Bearing of Cytology on the Systematic Position of *Ixiolirion* (Fischer) Herb. (Amaryllidaceae)

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Despite the fact that Amaryllidaceae has been a favourite plant group for cytotoxic studies, little attention has been paid to *Ixiolirion* (Fischer) Herb., a small but distinct genus of the family. On the basis of morphological description, *Ixiolirion* was placed in subfamily Agaveae of Amaryllidaceae by Herbert (1837). Subsequently, Benthem and Hooker (1883) and Baker (1888) referred it to subfamily Alstroemerieae of the same family. Hutchinson in 1934 raised, among others, orders Agavales and Alstroemeriales distinct from Amaryllidales and placed *Ixiolirion* within the latter as the only member of tribe Ixioliriae of family Amaryllidaceae (Hutchinson 1959). Traub (1957) followed Hutchinson in placing the genus in Amaryllidaceae but recognizing its somewhat different morphology from other members of the family, raised it to the status of a subfamily, Ixiolirioideae. This position has been maintained by Takhtajan in a more recent classification of angiosperms (Takhtajan 1980).

Species delimitation in *Ixiolirion* has been quite controversial. While some systematists have named as many as five species and several varieties (see for example Komarov 1935, Traub 1942, Wendelbo 1970, Nasir 1980), Traub (1942) recognized just one, *I. tataricum*.

The two problems that seek solution are: phylogeny of the genus *Ixiolirion*, and its species composition. Hitherto the only information available on cytology of the genus is chromosome numbers of two taxa (Traub 1942, Murin and Sheikh 1971). These reports put the $2n$ number at 24. During the present study chromosome complement and male meiosis of *I. tataricum* (Pall.) Herb., growing wild in saffron fields of Kashmir, were studied for eliciting information on the two problems pointed above.

Materials and methods

Plants of *I. tataricum* were collected from saffron fields in Pampore, Kashmir. Root tips were obtained by sowing bulbs in moist sand. About 1 cm long tips were prefixed in a saturated solution of paradichlorobenzene for 3 hr at 10°C and later fixed in 1:3 acetic alcohol solution. The root tips were hydrolysed in 9:1 aceto-orcein:NHCl mixture and squashed in 1% aceto-orcein. For meiosis flower buds were fixed in a solution of 1 acetic acid:1 alcohol:1 chloroform for 4-12 hr. Chromosome classification proposed by Battaglia (1955) has been followed. The terms metacentric (chromosomes with median centromere), submetacentric (chromosomes

with submedian centromere) and acrocentric (chromosomes with subterminal centromere) have also been used.

Figs. 1-2. 1, somatic chromosomes; 1', explanatory diagram to Fig 1. 2, karyoidiogram of hexaploid population of *Ixiolirion tataricum* showing 72 chromosomes. Scale 10 μ .

Observations

Morphology: Plants 25-48 cm tall bulbous perennials, bulbs ovoid, covered with dark brown coriaceous coats; basal leaves 3-5, forming a tuft at the soil surface, linear, flat, slightly grooved; scape 21-31 cm long with 0-2 leaves; flowers 2-5 in

1-2 small and loose umbels, spathaceous, bracts lanceolate, each subtending a pedicellate flower; flowers broadly infundibuliform, 2.3-2.8 cm long and 2.4-3.1 cm across; perianth tube absent, lobes reflexed above, blue violet with prominent white band running down the whole length on the inner side; stamens fixed near the base of

Figs. 3-6. Diplotene-diakinesis stages in the male meiosis of *Ixiolirion tataricum*. 3, 3 VI+3 IV+1 III+19 II+1 I. 4, 2 VI+2 IV+26 II. 5, 1 VI+1 IV+31 II. 6, 1 VI+1 IV+30 II+2 I. Scale 10 μ .

perianth; anthers basifixed, ovary inferior, ovules 12-15 per locule.

Karyotype: Root tip cells of all plants scanned for cytology contained 72 chromosomes (Figs. 1-2) ranging between 0.9-5.8 μ . On the basis of their length and arm ratio the chromosomes form 12 groups of 6 chromosomes each. The first two

groups include 12 long chromosomes designated here as L type, the remaining 10 groups consisting of short chromosomes are collectively referred to as the S type.

