Cytology of *Wolffia microscopica* Kurz.

R. P. Roy and Bithi Dutt

Botany Department, Patna University, Patna-5, India

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Introduction

The genus *Wolffia* of the Lemnaceae is characterised by very small plants. Only one species *W. arrhiza* was cytologically investigated by Blackburn (1933) and later by Lawalrée (1943). Even these reports are only short notes on the chromosome number of the species. The other species *W. microscopica* Kurz., which is the minutest flowering plant so far known, has not been cytologically investigated at all. Before an attempt to establish interrelationship between species is made, the cytological situation in the individual species has to be critically analysed. The fragmentary and meagre cytological data that we have about the genus prevents us from drawing any phylogenetic conclusion. It was, therefore, considered desirable to investigate as many species of the genus as available to make use of them later for phylogenetic considerations. The first account in this attempt on *W. microscopica*, the smallest flowering plant, is presented in this paper.

Several strains of *W. microscopica* are being used by different workers for tissue culture studies. One strain M8 of this species has been described by Maheswhari and Chauhan (1963). This is characterised by indistinct stem, leaf and root and the whole plant simply consists of a disc-like structure, flat above and conical on the underside. Flowering in nature has been observed very rarely in a few cases only, but the authors mentioned above have very successfully induced flowering in culture media. The plants of this strain in flowering condition, grown in culture media by the above named authors were very kindly supplied to us for the cytological studies reported herein.

Materials and methods

Due to the minute nature of the plant, it was not possible to dissect out the anthers. So the whole plants were fixed for more than 24 hours in a mixture of glacial acetic acid and absolute alcohol in 1:3 ratio and a few drops of ferric chloride were added. The plants as such were stained in 2.5% acetocarmine by heating gently and squashed in 45% acetic acid. The chromosomes during meiosis were studied and photographs of temporary slides were taken. The slides were made permanent in n-butyl alcohol series and mounted in Euparal.

Observations

A cytological observation revealed that 35 bivalents are formed at metaphase I (Fig. 1), and majority of them are of ring type with only a few rod bivalents. Their size variation indicates that there is no uniformity among the chromosomes. Most of the bivalents are very small, but a few of them are large enough in comparison to the others. The mean numbers of ring and rod bivalents per pollen mother cell are 30 and 5 respectively. At anaphase I, equal number of chromosomes are seen oriented towards each pole (Fig. 2). The second division is also regular, resulting in the formation of 4 isobilateral microspores. The chiasma-frequency data for the whole nucleus and terminalization coefficient is shown in Table 1.

Figs. 1-4. Meiotic and mitotic stages in *W. microscopica*. 1, metaphase I showing 35 bivalents ($\times 1250$). 2, anaphase I showing equal number of chromosomes towards each pole. 3, pollen mitosis showing 35 chromosomes ($\times 3750$). 4, diagrammatic representation of Fig. 3.

Table 1. Chiasma frequency and terminalization coefficient in *W. microscopica*

Stage of meiosis	No. of nuclei	Chiasma frequency per nucleus	No. of chiasma		Terminalisation coefficient
			Total chiasmata	Terminal chiasmata	
Mataphase I	20	64.7	1294	1166	0.90

Pollen mitosis (Fig. 3) further confirms that the haploid chromosome number is 35. From the somatic chromosomes it is clear that some of the chromosomes have terminal centromeres and a few of them have sub-median ones. Due to undifferential staining of the pollen grains, it was not possible to exactly locate the position of centromeres in all the chromosomes,

Discussion

It has been mentioned earlier that no cytological work has been done on this species. Minute structure of the plant body presumably appears to be one of the reasons for this. However, Lawalrée (1943) observed that in *W. arrhiza* the diploid chromosome number is approximately 50. Our study shows clearly that the diploid chromosome number in *W. microscopica* (Strain M8) is 70. Thus the two species *W. arrhiza* and *W. microscopica*, whose cytology is now known seem to possess two different chromosome numbers and apparently it appears that there is a polyploid series in the genus. But it must be mentioned at this stage that *W. microscopica* [also *W. arrhiza* (Lawalrée 1943)] reproduces vegetatively and hence, there is every possibility that different clones of this species may have different chromosome numbers. Unless this probability is explored in detail, no final conclusion about the basic chromosome number can be made at present, although aneuploidy may have played an important role in the evolution of various vegetative clones. This fact is also supported by the other genus *Lemna* in which different species have been found to possess different chromosome numbers (Darlington and Wylie, 1955). However, the high chromosome numbers in the worked out members of the family indicate that the taxa are not primitive but reduced ones.

Summary

Cytological studies in the species *W. microscopica* Kurz. of the family Lemnaceae have been made. Meiotic chromosome behaviour is quite regular. 35 bivalents were clearly visible. Pollen mitosis further confirms the haploid chromosome number to be 35. The high chromosome number in the species indicates that it is not a primitive but a reduced taxon. Any opinion about the interrelationship of the various species has to await detailed cytological studies of the different taxa.

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