Cytogenetic Studies of Some Members of Polygonaceae of Kashmir

III. *Rheum* L.

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Commonly called rhubarb, species of *Rheum* L. are important source for a number of chemical compounds like emodin, chrysophanic acid and rhaponticin, used as purgatives (Tutin and Clewer 1911). Although these chemicals are present in nearly all the species of *Rheum* whose chemical analysis has been done, *R. emodi* and *R. frazenbachii* are very rich in them. The two species of this genus namely *R. emodi* and *R. webbianum*, growing wild in Kashmir Himalaya, are used as edibles and medicinals both by the people inhabiting this region and the nomads. In view of their economic importance it was decided to study these species critically. The present paper puts on record meiotic details of these two species.

Materials and methods

The material of *R. emodi* was collected from Mahadev (3,900 m) and Baltal (2,900 m) and that of *R. webbianum* from Kangan (1,800 m). For the study of male meiosis anthers from the floral buds, fixed in modified Carnoy's 1 : 1 : 1 (acetic acid, absolute ethanol and chloroform) fluid were squashed in 1% acetocarmine. For studying pollen fertility, pollen grains from fresh, ripe but undehiscent anthers were stained in acetocarmine. The shrivelled and unstained pollen grains were scored as sterile. All the observations were made from temporary mounts which were later made permanent by passing through acetic acid-n-butyl alcohol series with euparal as the mounting medium.

Observations

Rheum emodi: All the plants collected from Baltal and Mahadev were diploid with different anthers of a bud at different stages of development. In addition to the 22 chromosomes of this species forming eleven bivalents, in the pollen mother cells, an accessory chromosome was also observed in 96.6% of the cells scored (Fig. 1). This accessory chromosome was very small as compared to other chromosomes.

At diplotene one or two bivalents remained attached to a large, round and

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Table 1. Mean chiasmata frequency in *R. emodi*

		Interstitial	Terminal	Total
Diplotene	per cell	6.50	14.00	20.50
	per bivalent	0.59	1.27	1.86
Metaphase I	per cell	0.00	18.60	18.60
	per bivalent	0.00	1.69	1.69

Figs. 1-7. Chromosome behaviour during reduction division in *Rheum emodi* (for details see text). Scale 10 μ m.

uniformly stained nucleolus. In a few cells the B chromosome was also found attached to the nucleolus (Fig. 1). Even at diplotene most of the chiasmata in this species were terminal, with the result that majority of the bivalents had only two chiasmata at this stage. However, in a few bivalents interstitial chiasmata were also observed. The average frequency of chiasmata at diplotene and metaphase I cells of this species is tabulated (Table 1).

At metaphase I too, in addition to the eleven bivalents, which oriented on the equatorial plate, many pollen mother cells had a single B chromosome (Fig. 2). In 27% pollen mother cells at metaphase I and even in some at diplotene the B chromosome was found attached to one of the bivalents by a very thin and faintly stained connection (Figs. 1, 3). The anaphase I segregation of chromosomes in this species was found to be slightly erratic. Various anaphase I segregation patterns met in 86 pollen mother cells of this species are given in Table 2.

Table 2. Anaphase I distribution of chromosomes in *R. emodi*

	Distribution pattern						
	11+B	10 B attached to bivalent	10 B attached to chromo- some	11 1-3 bridges	11 1	10 2	10 3 12
	11	10	11	11	10	10	9 10
No. of cells	42	1	1	11	10	10	9 2
Fig. no.	4			5			

As is evident from the above table, in nearly all the cells studied, the B chromosome moved to one of the poles and was not found to divide at this stage (Fig. 4). In 27% cells studied at anaphase I chromatin bridges or laggards ranging in number from 1-3 (Fig. 5) were also observed. Segregation abnormalities, like formation of chromatin bridges and laggards, were observed also during second anaphase and telophase (Figs. 6, 7). After sometime these laggards formed micronuclei. These plants produced enough pollen, 94% of which stained positively with acetocarmine and the seed set was also high.

R. webbianum: This species is tetraploid with $2n=44$ chromosomes. A large number of pollen mother cells were available at various stages of meiosis for critical studies. Male meiosis in this species is characterised by the presence of multivalent associations at diplotene and metaphase I followed by erratic anaphase segregations.