L_1 : Chromosomes 1–6, 4.8–5.8 μ long, arm ratio 1.09–1.22, meta-submetacentric;

L_2 : Chromosomes 7–12, 3.4–4.2 μ long, arm ratio 2.78–3.67, submeta-acrocentric;

S_1 : Chromosomes 13–18, 1.9–2.2 μ long, arm ratio 1.63–2.14, submetacentric;

S_2 : Chromosomes 19–24, 1.8–2.3 μ long, arm ratio 1.09–1.40, meta-submetacentric;

S_3 : Chromosomes 25–30, 1.5–2.0 μ long, arm ratio 1.38–2.33, metacentric;

Figs. 7–9. Metaphase I stages in the male meiosis of *Ixiolirion tataricum*. 7, 3 IV+30 II. 8, 1 IV+34 II. 9, 36 II. Scale 10 μ .

S_4 : Chromosomes 31–36, 1.5–1.8 μ long, arm ratio 1.0–1.5, meta-submetacentric;

S_5 : Chromosomes 37–42, 1.4–1.6 μ long, arm ratio 1.29–1.33, submetacentric;

S_6 : Chromosomes 43–48, 1.4–1.5 μ long, arm ratio 1.0–1.14, meta-submetacentric;

S_7 : Chromosomes 49–54, 1.3–1.5 μ long, arm ratio 1.25–1.60, submetacentric;

S_8 : Chromosomes 55–60, 1.2–1.4 μ long, arm ratio 1.0–1.17, meta-submetacentric;

S_9 : Chromosomes 61–66, 1.0–1.3 μ long, arm ratio 1.50–1.75, submetacentric;

S_{10} : Chromosomes 67–72, 0.9–1.2 μ long, arm ratio 1.25–1.75, submetacentric.

Karyotype formula : 6 L Me-Sm+6 L Sm-St+36 S Sm+24 S Me-Sm.

Male meiosis: Flower buds of *I. tataricum* undergo male meiosis some 2–3 weeks before their opening. On account of high number and small size of chromosomes, early stages of meiosis were not quite compromising.

At diplotene-diakinesis, majority of the chromosomes were involved in bivalent associations, nonetheless some multivalent associations were found in all well spread cells (Figs. 3–6). The 17 pollen mother cells scored for chromosome configurations

Table 1. Chromosome associations at diplotene-diakinesis in *I. tataricum*

S. no.	Associations						No. of cells	Fig. no.
	VI	V	IV	III	II	I		
1	3	—	3	1	19	1	1	3
2	2	—	3	—	24	—	1	
3	2	—	2	—	26	—	2	4
4	2	—	1	—	28	—	1	
5	1	1	1	1	26	2	1	
6	1	—	2	—	29	—	1	5
7	1	—	1	—	31	—	1	
8	1	—	1	—	30	2	1	6
9	—	—	4	1	26	1	1	
10	—	—	3	—	30	—	5	
11	—	—	2	1	30	1	1	
12	—	—	1	1	32	1	1	
Total	15	1	38	5	411	8	17	

Table 2. Chromosome associations at metaphase I in *I. tataricum*

S. no.	Associations						No. of cells	Fig. no.
	VI	V	IV	III	II	I		
1	1	—	1	—	31	—	3	
2	1	—	—	—	33	—	1	
3	—	1	—	—	33	1	1	
4	—	—	3	—	30	—	2	7
5	—	—	2	—	32	—	6	
6	—	—	1	—	34	—	11	8
7	—	—	1	—	32	4	1	
8	—	—	—	1	34	1	1	9
9	—	—	—	—	36	—	16	
10	—	—	—	—	34	4	7	
11	—	—	—	—	32	8	1	
Total	4	1	33	1	1691	42	50	

carried an average of 0.88 VI, 0.06 V, 2.24 IV, 0.29 III, 28.06 II and 0.47 I (Table 1). Upto 3 hexavalents and 4 quadrivalents were observed in cells at diplotene. The univalents ranged from 1 to 2 per cell. Chiasmata were found randomly distributed in all chromosome associations.

By the time metaphase I was reached, multivalent frequency reduced with a proportionate increase in bi- and univalent frequencies. The 50 metaphase spreads

scored for chromosome associations (Figs. 7-9) carried on an average 0.08 VI, 0.02 V, 0.66 IV, 0.02 III, 33.94 II and 0.84 I (Table 2). The maximum number of hexavalents and quadrivalents observed in any pollen mother cell was 1 and 3 respectively.

