns of the sinthesis of chromosomal proteins. YONEZAWA (1981a; 1981b; 1987; 1991) analysed the structural changes of chromosomes such as inversion and translocation and the correlated karyotypes. WERRY *et al.* (1977) analysed the relative position of mitotic metaphase chromosomes by direct observation of undisturbed methapases in root tip cells. Not only mitotic chromosomes were considered but also meiosis and pollen behaviour were studied in wild populations and Mexican race (JACKSON and JORDAN 1975; KARASAWA and VEDA 1983; JACKSON *et al.* 2000; 2002).

Levels of allozyme diversity and pattern of genetic structure within and among populations were assessed to estimate the average level of gene flow by FREILAY (1993) who evidenced that no apparent correlation between geographical positions and allozyme variational patterns was present. LEVI *et al.* (1983; 1984) analysed the effects of theophylline on cell cycle, elongation and some transport processes in root meristem and embryos as well as GALLI *et al.* (1979) studied the germination of seeds and the effects of abscisic acid and fusicoccin: abscisic acid inhibited the germination and this effect could be reversed by fusicoccin treatments. Later GALLI (1988) analysed the effects of fluorodeoxyuridine, an inhibitor of the DNA synthesis, on hypocotyl elongation in light and dark. The fluorodeoxyuridine seemed to be of particular importance when endomitotic processes are involved.

The technique of cell culture, protoplast and plant regeneration are of importance in plant improvement and many reports on chromosome instability has been performed just from 1960 on *H. gracilis*, because of the unsual citology, due to its low chromosome number. BLAKELY and STEWARD (1961) analysed the behaviour of cultured cells as well as MITRA and STEWARD (1961) the behaviour of the nucleus in growing cells. SING (1975) analysed the pattern of mitotic activity in suspension culture: karyotype changes with numerical and structural alteration commonly occurs in a tissue culture enviroment. Such alterations lead the the somaclonal variation in regenerated plant or may inhibit the morphogentic potential of cells. SINGH (1981) investigated the origin of aneuploid cells in tissue cultures. ASHMORE and SHAPCOTT (1989) showed the C-banded metaphase chromosomes complements of *H. gracilis* and investigated the karyotype changes in both callus and suspension cultures evidencing polyploidization. KARM *et al.* (1991) analysed plantlet populations generated in vitro from callus from immature flower heads and karyotype analysis was performed too. Later OGURA *et al.* (1999) compared the difference of changeability of two pair of homologous chromosomes and evidenced centromeric transposition in callus cells. From cell suspension culture DE LAAT and BLAAS (1984) and DE LAAT and SCHEL (1986), obtained isolated metaphase chromosomes by flow citometry, such method might be useful in genetic manipulation. Very few are the reports regarding molecular approaches on *H. gracilis*.

STAHLE *et al.* (1975) evidenced a DNA satellite with buoyant density of 1.699 g/ml and by in situ hybridization ribosomal cistrons were localized in the DNA satellite. RUFFINI CASTIGLIONE *et al.* (1998) analysed the DNA methylation pattern on metaphase chromosomes using monoclonal antibodies against 5-mC: 5-mC indirect immunolocalization was observed expecially in telomeric regions and in the secondary constriction of the nucleolar organizers.

Brachycome dichromosomatica - The *Brachycome* (synonym *Braschycome*) *lineariloba* complex occurs in a semi arid area in central south Australia. SMITH-WHITE and CARTER (1970) described in *B. lineariloba* (DC) Druce complex five different races differing in chromosme number (A, B, C, D and E) and reported that race A (epheneral daisy) with two pairs of chromosomes was distinguishable from the others. SMITH-WHITE and CARTER described inside the race A three different cytodemes: A_1, A_2 and A_3. WATANABE *et al.* (1975) described a fourth cytodeme (A_4) and the relationships among the four cytodemes due to the suppression, loss or translocation of chromosomal segments. CARTER (1978a) described the taxonomy of the *B. lineariloba* complex and two of the cytodeme, A (2n=4) and D (2n=8) have been

raised to specific rank as *B. dichromosomatica* C.R. Carter and *B. brescopis* C.R. Carter respectively. Moreover CARTER (1978b) reported the inheritance, frequency and distribution of B chromosomes.

Later, by experimental hybridization, WATANABE and SMITH-WHITE (1987) analysed phyletic and evolutionary relationships of *B. lineariloba* complex where *B. brescopis*, (2n=8), *B. lineariloba* (2n=10,12 and 16) and *B. dichromosomatica* (2n=4) were present. Species with a little number of chromosomes but clearly visible should be well suited to analyse karyotype evolution in cell colture. NAGL and PFEIFFER (1988), in a long-term suspension culture of *B. dichromosomatica* induced from a cotyledo-derived callus evidenced a stable diploid karyotype and one cell line with 2n=5 only.

