Cytogenetic Studies in *Withania somnifera* (L.) Dun. (Solanaceae)

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Summary Karyotype analysis (through Image Analyzing System) of *Withania somnifera* revealed 7 ($2n=48$: $4A_{sm}^{sc}+4B_m+14C_m+4D_{sm}+2E_{st}+18F_m+2G_{sm}$) morphologically distinct chromosome types. The karyotypes showed prevalence of chromosomes with median primary constrictions. Satellites were associated with short arm of 4A-type chromosomes. Chromosome length in the complement varies from 1.43 to 2.64 μm . The karyotype was symmetric in nature (TF%: 42.26). Root tip squash preparations revealed polysomatomy ($2n=12$: 5.8%, $2n=18$: 4.8%, $2n=24$: 2.9%, $2n=36$: 25.0%, $2n=48$: 57.7% and $2n=72$: 3.8%) with predominance of $2n=48$ chromosomes. The meiocytes had $2n=48$ chromosomes always with an average of $23.52 II+0.95 I$ per cell. The bivalents formed rods (rods: 20.64 ± 0.15 , rings: 2.90 ± 0.17) mostly at diplotene with mean chiasma of 26.45 ± 0.25 per cell. A persistent feature in 76.2% metaphase I cells was the presence of secondary association of chromosomes and the chromosomes tended to form groups of 3 (8.0%), 6 (32.0%), 9 (16.0%) and 12 (44.0%). Secondary polyploidy has been attributed as the possible cause of secondary association of chromosomes and the basic chromosome number of the species has been suggested to be $x=12$.

Key words *Withania somnifera*, Karyotype, Meiosis, Secondary chromosome association, Basic chromosome number.

Withania somnifera (L.) Dun. (family Solanaceae) is a much-valued medicinal plant with immense therapeutic applications including anticancerous (Leyon and Kuttan 2004) and antioxidant (Bhattacharya *et al.* 2001) properties. Cytogenetical studies, which is a prerequisite for genetic improvement in plant species, is restricted to chromosome counts only in *W. somnifera* and has been found to be variable as $2n=24$ (Mohan Ram and Kamini 1964), $2n=48$ (Bhaduri 1933, Gottschalk 1954, Baquar 1967, Bir and Sidhu 1980) and $2n=72$ (Bir and Neelam 1980). Ray and Jha (2002) reported 21 pairs of bivalent formation in regenerated plants of *W. somnifera*. In view of the fact that chromosome number alone does not provide any cytogenetical criteria, present investigation is aimed to collect cytogenetical information about *W. somnifera* and this research communication reports on the karyotype analysis and meiotic chromosome behaviour which were previously undescribed in the species.

Materials and methods

Plant material

Seed sample of *Withania somnifera* (L.) Dun. was collected from Agri-Horticultural Society of India, Kolkata (MPST No.–NHII/XVIII/946, CST No.–NHII/XVIII/596) and plants were raised in the Experimental Garden, Kalyani University. Voucher specimen has been deposited in the Herbarium, Department of Botany, Kalyani University.

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Karyotype analysis

Root tips were pretreated for 4 h in aqueous solution of PDB-aesculin at $16 \pm 1^\circ\text{C}$, fixed in 1 : 3 propionolalcohol (v/v) overnight and stained in orcein-1(N) HCl mixture (9 : 1). Slides were prepared using squash technique (45% acetic acid). Suitable metaphase plates (5 properly condensed plates) prepared for the species were analyzed through Image Analyzing System as was suggested by Fukui (1986).

Metaphase plates observed under the microscope (Olympus CH 40) were photographed using Olympus C-5060 Wide Zoom Camera and these photographs were transferred directly to computer and the chromosome images were analyzed through Micro Image™ Lite Software (Version 4.0 for Windows, 47N 40155 2000 0515 MAN VG MIX). Chromosome images were measured from 5 plates in pixel unit and were converted to micrometer with reference to a standard scale (stage micrometer) and idiogram was prepared.

The chromosomes were classified as median (m) submedian (sm) and subterminal (st) according to the position of the centromere (Hirahara and Tatuno 1967) and on the basis of chromosome length (long: 2.25 to 3.00 μm , medium: 1.75 to <2.25 μm and small: <1.75 μm), centromeric position and the presence of secondary constriction (sc), the chromosomes were morphologically graded into the following type: type A: long submedian chromosomes with satellites, type B: long median chromosomes, type C: median chromosomes of medium sizes, type D: submedian chromosomes of medium sizes, type E: medium subtelocentric chromosomes, type F: small median chromosomes, type G: small submedian chromosomes, type H: small subtelocentric chromosomes. Total chromatin length, TF% (proportion of short arm in the total chromatin length) and S% (relative length of shortest chromosome compared to the longest) were also analyzed.

Meiotic analysis

Flower buds of suitable sizes from 10 randomly selected plants were fixed (5.30 to 6.30 AM) for 48 h in Carnoy's fluid at $28 \pm 2^\circ\text{C}$ and 1–2 drops of iron alum were added in the fixative for proper staining. At least 2 changes were given in fixative for removal of oil precipitates. Pollen and pollen mother cells were assessed from anther squash preparations in 1% propionocarmine solution. Fully stained pollen grains were considered fertile. Meiotic data over the plants has been pooled and represented in the text.

