Cytology of the Genus *Alysicarpus* Neck. ex Desv. (Fabaceae)

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Received April 21, 1976

The genus *Alysicarpus*, represented by 15 species is widely distributed in the tropics of the old world and Australia (Cooke 1903). The present work considers 6 species, of which five are from different localities in India and one is from Zambia.

The cytological work on the genus was undertaken by Frahm-Lelived for the first time in 1957. Thereafter several others viz. Miede (1960), Gadella and Kliphuis (1964), Bir and Sidhu (1967), Mitra and Datta (1967), Baquar and Warsi (1968), Mehra and Dhawan (1971), Bir and Kumari (1973), Bhatt (1974) and Sareen and Singh (1975) have reported from time to time the chromosome number of various species of the genus. It is only in the work of Bhatt (1974), that a preliminary attempt to describe the karyotypes was made. The close scrutiny of the karyotypes revealed more details. Moreover, the meiotic studies of the taxa yielded information concerning the normal behaviour of chromosomes and some abnormalities.

Material and methods

Source of the material:

Taxa	Source
<i>A. longifolius</i> Wt. and Arn.	Dangs, Gir, Rajpipla.
<i>A. procumbens</i> (Roxb.) Schindler (= <i>A. hamosus</i> Edgew.)	Baroda, Chhotaudaipur, Devgadhbaria.
<i>A. monilifer</i> (Linn.) DC.	Baroda, Aurangabad, Dangs.
<i>A. tetragonolobus</i> Edgew.	Pavagadh, Dangs.
<i>A. ovalifolius</i> (Schumacher) J. Léon.	Zambia
<i>A. vaginalis</i> (Linn.) DC.	Baroda, Aurangabad, Dangs, Dehra Dun.

The seeds collected from different localities were given separate voucher numbers and voucher specimens were deposited in departmental Herbaria. All collections were grown in Botanical Gardens, The M. S. University of Baroda, for cytological investigations. Seeds were treated with conc. H_2SO_4 for 3-5 minutes and then thoroughly washed in running water before germination. Tjio and Levan's (1950) oxyquinoline aceto-orcin squash technique was adopted for mitotic preparations from root tips. Flower buds of suitable size, were fixed in modified Carnoy's fixative (6:3:1) for 24 hours before smearing in aceto-carmine.

Drawings were made at table level on a Steindroff research microscope using E. Leitz Wetzler Camera lucida apparatus at 30 or 15 $\times$ 120 (apochromat) magnification. Histograms represent the total length of haploid set of chromosomes. Chromosomes with satellites and secondary constriction are represented by out line.

Observations

For better comparison of karyotypes, uniform categorization based on size dif-

Figs. 1-6. Somatic metaphase plates of *Alysicarpus longifolius*, *A. procumbens*, *A. tetragonolobus*, *A. monilifer*, *A. ovalifolius* and *A. vaginalis*.

ference and the ratios of short arm/long arm (R_1) and long arm/short arm (R_2) (Adhikary 1974) was made. The chromosomes of all the investigated species can be classified into the following types:

Figs. 1a-6a. Comparative idiograms.

Type:

A—Chromosomes less than 2μ in length with nearly median centromere (R_1 between 0.99-0.61 and R_2 between 1.01-1.63).

B—Chromosomes less than 2μ in length with nearly sub-median centromere (R_1 between 0.66-0.34 and 0.32-0.23 and R_2 between 1.64-2.99 and 3.01-

4.26).

C—Chromosomes $2-4\mu$ in length with nearly median centromere (R_1 between 0.99–0.61 and R_2 between 1.01–1.63).

D—Chromosomes $2-4\mu$ in length with nearly submedian centromere (R_1 between 0.66–0.34 and 0.32–0.23 and R_2 between 1.64–2.99 and 3.01–4.26).

Superscript S denotes Secondary constriction.

„ S' denotes satellite.

