Cytological Studies on *Bolbitis quoyana* (Gaud.) Ching

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Bolbitis quoyana is a tropical leptosporangiate fern, belonging to the family Aspidiaceae (Copeland 1947). A preliminary report on the somatic chromosome number ($2n=123$) of this species was made earlier by the present author (Bhavanandan 1968). A perusal of cytological literature on the genus *Bolbitis* reveals that species of the genus studied so far from different geographical regions (Manton 1954 1959, Manton and Sledge 1954, Walker 1966) including India (Abraham *et al.* 1962) are either diploids ($n=41$) or tetraploids ($n=82$) except *B. quoyana* which is a triploid based on $x=41$. A detailed meiotic and karyomorphological study of this triploid species has been made with a view to shed light on the nature and origin of this taxon.

Material and methods

Material for the present study was collected from the Public Gardens, Trivandrum. Young sporophylls were fixed in 3:1 ethyl alcohol and acetic acid for meiotic chromosome studies. The somatic chromosome studies were made from root tip cells. For this, actively growing root tips were collected in water and kept them in the refrigerator at about 4°C for overnight prior to fixation in 3:1 ethyl alcohol and acetic acid. The acetocarmine squash technique was followed for chromosome studies. Addition of a few drops of ferric ammonium sulphate to the fixative increased the stainability of the chromosomes. The systems of Levan *et al.* (1964) and of Stebbins (1958) were followed for karyotype analysis. TF% has been calculated as given by Huziwara (1962).

Results

The spore mother cells showed 41 bivalents and 41 univalents at metaphase I (Fig. 1). The subsequent stages of divisions were irregular. The univalents were scattered throughout the cytoplasm. Those univalents which were not included in any of the poles formed laggards at anaphase I (Fig. 2) and later they organized to form micronuclei of varying sizes at telophase I (Fig. 3). The laggards and micronuclei were observed at anaphase II and telophase II respectively (Fig. 4). Micronuclei were also seen in the tetrads (Fig. 5). This meiotic irregularity resulted in almost total spore sterility.

The somatic chromosome studies were carried out from the root tip cells, which showed 123 chromosomes at metaphase (Fig. 6). A detailed karyotype analysis

of chromosomes was made, and the data shown in Table 1. The chromosomes were all medium sized and the length varied from $3.5\text{ }\mu$ to $7\text{ }\mu$.

From the data obtained from the detailed karyotype analysis of the somatic

Figs. 1-6. *Bolbitis quoyana*. 1, a spore mother cell showing 41 bivalents and 41 univalents at metaphase I. 2, a spore mother cell showing laggards at late anaphase I. 3, micronuclei of varying sizes are seen in a spore mother cell at telophase I. 4, a spore mother cell showing micronuclei at telophase II. 5, tetrad with micronuclei. 6, a root tip cell showing 123 chromosomes at metaphase. All figs. $\times 760$.

chromosomes it is found that the chromosomes in the complement could be grouped into two distinct sets, one diploid represented by 41 homologous pairs (AA) and the other, by 41 non-homologous (B) chromosomes (Fig. 7). The chromosome length in the A genome varied from 3.83 $m\mu$ to 7 $m\mu$ and it consisted of 3 m types, 7 sm types, 14 st types and 17 t types of chromosomes. The chromosomes of the B