Of the 40 pollen mother cells scored at anaphase I (Table 3), 18 followed regular (36:36) chromosome segregation. In 12 cells the distribution was 35:37; other deviant patterns were rare. Occasionally 1-3 chromosomes were observed lagging at the equatorial plate. Although mature pollen was uniform in size; some micro-pollen grains (0.67%) were also recorded. The latter have their origin in the persistent laggards which first form micronuclei and subsequently differentiate as micro-pollen grains. Pollen stainability was as high as 90.5%.

Table 3. Anaphase I distribution of chromosomes in *I. tataricum*

	32	34	35	36	34	34	34	35
Distribution pattern	:	:	:	:	:	:	:	:
	40	38	37	36	37	35	36	36
Number of cells	1	4	12	18	1	1	1	2

Discussion

Chromosome number

Except for two reports on chromosome numbers of *I. tataricum* (Traub 1942) and *I. montanum* (Murin and Sheikh 1971), little is known about the cytology of genus *Ixiolirion*. These reports indicate that both species are diploid with $2n=24$.

Regarding 12 as the base number for the genus, the present population of *I. tataricum* having somatic complement of 72 chromosomes represents a hexaploid. Polyploid nature of this population is reflected in the idiogram wherein chromosomes fall into groups of six. Chromosomes constituting each group exhibit minor size differences, more prominently among the long (L_1 and L_2) than in the short members. As to their arm ratio, chromosomes within each group demonstrate good agreement; L_2 and S_3 being the only exceptions. When studied in respect of overall size and arm ratios of the chromosomes, Kashmir population of *I. tataricum* seems to represent a segmental allopolyploid. Meiotic pairing whereby only 22.06% chromosomes at diplotene-diakinesis are seen involved in multivalent configurations, the rest remaining as bivalents, supports this conclusion. However, reliability of chromosome morphology and meiotic pairing should not be overemphasized in assessing the nature of polyploidy. Stebbins (1971) regards mitotic chromosomes as mere shells of the genetic material on which too much reliance cannot be laid for interpreting phylogeny. Multivalent frequency as well, does not necessarily speak of the extent of genetic homology between constituent genomes of a polyploid because chromosome pairing is controlled by several other factors such as chromosome length (Stebbins 1950), chiasma localization (Levan 1940), genic multivalent suppression system (Riley and Chapman 1958) B chromosomes (Evans and Macefield 1973) and period of

establishment (Gilles and Randolph 1951).

Systematic position and interrelationships

With its coloured, trimerous and epigynous flowers borne in scapose umbels, *Ixiolirion* has rightly been placed in Amaryllidaceae (*sensu lato*). However, its intrafamilial placement is still unresolved. The confusion stems from its unique morphological features in some of which, like the arrangement of flowers in loose umbels or clusters and presence of leaves on the scape, it resembles Alstroemerieae and Agaveae.

Benthem and Hooker (1883) include *Ixiolirion* in Alstroemerieae along with *Alstroemeria*, *Bomarea* or *Leontochir*. Members of the tribe have rhizomatous or tuberous root stock but *Ixiolirion* has bulbous root system. Karyotype of *Ixiolirion* has little in common with typical Alstroemerieae. Chromosome numbers of the tribe (Alstroemerieae of Darlington and Wylie 1955) are in multiples of 8 and 9. The chromosomes of *Alstroemeria* are relatively long (4.1–18.6 μ as calculated from figures drawn by Satô 1938) with 8 of the 16 chromosomes bearing subterminal centromere. The 18 chromosomes of *Bomarea* group into 4 metacentric and 14 acrocentric. In contrast, *Ixiolirion* has base number 12, chromosomes are small (0.9–5.8 μ), and as many as 11 chromosomes of the basic set bear median-submedian centromere. On the basis of root system and karyotype there is hardly any justification for placing *Ixiolirion* in Alstroemerieae.