In some pollen mother cells at early diplotene the chromosome associations were so complex that these could not be resolved (Fig. 8). At this stage the cells had a deeply stained nucleolus with one or two bivalents attached to it. In the cells at late diplotene, however, the number of these complex associations decreased and the frequency of bivalents was more than the multivalents (Fig. 9). At this stage most of the bivalents were of ring type with two terminal chiasmata per biva-

lent. At metaphase I the multivalents and the bivalents get oriented at the equatorial plate (Fig. 10). The frequency of different chromosome associations observed in 98 metaphase I pollen mother cells of this species is summed up in Table 3.

Figs. 8-14. Chromosome behaviour during reduction division in *Rheum webbianum* (for details see text). Scale 10 μ m.

Table 3. Metaphase I associations in *R. webbianum*

Configurations		No. of cells	Fig. no.
II	IV		
22	—	38	10
20	1	58	
16	3	1	
14	4	1	

Of the 4312 chromosomes scanned at metaphase I, only 260 (6%) formed quadrivalents and the remaining (94%) formed bivalents only. The maximum number of quadrivalents found in any cell was 4 only. In 38.7% cells at metaphase I of this species 22 bivalents only were observed.

Although the anaphase I segregation of chromosomes in most of the pollen mother cells studied in this species was found to be normal with 22 chromosomes

going to either pole (Fig. 11), in some cells it was unequal (Fig. 12) and in some others laggards (Fig. 13) and chromatin bridges (Fig. 14) were also observed. The distribution pattern met in 35 clear cells at anaphase I in this species is given in Table 4.

As is evident from the above table, in 62.8% cells the chromosome segregation was normal. The chromatin bridges, whenever present, broke into two after some time and the laggards formed micronuclei. Such anomalies were also observed at anaphase II and telophase II. Despite all the anomalies observed in this species, the pollen stainability was as high as 88.8% and the seed set in these plants also was good.

Table 4. Anaphase I distribution of chromosomes in *R. webbianum*

	Distribution pattern				
	22	21	22 1	22 2	22 1-2 bridges
	22	23	21	20	22
No. of cells	22	3	1	1	8
Fig. no.	11	12		13	14

Discussion

The genus *Rheum* comprising about 50 species is mostly restricted to Asia (Allayarov 1972, Airy-Shaw 1973). In the Indian subcontinent the genus is represented by 10 species (Hooker 1885, Santapau and Henry 1973) with five of these reported from Kashmir (Stewart 1972).

Of the two species studied, *R. emodi* is a diploid with $2n=22$ and *R. webbianum* a tetraploid with $2n=44$ chromosomes. The genus is monobasic with all the species having $x=11$ (Darlington and Wylie 1955, Fedorov 1969). While there are reports of the occurrence of tetraploid forms also in *R. emodi*, in *R. webbianum* only a single chromosome number ($4x$) has been recorded (Fedorov, 1969). A perusal of literature reveals that *Rheum* is one of the best studied genus of the family Polygonaceae from cytogenetical point of view. The chromosome numbers in this genus range from $2n=22$ to 88 (Darlington and Wylie 1955, Fedorov 1969, Allayarov 1972, Moore 1972, 1974, 1977). In the genus *Rheum* chromosome numbers of 32 species (64%) are on record. These 32 species exist in 48 cytotypes. Of these diploids and tetraploids are equally common (45.8% each), followed by hexaploids and octaploids (4.16% each). As such in *Rheum* polyploidy is dominant over diploidy meaning thereby that this has been one of the important factors in the speciation and evolution within this group. Although, such a high frequency of polyploidy agrees with the rhizomatous nature of its species, the total absence of oddploidy ($3x$, $5x$, etc) in *Rheum* is significant and can only be explained on the basis of incompatibility between cytotypes at different ploidal levels, otherwise, once formed, oddploids will be able to survive and propagate. At the same time the number of

marker chromosomes in diploid and tetraploid species of the genus indicates that amphidiploidy has been responsible for the evolution of some of its species (Chin and Youngken 1947). As such another reason for this could be the failure of the oddploids to survive competition posed by long established evenploids. *R. ribes* is the only species of the genus where, besides the tetraploid cytotypes ($2n=44$), plants having 38, 39 and 41 chromosomes have also been isolated (Schratz and Schnelle 1964).