Alysicarpus longifolius Wt. and Arn.

$$(2n=16=C_8+D_6+D_2^s)$$

The earlier reports of n and $2n$ numbers are confirmed in the present study. The taxon studied has equal numbers of nearly median and nearly submedian types of chromosomes. Moreover, one pair of nearly submedian type (D^s) with secondary constriction on long arm is observed. This is in contrast to earlier karyotypic study (Bhatt 1974), in which 1 pair of median and 7 pairs of submedian chromosomes were noticed. The chromosome length and F% ranges from 2.04μ to 3.07μ and 33.33 to 41.46 respectively (Fig. 1 and 1a).

The normal meiosis showing 8 distinct bivalents at diakinesis was observed in a majority of the PMCs. However, cytomixis, a tendency of association of bivalents, non-synchronised distribution at anaphase II and persistent micronuclei were recorded in some PMCs (Figs. 7a-b).

Alysicarpus procumbens (Roxb.) Schindler

=(*A. hamosus* Edgew.)

$$(2n=16=A_4+C_4+D_6+D_2^s)$$

The present investigation confirms the earlier reports of the chromosome number. The karyotype analysis reveals the presence of 2 pairs of short chromosomes (A type) and 6 pairs of long chromosomes (C and D types). However, 1 pair (D^s type) with secondary constriction was also observed. Earlier studied taxon (Bhatt 1974) showed 4 pairs of long and short chromosomes each, without any secondarily constricted chromosomes. The chromosome length and F% ranges from 1.78μ to 3.15μ and 30.15 to 42.69 respectively (Figs. 2 and 2a).

The meiosis was regular showing 8 distinct bivalents at diakinesis and metaphase I. In some cases association of bivalents were recorded (Figs. 7f-h).

Alysicarpus tetragonolobus Edgew.

$$(2n=16=C_4+D_8+D_2^s+D_2^s)$$

Earlier reports of $n=8$ and $2n=16$ are confirmed. The taxon investigated has within its complement, 2 pairs of nearly median and 6 pairs of nearly submedian types of chromosomes. A pair of chromosomes with secondary constrictions and a pair

Figs. 7a-s. Magnification of the drawings. $1800\times$. 7a-d, diakinesis, metaphase I, anaphase I and anaphase II (non-synchronisation) of *A. longifolius*. 7e, metaphase I (side view) of *A. tetra-*

gonolobus. 7f-h, anaphase I, metaphase I (polar view) showing 2 groups of associations of bivalents and metaphase I (side view) of *A. procumbens*. 7i-k, diakinesis, metaphase I (polar view) associations of bivalents in 2 groups and unequal distribution (7+9) of *A. monilifer*. 7l-m, diakinesis, metaphase II (polar view) normal of *A. ovalifolius*. 7n-q, diakinesis, metaphase I (polar view) with interconnections of bivalents, metaphase I (polar view) with 2 groups of associations of bivalents. Metaphase I (side view) normal of *A. vaginalis*. 7r-s, cytomixis and micronuclei.

with satellites, not recorded in the earlier work (Bhatt 1974), were observed. The material examined differs from the earlier analysis in having comparatively shorter chromosomes. The chromosome length and F% ranges from 2.37μ to 3.40μ and 26.38 to 48.24 respectively (Figs. 3 and 3a).

During meiosis the chromosome behaviour is fairly regular, showing presence of 8 distinct bivalents, at metaphase I and in their subsequent divisions. Presence of micronuclei is common (Fig. 7e).

Alysicarpus monilifer (Linn.) DC.

$$(2n=16=C_2+D_{10}+D_2^s+D_2^{s'})$$

The present report is in agreement with the earlier reports of n and $2n$ numbers for the taxon. Comparison of the presently and previously analysed (Bhatt 1974), karyotypes, showed similarity in types of chromosomes. One pair of chromosomes with secondary constrictions (D^s type) and a pair with satellites ($D^{s'}$ type) observed presently were not reported in the earlier work. The chromosome length and F% ranges from 2.46μ to 3.80μ and 28.61 to 46.86 respectively (Figs. 4 and 4a).

The PMCs showed regular behaviour of chromosomes during meiosis. However, unequal distribution and associations of bivalents were recorded in a few cases only (Figs. 7i-k).