ADACHI *et al*. (1997) reported chromosomal location of 45S and 5S rDNA in all the species of *B. lineariloba* complex. In *B. dichromosomatica* each of the 5S and 45S rDNA loci occurred at two sites on chromosomes even if chromosome 2 has been subjected to some changes including very small pericentric inversion. HOUBEN *et al*. (2000) isolated a repetitive sequence specific to the polymorphic chromosome segments of *B. dichromosomatica*. A single repeated unit was 92 bp long and was organised in tandem arrays at three different polymorphic segment sites on the chromosomes.

The B chromosomes or dispensable supernumerary are present in many individuals within wild population of animal and plant species and these chromosomes represent one of the many causes of numerical chromosome variation. These chromosomes are smaller than chromosomes of the usual complement (A chromosomes) and are heterochromatic and able to get genome size polymorphism within a species. They differ from the chromosomes of the usual set in pairing behaviour and are not necessary for normal growth and development (CAMACHO *et al*. 2000 and references therein). Normally only one type of B chromosomes is found in a given species but two morphological forms of B chromosomes are known in *B. dichromosomatica*. In additionto the large B chromosomes dot-like micro B chromosomes have been described (CARTER and SMITH WHITE 1972). The larger B chromosomes are somatically stable, on the contrary the smaller are somatically instable.

JOHN *et al.*(1991) reported the isolation and characterisation of sequences that included a B chromosome specific tandemly repeated DNA sequences suggesting that B chromosomes did not arise directly from one of the A complement chromosomes.

By fluorescence in situ hybridization DONALD *et al.* (1995) revealed the presence of an rRNA gene cluster on both the A and B chromosomes of *B. dichromosomatica*. An highly methylated tandem repeats sequence was localised at the centromere region of the B chromosomes (LEACH *et al.* 1995 and FRANKS *et al.* 1996).

HOUBEN *et al.* (1997) evidenced that the micro B chromosomes contained both a number of DNA sequences that were also present on the A chromosomes and a highly repeated sequence present in lower copy on the larger B chromosomes and almost absent on the A chromosomes and the hypothesis that micro B chromosomes could be derived from A chromosomes by a simple excision was excluded (HOUBEN *et al.* 2001).

DONALD *et al.* (1997) localized rRNA genes in the B chromosomes and HOUBEN *et al.* (1999) suggested a monophyletic origin of the B chromosomes by computer-aided chromosomes image analysis and fluorescence in situ hybridization of ribosomal DNA and B chromosome-specific sequence. In this contest it is also necessary to recall two recent reviews by JONES and HOUBEN (2003) and LEACH *et al*. (2004).

Ornithogalum tenuifolium - Very few are the reports in the literature regarding *Ornitogalum tenuifolium*. This plant is distributed from South Africa to tropical East Africa and it includes seven chromosome races 2n= 4, 6,8,10,12 and 16 (STEDJE 1988) with bimodal karyotype. According to the hypothesis that 2n=12 is the most primitive karyotype (STEDJE 1989) by successive unequal translocation and loss of the resulting microchromosomes it is possible to explain 2n=6. By further loss of chromosomal fragments and Robertsonian translocation it is possible to explain the lower chromosome number. The hypothesis is confirmed by means of measurements of nuclear DNA content, studies of meiosis and pollen fertility of hybrids and comparison of karyotype morphology even if some reservation were pointed out by VOSA (1993) in his studies of hybrid meiosis.

Colpodium versicolor - *Colpodium versicolor* is one of the two plants with only two chromosomes in their haplid complement detected in the tribe *Poeae* of the *Poideae* subfamily (SOKOLOVSHAYA and PROBATOVA 1975) living on the mountains of Azeirbaijan on the Russian Caucasus, the other is

Zingeria biebersteiniana (Tsvelev and Zhukova 1974). Among the five species with 2n=4 *C. versicolor* is the only perennial species suggesting its more ancient origin since angiosperm evolution is from perennial to annual forms. In literature there is only one report on *Colpodium* (Kotseruba *et al*. 2005). The authors investigated the karyotype and tested whether *C. versicolor* represents a putative donor of one of the two parental genomes of the allotetraploid grass *Zingeria trichopoda* (Boiss) P.A. Smirnov (2n=8). Nuclear DNA content, chromosomal position of 5S and 45S rDNAs were determined. A comparison of the sequences of ITS1, 5,8 and ITS2 region between *C. versicolor* and *Zingeria trichopoda* and genomic in situ hybridication were performed. Although the analysed sequences of the two species were very similar, the results of in situ hybridization did not support the position of *C. versicolor* as direct ancestor of the allopolyploid species *Z. trichopoda*.

CONCLUDING REMARKS

Plant with very low chromosome number are useful for the analysis of the structure of their chromosmes and chromatin organization and these five angiosperms might be of interest for the study of the behaviour of homologous chromosomes. Karyotype studies suggest that low chromosome number in several plant genera might derived from tandem fusion with chromosomal rearrangments. Moreover B chromosomes are present in hundred of flowering plants and also in gimnosperms and in some lower form and have more than one function. Therefore the analysis of karyotype evolution and B chromosomes might provide further informatin in order to establish the evolutionary position of a species inside a genus and for a better understanding of the relationships among the species belonging to the same genus.

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