Studies on the Cytology of Legumes of Kashmir Himalaya

IV. Meiotic behaviour in 21 species of *Astragalus* L.

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The study of chromosome behaviour during the reduction division of a taxon is important for a number of reasons. This study besides providing an insight into the homology of the constituent genomes also helps in establishing its recombination potential. In view of the significance of this study it is surprising to find that in an easily accessible material, like the species of *Astragalus*, only scanty information regarding the reduction division of only 300 of its 1600 species (18.75% only) is on record (Senn 1938, Ledingham 1957, 1958, Bijok *et al.* 1972, Spellenberg 1974, 1976). This information too is limited to the number of bivalents per pollen mother cell (PMC). None of the earlier workers has given the details like extent of pairing, chiasmata formation or the patterns of anaphase disjunction. As such the present communication is the first compilation of detailed information on meiotic details in 21 diploid species of the genus *Astragalus*.

Materials and methods

Materials for the present work were collected from different parts of the Kashmir Himalaya. Table 1 lists the species of *Astragalus* worked out during the present investigation. For studying chromosome behaviour during microsporogenesis, in these plants, methods used earlier (Gohil and Ashraf 1984, Ashraf and Gohil, 1988) were followed.

The plant specimens of these species have been deposited in the Kashmir University Herbarium (KASH).

Observations and discussion

In all these 21 diploid species ($n=8$) chromosome pairing was perfect, resulting in the formation of bivalents only; followed mostly by regular anaphasic segregations. Salient features of PMC meiosis, as observed in these species, are given in Table 2.

Although in nearly all these species meiosis was by and large normal with some occasional anomalies observed in a few species only, in some plants of *A. subuliformis* and one collection of *A. tibetanus* (from Drass) most of the PMCs showed anomalous meiotic behaviour. Details of the collections showing aberrant meiosis will be described separately.

Although in most of the PMCs at diplotene, of all these species, at least one bivalent was invariably found associated with a deeply stained nucleolus, in some PMCs, however, 2–4 bivalents were found associated with it. In some PMCs upto 3 nucleoli were also observed; with or without any bivalent associated with them. The presence of a single nucleolar bivalent is in keeping with the observation of two secondarily constricted chromosomes in the karyotypes of most of these species (Ashraf and Gohil comm.). The presence of more than one bivalent apparently attached to the nucleolus can be explained either on the basis of chance

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associations or the presence of some cryptic nucleolar organising regions in the karyotypes of these species, which could not be detected during the study of their somatic chromosomes.

Commensurate with the size of the somatic chromosomes, in majority of these species, no significant difference was observed in the size of the bivalents, both at diplotene and metaphase-I. In *A. munroi*, like the somatic chromosomes (Ashraf and Gohil comm.), the bivalents were of different sizes and a gradual decrease in size of bivalents was noticed. In this species the largest and the smallest bivalents were clearly distinguishable (Fig. 11).

As is clear from Table 2 chiasma frequency at diplotene, in these species, ranges from 16.70 (*A. bakeri*) to 25.95 (*A. subuliformis*) per PMC while at metaphase-I it ranges from 9.97 (*A. himalayanus*) to 15.85 (*A. scorpiurus* var. *scorpiurus*) per PMC. While at diplotene chiasma terminalization coefficient ranges from 0.34 (*A. zanskarensis*) to 0.68 (*A. tribulifolius*) at metaphase-I it ranges from 0.82 (*A. leucocephalus*) to 0.99 (*A. chlorostachys*, *A. coluteocarpus* var.

Table 1. List of *Astragalus* species studied during the present work.

S. No.	Species	Place(s) of collection	Altitude (m)
1	<i>Astragalus bakeri</i> Ali	Dawar	2,700
2	<i>A. candolleanus</i> Royle ex Benth.	Tragbal	3,100
3	<i>A. chlorostachys</i> Lindl.	Ferozpur Nullah	1,950
4	<i>A. coluteocarpus</i> Boiss. var. <i>glaber</i> Ali	Drass	3,750
5	<i>A. grahamianus</i> Royle ex Benth.	Beru	1,850
6	<i>A. graveolens</i> Buch. ex Benth.	Manasbal	1,650
7	<i>A. himalayanus</i> Kl.	Tragbal	3,200
8	<i>A. hoffmeisteri</i> (Kl.) Ali var. <i>hoffmeisteri</i>	Kargil	2,750
9	<i>A. leucocephalus</i> Grah. ex Benth.	Beru	1,850
10	<i>A. macropterus</i> DC.	Heniskut	3,700
11	<i>A. munroi</i> Benth. ex Bunge	Heniskut to Fotula	3,750
12	<i>A. nivalis</i> Kar. & Kir.	Fotula	4,100
13	<i>A. peduncularis</i> Royle ex Benth.	Near Kharbu	3,100
14	<i>A. rhizanthus</i> Royle ex Benth.	Meenamarg	3,425
15	<i>A. scorpiurus</i> Bunge var. <i>scorpiurus</i>	Kangan	1,800
16	<i>A. stewartii</i> Baker	Sonamarg to Sarbal	2,750
17	<i>A. subuliformis</i> DC.	Drass	3,175
18	<i>A. tibetanus</i> Benth. ex Bunge	I. Heniskut to Fotula II. Drass	3,775 3,150
19	<i>A. tribulifolius</i> Benth. ex Bunge	Meenamarg	3,325
20	<i>A. webbianus</i> Grah. ex Benth.	Drass	3,150
21	<i>A. zanskarensis</i> Benth. ex Bunge	Fotula	4,100