Although somatic chromosomes of the two species studied could not be analysed in detail, it appears that the chromosomes of *R. emodi* are larger than those of *R. webbianum*.

The behaviour of chromosomes during meiosis in the two species included in the present investigation varied a lot. While in case of *R. emodi* meiosis was characterised by the presence of a single B chromosome in 96.6% of the pollen mother cells studied, followed by anaphase irregularities, in *R. webbianum* multivalent associations were observed. The behaviour of B chromosome in pollen mother cells of *R. emodi* varied; in nearly 73% of the cells studied it remained separate from rest of the chromosomes and in remaining (27%) pollen mother cells it was attached to one of the bivalents by means of a thin lightly stained connection. A somewhat similar behaviour has earlier been reported in grasshopper (Henderson 1961) and in *Petunia hybrida* (Gohil and Kaul 1980). In addition to this, even the anaphase segregation of this B chromosome was peculiar. In none of the cells analysed was it found to divide or remain as a laggard at anaphase I. In all these cells at this stage it was found to move towards one of the poles only. Grun (1959) has observed similar behaviour of B chromosome in *Allium cernuum*. It would be worth pointing out here that this is the first report of the presence of a B chromosome in this genus. In fact the only other genus and species of this family in which B chromosomes have been reported is *Rumex acetosa* (Haga 1961). As such this is the second report of the presence of B chromosome for the entire family Polygonaceae as a whole.

In the diploid *R. emodi*, in addition to the apparent unequal segregation of chromosomes at anaphase I, because of the presence of B chromosome, other segregational anomalies were also observed. As is clear from Table 2, only in 51.16% of the pollen mother cells observed at anaphase I of this species was the segregation normal. In the rest (48.84) of the cells it was erratic characterised by the presence of chromatin bridges, fragments and lagging chromosomes. In view of the perfect pairing of the chromosomes of this species into 11 bivalents these anomalies appear rather difficult to explain. The presence of bridges and fragments in 12.85% cells could be an indicator of paracentric inversions in this species. This assumption remains to be confirmed from the study of pollen mother cells at pachytene of meiosis. Similar anomalies have been recorded from other taxa also which have B chromosomes (Jones 1975, Gohil and Kaul 1980).

Multivalent associations were observed in the pollen mother cells of *R. webbianum*. Although, in only 38.8% pollen mother cells studied, the 44 chromosomes of this species paired to form 22 bivalents, the frequency of quadrivalents per cell in the rest (61.2%) of the pollen mother cells is also not high. The highest number of quadrivalents (4) was observed in one cell only. In 58 cells only a single quadri-

valents was observed. The presence of quadrivalents in such a low frequency can be explained on the basis of probable amphidiploid nature of this species with little homology between the constituent genomes of this tetraploid. Another reason could be that this species might have been autoploid to start with, whose constituent genomes have developed dissimilarities during the course of evolution. This is possible either through the repatterning of chromosomes or spontaneous mutations. Since the somatic chromosomes of this species could not be studied in detail, it is not possible at this time to comment more on the nature of ploidy involved in its evolution. At the same time, so far, no diploid cytotype of this species has been described with which the comparison can be made. Chin and Youngken (1947) had also observed 22 bivalents only in majority of the cells of this species studied by them. In the material studied by them (Chin and Youngken 1947) also, occasionally a single quadrivalent was observed. As such the present cytotype appears similar to the one studied earlier.

As already pointed out, all the species of this genus have stout rhizomes and are mostly vegetatively propagating. As such these plants are able to efficiently bear the ill effects of chromosomal alterations, both numerical and structural.

Summary

Meiotic details of two species of *Rheum* have been studied. *R. emodi*, a diploid species with $2n=22$ chromosomes, an accessory chromosome was observed in most of the pollen mother cells studied. In 27% pollen mother cells this B chromosome was observed attached to one of the bivalents by a faintly staining connection. It is the first report of the presence of an accessory chromosome in this genus.

Meiosis in both *R. webbianum*, a tetraploid species with $2n=44$, and diploid *R. emodi* was mostly normal. The frequency of multivalents in *R. webbianum* was very low, which may probably be due to its amphidiploid nature.

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