With Agaveae too *Ixiolirion* does not seem to bear a close natural link. Agaves are mostly woody with dry and persistent perianth. Bulk of Agaveae (Agavaceae of Darlington and Wylie 1955) have chromosome numbers in multiples of 30 with characteristic *Yucca-Agave* type karyotype (Satô 1938) consisting of 5 long and 25 short acrocentric chromosomes. The ratio between long and short chromosomes is as high as 13:1. In these features *Ixiolirion* differs from majority of Agaveae. The overall evidence of cytology and plant morphology does not, therefore, justify inclusion of *Ixiolirion* in either Alstroemerieae or Agaveae.

Among the remaining epigynous Amaryllidaceae (*sensu* Hutchinson) *Ixiolirion* holds an isolated and primitive position because of its subumbellate inflorescence and leafy scape; all other genera have flowers solitary or borne in strict umbels and leafless scape. This position is reflected in its karyotype. Chromosomes of *Ixiolirion* are quite small unlike most other amaryllids which bear large chromosomes (Mehra and Sachdeva 1976). Its karyotype is symmetric with all except one chromosome of the basic set bearing median or submedian centromere. Obviously, creation of a distinct tribe (Ixiolirieae) or subfamily (Ixioliriodeae) to accommodate the single genus *Ixiolirion* seems to be justified.

Hutchinson (1959) has treated the group Hemerocallideae as a connecting link between Liliaceae and Amaryllidaceae. Regarding *Ixiolirion* as a primitive genus of the latter it should bear some similarity with Hemerocallideae. This seems to be the case. Hemerocallideae are characterized by plants with rhizomatous or bulb-like root stock, basal leaves and a leafy scape bearing hypogynous flowers in a racemose or paniculate inflorescence. The difference in position of ovary notwithstanding, *Ixiolirion* differs from Hemerocallideae only in the degree of leafiness of

scape and restriction of flowers to more terminal clusters. Cytologically also the two are quite comparable. Karyotype of *Hemerocallis*, a well worked out genus of Hemerocallideae, consists of a basic set of 11 relatively small chromosomes 8 to 9 of which bear median-submedian centromere (Zadoo *et al.* 1976). These similarities without necessarily speaking of any direct relationship between the two taxa certainly favour the derivation of at least some primitive epigynous Amaryllidaceae from Hemerocallideae of the Liliaceous stock.

As mentioned earlier species delimitation in *Ixiolirion* has been quite confusing. Commenting upon *I. tataricum* Wendelbo (1970) writes "... is a variable species. There are all kinds of transitions, and the distribution pattern of different forms does not make any sense". The present record of a hexaploid population throws light on the cause of this infrageneric confusion. With its vegetative mode of reproduction *Ixiolirion* can accumulate polyploidy regardless of its impact on fertility. Thus a range of euploids upto at least 6x should exist in nature. Being a segmental allopolyploid the genetic constitution of the basic diploids of the hexaploid must be somewhat different. Hybridization and backcrossing in polyploids can give rise to complexes which range in morphology all the way from one diploid parent to the other. Occasionally they even acquire characters vastly different from either parent (Davis and Heywood 1963). Further, if anaphase I chromosome distribution in the present material is any index, polyploidy widens the spectrum of variability by producing gametes with deviant numbers, some of which on involvement in fertilization may lead to production of aneuploids. Probably a combination of all these factors has resulted in a highly variable genus. Its resolution will require detailed cytomorphological studies on populations from various locations.

Summary

Genus *Ixiolirion* is represented in Kashmir by a single species, *I. tataricum*. Plants collected from Pampore are hexaploid with 72 chromosomes. Karyology and mode of chromosome synapsis during male meiosis indicate that the population is a segmental allopolyploid. Comparison of karyotypes reveals that the chromosomes of *Ixiolirion* hardly bear any structural homology with members of either Alstroemerieae or Agaveae. Presence of leafy scape and subumbellate inflorescence along with cytological features like very small chromosomes and symmetric karyotype give *Ixiolirion* a unique and primitive position in Amaryllidaceae (*sensu* Hutchinson) and justify its treatment into a distinct tribe Ixiolirieae or subfamily Ixiolirioideae. Similarity between *Ixiolirion* and Hemerocallideae in both plant morphology and chromosome complement supports their placement close to one another. Polyploidy and segregational irregularities during meiosis explain the existence of morphological diversity and consequent taxonomic confusion in the genus.

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* Not seen in original.