Table 1. Karyotype analysis of *B. quoyana*

Chromosome No.	Length of short arm $m\mu$	Length of long arm $m\mu$	Total length $m\mu$	Arm ratio L/S	Relative length $m\mu$	F %	Chromosome designation
Diploid A genome							
1	1.33	5.67	7.00	4.26	3.44	19	st
2	1.42	5.00	6.42	3.52	3.15	22	st
3	0.67	5.67	6.34	8.46	3.12	11	t
4	1.00	5.33	6.33	5.33	3.11	15	st
5	1.00	5.07	6.07	5.07	2.98	16	st
6	2.00	4.00	6.00	2.00	2.94	33	sm
7	1.67	4.33	6.00	2.59	2.94	27	sm
8	0.33	5.67	6.00	17.18	2.94	5.5	t
9	0.33	5.5	5.83	16.67	2.86	5.6	t
10	2.00	3.67	5.67	1.84	2.78	35	sm
11	1.33	4.33	5.66	3.26	2.78	23	st
12	1.33	4.33	5.66	3.26	2.78	23	st
13	0.67	4.67	5.34	6.97	2.62	12	st
14	0.67	4.67	5.34	6.97	2.62	12	st
15	0.17	5.00	5.17	29.41	2.54	3.28	t
16	1.00	4.00	5.00	4.00	2.45	20	st
17	0.67	4.33	5.00	6.46	2.45	13	st
18	0.33	4.67	5.00	14.15	2.45	6.6	t
19	0.33	4.67	5.00	14.15	2.45	6.6	t
20	0.67	4.08	4.75	6.09	2.33	14	t
21	0.58	4.17	4.75	7.19	2.33	12	t
22	2.00	2.67	4.67	1.34	2.29	42	sm
23	2.00	2.67	4.67	1.34	2.29	42	sm
24	0.67	4.00	4.67	5.97	2.29	14	st
25	1.33	3.33	4.66	2.50	2.28	28	sm
26	0.67	3.83	4.5	5.72	2.21	14	st
27	0.44	4.00	4.44	9.09	2.18	9.9	t
28	0.33	4.1	4.43	12.42	2.17	7.4	t
29	1.67	2.67	4.34	1.60	2.13	38	m
30	1.67	2.67	4.34	1.60	2.13	38	m
31	0.67	3.67	4.34	5.48	2.13	15	st
32	0.33	4.00	4.33	12.12	2.12	7.6	t
33	0.33	4.00	4.33	12.12	2.12	7.6	t
34	0.33	4.00	4.33	12.12	2.12	7.6	t
35	1.5	2.67	4.16	1.78	2.04	36	sm
36	0.33	3.67	4.00	11.12	1.96	8.25	t
37	0.33	3.67	4.00	11.12	1.96	8.25	t
38	0.17	3.83	4.00	22.53	1.96	4.25	t
39	0.17	3.67	3.84	21.59	1.88	4.42	t
40	1.83	2.00	3.83	1.09	1.88	47	m
41	0.33	3.5	3.83	10.61	1.88	8.6	t

Table 1. (continued)

Chromosome no.	Length of short arm $m\mu$	Length of long arm $m\mu$	Total length $m\mu$	Arm ratio L/S	Relative length $m\mu$	F %	Chromosome designation
Haploid B genome							
1	0.67	6.33	7	9.45	3.38	9.57	t
2	1.67	5.33	7	3.19	3.38	23	st
3	1.53	5.33	6.83	3.48	3.30	24	st
4	1.08	5.58	6.66	5.17	3.22	16	st
5	1.75	4.42	6.27	2.53	3.03	27	sm
6	1.17	4.67	5.84	3.99	2.82	20	st
7	0.67	5.00	5.67	7.46	2.74	11	t
8	1.08	4.5	5.58	4.17	2.69	19	st
9	1.25	4.33	5.55	3.46	2.68	22	st
10	0.33	5.17	5.5	15.67	2.66	6.00	t
11	0.17	5.33	5.5	31.35	2.66	3.09	t
12	1.00	4.33	5.33	4.33	2.57	18	st
13	2.25	3.00	5.25	1.33	2.53	42	m
14	0.75	4.5	5.25	6.00	2.53	14	st
15	1.17	4.00	5.17	3.42	2.50	22	st
16	0.5	4.60	5.17	9.34	2.50	9.67	t
17	1.42	3.67	5.09	2.58	2.46	19	sm
18	2.00	3.00	5.00	1.5	2.41	40	sm
19	1.50	3.50	5.00	2.33	2.41	30	sm
20	1.33	3.67	5.00	2.76	2.41	36	sm
21	0.17	4.83	5.00	28.41	2.41	3.40	t
22	0.33	4.58	4.91	13.87	2.37	6.72	t
23	1.44	3.4	4.84	2.36	2.34	29	sm
24	1.17	3.67	4.84	3.13	2.34	21	st
25	0.84	4.00	4.84	4.76	2.34	17	st
26	0.17	4.67	4.84	27.47	2.34	21	t
27	1.75	3.00	4.75	1.71	2.29	36	sm
28	0.25	4.42	4.67	17.68	2.25	5.35	t
29	0.33	4.33	4.66	13.12	2.25	7.08	t
30	1.5	3.08	4.58	2.05	2.21	32	sm
31	2.00	2.5	4.5	1.25	2.17	44	sm
32	1.17	3.33	4.5	2.85	2.17	26	sm
33	1.00	3.5	4.5	3.5	2.17	22	st
34	0.67	3.83	4.5	5.72	2.17	14	st
35	0.33	4.17	4.5	12.64	2.17	7.33	t
36	0.92	3.42	4.34	3.71	2.09	21	st
37	0.25	4.08	4.33	16.32	2.09	5.77	t
38	1.08	3.08	4.16	2.85	2.01	25	sm
39	0.33	3.62	3.95	10.9	1.91	8.35	t
40	1.00	2.92	3.92	2.92	1.89	25	sm
41	0.17	3.33	3.5	19.59	1.69	4.85	t

genome varied in length from 3.5 $m\mu$ to 7 $m\mu$, and it consisted of 1 m type, 11 sm types, 14 st types and 15 t types of chromosomes. However, both the genomes are almost similar with regard to TCL, TSL, range of F% and TF_{∞} (Table 2). It has been found that 33 chromosomes (85%) in the A genome and 37 chromosomes

(90.2%) in the B genome have the arm ratio more than two. It has been observed that both the A and B genomes are asymmetric and come under 3A and 3B category respectively.