glaber, *A. nivalis* and *A. tibetanus*). Recombination index, in these species, varies between 24.70 (*A. bakeri*) and 33.95 (*A. subuliformis*). As is clear from Fig. 24 chiasma frequencies at diplotene and recombination indices show a positive correlation with total chromatin lengths (Ashraf and Gohil comm.), in these species (coefficients of correlation=0.68). The coefficient of correlation between chiasma frequencies at diplotene and metaphase-I, although positive, is insignificant (coefficient of correlation=0.28). Such a high frequency of chiasmata formed in these species is, as expected, a pointer towards the complete homology between the constituent genomes of these species. It also indicates that these species have a high recombination potential.

Anaphase-I separations, in these species, were also found to be mostly normal with 8 chromosomes going to either pole. However, occasionally some segregational anomalies were observed in a few PMCs of some species. In two species namely, *A. nivalis* and *A. scor-*

Table 2. Salient features of meiosis in 21 species of *Astragalus*.

S. No.	Taxa	Fig. Nos.	Diplotene				Metaphase-I			
			No. of nucleoli per PMC	No. of bivalents attached to nucleolus/nucleoli per PMC	Mean chiasma frequency per PMC $\pm$ Sx	Terminalization coefficient	Mean frequency per PMC $\pm$ Sx	Terminalization coefficient	Recombination index	Pollen viability (%)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1.	<i>A. bakeri</i>	1	1-3	1-3	16.70 $\pm$ 0.41	0.61	11.42 $\pm$ 0.29	0.98	24.70	98.50
2.	<i>A. candolleanus</i>	2	1-3	1-3	17.65 $\pm$ 0.35	0.51	15.20 $\pm$ 0.23	0.85	25.65	98.23
3.	<i>A. chlorostachys</i>	3	1-2	1-2	17.70 $\pm$ 0.37	0.56	14.77 $\pm$ 0.19	0.99	25.70	98.95
4.	<i>A. coluteocarpus</i> var. <i>glaber</i>	4	—	—	—	—	14.95 $\pm$ 0.13	0.99	—	97.93
5.	<i>A. grahmanianus</i>	5	1-3	1-3	18.75 $\pm$ 0.30	0.43	15.02 $\pm$ 0.17	0.98	26.75	98.24
6.	<i>A. graveolens</i>	6	1	1	19.35 $\pm$ 0.19	0.42	15.42 $\pm$ 0.15	0.98	27.35	94.26
7.	<i>A. himalayanus</i>	7	1-2	1-3	21.20 $\pm$ 0.52	0.55	9.97 $\pm$ 0.23	0.97	29.20	99.23
8.	<i>A. hofmeisteri</i> var. <i>hofmeisteri</i>	8	1	1	22.20 $\pm$ 0.17	0.52	14.27 $\pm$ 0.12	0.92	30.30	99.16
9.	<i>A. leucocephalus</i>	9	1-2	1-2	18.00 $\pm$ 0.18	0.39	12.77 $\pm$ 0.29	0.82	26.00	99.20
10.	<i>A. macropteris</i>	10	1	1	20.80 $\pm$ 0.23	0.50	14.22 $\pm$ 0.27	0.86	28.80	99.21
11.	<i>A. munroi</i>	11	1	1	22.25 $\pm$ 0.19	0.45	15.00 $\pm$ 0.13	0.86	30.25	95.11
12.	<i>A. nivalis</i>	12	1	1-3	20.62 $\pm$ 0.30	0.57	14.85 $\pm$ 0.19	0.99	28.62	94.93
13.	<i>A. peduncularis</i>	13	1-2	1-3	20.87 $\pm$ 0.20	0.46	11.32 $\pm$ 0.19	0.89	28.87	97.77
14.	<i>A. rhizanthus</i>	14	1	1	20.67 $\pm$ 0.23	0.39	14.82 $\pm$ 0.16	0.95	28.67	98.70
15.	<i>A. scorpiurus</i> var. <i>scorpiurus</i>	15	1-3	1-2	25.12 $\pm$ 0.27	0.45	15.85 $\pm$ 0.33	0.86	33.12	98.11
16.	<i>A. stewartii</i>	16	1-2	1	17.70 $\pm$ 0.36	0.56	13.65 $\pm$ 0.27	0.97	25.70	99.09
17.	<i>A. subuliformis</i>	17	1	1-3	25.95 $\pm$ 0.20	0.34	15.80 $\pm$ 0.14	0.95	33.95	95.14
18.	<i>A. tibetanus</i>	18	1	1-2	21.72 $\pm$ 0.36	0.34	14.85 $\pm$ 0.23	0.99	29.72	98.50
19.	<i>A. tribulifolius</i>	19	1-2	1-3	17.12 $\pm$ 0.42	0.68	15.35 $\pm$ 0.13	0.97	25.12	98.69
20.	<i>A. webbianus</i>	20	1	1-3	19.55 $\pm$ 0.49	0.37	15.35 $\pm$ 0.13	0.97	27.55	99.03
21.	<i>A. zanskarensis</i>	21	1-2	1-2	20.27 $\pm$ 0.24	0.34	15.80 $\pm$ 0.07	0.98	28.27	98.07