Results and discussion

Mitotic analysis

Karyomorphological studies revealed 7 ($2n=48$: $4A_{sm}^{sc}+4B_m+14C_m+4D_{sm}+2E_{st}+18F_m+2G_{sm}$) morphologically distinct chromosome types (Table 1, Figs. 1, 2). The karyotypes showed

Table 1. Karyotypic details in *W. somnifera*.

Chromosome type	No. of chromosomes	Length (μm)	F%	Centromeric nature
A*	4	2.36–2.64	35.20–36.40	sm
B	4	2.28–2.35	48.5–50.00	m
C	14	1.86–2.00	43.00–50.00	m
D	4	1.85–1.99	34.60–35.70	sm
E	2	1.93	24.90	st
F	18	1.43–1.72	40.00–50.00	m
G	2	1.71	33.30	sm

* Chromosomes with satellites.

Figs. 1–7. Mitosis (1–2) and meiosis (3–7) in *W. somnifera*. 1) $2n=48$ chromosomes at metaphase (bar= $3.0\ \mu\text{m}$). 2) Idiogram showing the chromosome types (bar= $1.14\ \mu\text{m}$). 3) 24 II at diplotene. 4) 24 II at MI. 5–7) Metaphase I configurations showing secondary groupings. 5) 9 group class. 6–7) 12 groups.

prevalence of chromosomes with median (F%: 40.00 to 50.00) and submedian (F%: 33.30 to 36.40) primary constrictions though there were a few subterminal (F%: 24.90) chromosomes. Chromosome length was found to vary from $1.43\ \mu\text{m}$ to $2.64\ \mu\text{m}$. Four chromosomes in A chromosome type were with satellites and the satellites were associated with short arm of the chromosomes. Total

haploid chromatin length and S% noted in the species were 45.41 and 64.77% respectively. The karyotypes were symmetric in nature (TF%: 42.26). Squash preparations also showed polysomatomy ($2n=12$: 5.8%, $2n=18$: 4.8%, $2n=24$: 2.9%, $2n=36$: 25.0%, $2n=48$: 57.7%, and $2n=72$: 3.8%) with predominance of $2n=48$ chromosomes (104 cells analyzed).

Meiotic analysis

Meiocytes formed $2n=48$ chromosomes always (Figs. 3, 4). Predominant chromosomal association had been 24 II in 70.73% metaphase I (MI) cells (164 cells scored); while the rest had 23 II+2 I (20.12%), 22 II+4 I (5.48%), 21 II+6 I (0.61%), 20 II+8 I (1.22%), 19 II+10 I (1.22%) and 18 II+12 I (0.61%) with an average of 23.52 II (23.17–23.74) and 0.95 I (0.53–1.67) per cell. Bivalents showed prevalence of rod (rods: 20.64 ± 0.15 , rings: 2.91 ± 0.17) configuration at diplotene (33 cells scored) with mean chiasma of 26.45 ± 0.25 per cell. About 63.85% anaphase I cells (166 cells analyzed) were cytologically balanced (24/24), while the rest showed unequal separation of chromosomes (23/25) and formed bridges and laggards (1 to 2). Average pollen fertility studied in the species (1939 pollen grains assessed) was 70.25% (67.9–72.6%).

Secondary association of chromosomes

It is interesting to note that bivalents and univalents in the species lie in close proximity without any distinct material connection forming groups (Figs. 5–7) of 3 (8.0%), 6 (32.0%), 9 (16.0%) and 12 (44.0%) consistently in 76.2% MI cells. Such groupings have been referred to as secondary association or secondary pairing of chromosomes (Darlington 1965) and have been used to study basic chromosome number and polyploid nature of plant species (Gupta and Roy 1973, Agarwal 1983, Singh 1986, Sengupta and Datta 2003, Mukherjee and Datta 2005). In the present investigation a total of 9 different types of associations could be analyzed in the group classes of which 18 II(1)+5 II(1)+1 II(1) under 3 group class (6.86%), 9 II(1)+4 II(2)+3 II(2)+1 II(1) under 6 group class (11.76%), 6 II(1)+4 II(2)+3 II(3)+2 II(2)+1 II(3) under 9 group class (9.80%) and 9 II(1)+3 II+1 I(1)+3 II(1)+2 II(1)+1 II(5)+1 I(3) under 12 (Fig. 6) group class (24.51%) were most frequent. Computation of χ^2 test revealed heterogeneity between the group classes ($\chi^2=39.0$ at 3DF, $p<0.001$), thereby suggesting that the chromosomes have assorted themselves more preferentially into certain number of groups (predominantly 12 group class) than they do into others much against random distribution. Although observed cell frequencies of 12 and 6 group classes were higher than expected, only cell frequency of 12 group class was significant and it was verified against the pooled frequency of rest of the classes (observed: 12 group class–55, rest–70; expected 12 group class–31.25, rest–93.75; total–125; $\chi^2=24.07$ at 1DF, $p<0.001$).

Persistent occurrence of secondary association of chromosomes has suggested secondary polyploid nature of the species and analysis of cytological data revealed that the probable basic chromosome number is 12. The species might have undergone cytological diploidization in course of evolution to form diploid like meiotic behaviour.

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