Fig. 7. Idiogram of *Bolbitis quoyana*.

Discussion

The meiosis in this taxon is characterized by the formation of 41 bivalents and 41 univalents. This appears to indicate that the somatic complement of this material is composed of two different genomes, one of which is diploid consisting of 41 homologous pairs (A genome) and the other with 41 non-homologous chromosomes (B genome). Further, the total absence of trivalents or higher associations at meiosis may be considered to indicate that there is apparently no homology between the chromosomes of the A and B genomes of the taxon.

Table 2. Summary of karyomorphological data of *B. quoyana*

Name of genomes	Number of chromosomes (haploid set)	Number of chromosomes according to centromere position				Range of chromosome length in m μ	Total chromosome length (TCL) m μ	Total short arm length (TSL) m μ	Range of F%	TF %	Karyotype asymmetry
		m	sm	st	t						
A	41	3	7	14	17	3.83 to 7	203.51	36.6	3.28 to 47	17.98	3 A
B	41	1	11	14	15	3.5 to 7	206.7	39.5	3.09 to 44	19.13	3 B

TCL = Total chromosome length	TSL = Total short arm length
$F\% = \frac{\text{Short arm length}}{\text{Chromosome length}} \times 100$	$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$

Evidence from karyotype analysis shows that, the somatic complement of the taxon could be divided into two groups such as a diploid set of 41 pairs of chromosomes and a haploid set of 41 chromosomes. It may be noted that the chromosome belonging to the two sets showed no recognizable homology. This together with the type of meiotic behaviour observed appears to indicate that the taxon possesses an allotriploid constitution originated from two different species. A condition like this can arise spontaneously either by crossing between two species, one of which is a tetraploid and the other a diploid or by fusion between the unreduced gamete (2n) of a diploid species and a normal gamete (n) of yet another diploid species. It may be noted that, so far no tetraploid species of *Bolbitis* has been reported from India and hence the latter possibility appears to be more plausible for origin of the present triploid taxon.

Summary

Detailed cytological study of *B. quoyana* (2n=123) was made. Regular formation of 41 bivalents and 41 univalents was consistently noticed in the spore mother cells.

Detailed karyomorphology of the material was studied and the data indicated that the karyotype is composed of two distinct sets, a diploid one consisting of 41 pairs, and a haploid set consisting of 41 non-homologous chromosomes.

The result appeared to indicate that this is an allotriploid species possibly ori-

ginated spontaneously by the fusion between the unreduced (2n) and normal (n) gametes of two diploid species of *Bolbitis*.

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References

- Abraham, A., Ninan, C. A. and Mathew, P. M. 1962. Studies on the cytology and phylogeny of the pteridophytes. VII. Observations on one hundred species of South Indian ferns. *Jour. Ind. Bot. Soc.* **41**: 339–421.
- Bhavanandan, K. V. 1968. Studies on the cytology of sixteen species of South Indian ferns. *Caryologia* **21**: 333–338.
- Copeland, E. B. 1947. *Genera Filicum*. Waltham, Mass. Chronica, Botanica Co.
- Huziwara, Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosome of aster. *Amer. J. Bot.* **49**: 116–119.
- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromere position on chromosomes. *Hereditas* **52**: 201–220.
- Manton, I. 1954. Cytological notes on one hundred species of Malayan ferns. Appendix to "Flora of Malaya". Vol. II. Ferns. Holtum. Govt. Press, Singapore.
- 1959. Cytological information on the ferns of West Tropical Africa. In Alston pp. 75–81. Crow Agents for Overseas Governments and Administrations, London.
- and Sledge, W. A. 1954. Observations on the cytology and taxonomy of the pteridophyte flora of Ceylon. *Phil. Trans. Roy. Soc. Lond. Series B. Biol. Sci.* **238**: 127–185.
- Stebbins, G. L. Jr. 1958. Longivity, habitat and release of genetic variability in higher plants. Cold Spring Harb. Symp. Quant. Biol. **23**: 365–378.
- Walker, T. G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. *Trans. Roy. Soc. Eding.* **66** (9): 169–237.
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