piurus var. *scorpiurus* one bivalent lagged behind at anaphase-I. While in the former 14% PMCs exhibited this anomaly in the latter this anomaly could be observed in a few PMCs only. In keeping with this anomalous behaviour, some PMCs of *A. nivalis* had micronuclei also, at subsequent stages. While a few PMCs each of *A. rhizanthus* and *A. stewartii* at

Figs. 1-11. PMCs, at diakinesis/metaphase-I, in species of *Astragalus*. 1, *A. bakeri*. 2, *A. candolleanus*. 3, *A. chlorostachys*. 4, *A. coluteocarpus* var. *glaber*. 5, *A. grahamianus*. 6, *A. graveolens*. 7, *A. himalayanus*. 8, *A. hoffmeisteri* var. *hoffmeisteri*. 9, *A. leucocephalus*. 10, *A. macropterus*. 11, *A. munroi*. (Scale=10 μ m).

anaphase-I had a single lagging chromosome, in some PMCs of *A. macropterus*, at this stage, 2 or 4 lagging chromosomes (Fig. 22) were observed. Apart from these anomalies a chromatin bridge without any fragment, at anaphase-I was observed in a few PMCs each of *A. scorpiurus* var. *scorpiurus* (Fig. 23), *A. webbianus* and *A. macropterus*. Besides these abnormalities

some PMCs each of *A. hoffmeisteri* var. *hoffmeisteri*, *A. bakeri* and *A. coluteocarpus* var. *glaber* had 1 or 2 late separating bivalents at anaphase-I. The frequency of such PMCs, however, was so low that these deviations, from the normal segregation pattern, do not appear to be due to any structural aberrations in the constituent chromosomes and can be explained on the basis of either partial failure of spindle apparatus leading to the presence of lagging chromosome/

Figs. 12–21. PMCs, at diakinesis/metaphase-I, in species of *Astragalus*. 12, *A. nivalis*. 13, *A. peduncularis*. 14, *A. rhizanthus*. 15, *A. scorpiurus* var. *scorpiurus*. 16, *A. stewartii*. 17, *A. subuliformis*. 18, *A. tibetanus*. 19, *A. tribulifolius*. 20, *A. webbianus*. 21, *A. zanskarensis*. Fig. 22. A PMC of *A. macropterus*, at late anaphase-I, showing 4 lagging chromosomes. Fig. 23. A PMC of *A. scorpiurus* var. *scorpiurus*, at late anaphase-I, showing a chromatin bridge. (Scale=10 μ m).

bivalents or stickiness of chromosomes resulting in the formation of what appear like chromatin bridges. None of the earlier workers have reported any such anomaly in the species of *Astragalus* studied by them (Senn 1938, Ledingham 1957, 1958, Bijok *et al.* 1972, Spellenberg 1974, 1976). Despite these anomalies the pollen stainability in these species was as high as 99.16% (*A. hoffmeisteri* var. *hoffmeisteri*).

Since none of the earlier workers, as pointed out earlier, has given details of reduction division in any species of this genus, it is not possible to make any comparisons.

Fig. 24. Relationships among total chromatin lengths, chiasma frequencies at diplotene and metaphase-I and recombination indices in 17 species of *Astragalus*.

Summary

The present communication puts on record the chromosome behaviour during reduction division in the pollen mother cells of 21 diploid species of *Astragalus*, growing wild in the Kashmir Himalaya. In all these species meiosis was by and large normal. The occasional segregational anomalies, observed in a few species, have also been described. The low frequency of PMCs with these anomalies indicate that these are not due to any genetic change but can be explained on the basis of occasional segregational errors only. It has been pointed out that there is a positive correlation between the recombination indices and the total chromatin lengths in these species.

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