Alysicarpus ovalifolius (Schumach) J. Léon.

$$(2n=16=A_2+B_8+C_2^{s'}+D_2+D_2^s)$$

This is the first report of n and $2n$ numbers and karyotype analysis for the species. The complement showed the presence of 2 pairs of nearly median and 6 pairs of nearly submedian types of chromosomes, within which a pair of secondarily constricted (D^s type) and a pair of chromosomes with satellites ($C^{s'}$ type) are also noticed. The chromosome length and F% ranges from 1.36μ to 2.46μ and 29.06 to 45.45 respectively (Figs. 5 and 5a).

Meiosis is regular showing 8 bivalents at diakinesis. Occurrence of micronuclei and associations of bivalents are observed along with the normal separation of chromosomes (Figs. 7l-m).

Alysicarpus vaginalis (Linn.) DC.

$$(2n=16=A_2^{s'}+B_6+D_6+D_2^s)$$

Earlier reports of other workers except that of Gadella and Kliphuis (i. e. $2n=20$), are confirmed in the present study. The presently analysed karyotype resembles the earlier one (Bhatt 1974) in types of chromosomes (1 pair of nearly median and 7 pairs of nearly submedian), but differs in having a pair of secondarily constricted (D^s type) and a pair of chromosomes with satellites ($A^{s'}$ type) in the complement. The chromosome length and F% ranges from 1.44μ to 2.38μ and 30.25 to 44.44 respectively (Figs. 6 and 6a).

The PMCs showed regular meiotic behaviour. Along with this micronuclei, associations of bivalents and interbivalent connections were recorded (Figs. 7n-q).

Discussion

The observations concerning number, morphology and meiotic behaviour of chromosomes of 6 species of the genus *Alysicarpus*, are included in the present paper. The occurrence of $2n=16$ and $n=8$ for all the taxa supports the claim of considering $x=8$ as the basic number for the genus. The genus as a whole shows consistency of chromosome number ($2n=16$), of which majority of them are of 'C' and 'D' types (i. e. $2-4\mu$). The number of each type varies from species to species (Figs. 1a-6a and Table 2). Moreover, all the investigated taxa shows a common feature of presence of a pair of secondarily constricted chromosomes (D type), which clearly indicates that the genomes of different species are related.

Table 1. Chromosome numbers in investigated taxa of *Alysicarpus*

Taxa	Present report		Previous reports
	2n	n	
<i>Alysicarpus longifolius</i>	16	8	Bir and Kumari (1973) $n=8$; Bhatt (1974) $2n=16$
<i>Alysicarpus procumbens</i>	16	8	Bir and Kumari (1973) $n=8$; Bhatt (1974) $2n=16$; Sareen and Singh (1975) $n=8$
<i>Alysicarpus tetragonolobus</i>	16	8	Bhatt (1974) $2n=16$; Sareen and Singh (1975) $n=8$
<i>Alysicarpus monilifer</i>	16	8	Bir and Sidhu (1967); Baquar and Warsi (1968); $n=8$; Bhatt (1974) $2n=16$
<i>Alysicarpus ovalifolius</i>	16	8	—
<i>Alysicarpus vaginalis</i>	16	8	Frahm-Leliveld (1957) $2n=16$; Gadella and Kliphuis (1964) $2n=20$; Bir and Sidhu (1967) $n=8$; Mitra and Datta (1957) $n=8$; Bhatt (1974) $2n=16$

Precise determination of arm ratios (R_1 and R_2) of chromosomes of different taxa, shows the presence of nearly median and nearly submedian types of chromosomes. The nearly submedian types are always more in number as compared to nearly median types, indicating the advanced nature of the genus. The karyotypes are asymmetrical as the TF% is between 34.90 and 38.02 (Table 2). The degree of asymmetry varies with different taxa, depending upon the number of different types of chromosomes and the ratio of longest to the shortest pair (L/S) in the complement.

Karyotypes of *Alysicarpus longifolius* and *A. procumbens* resemble more closely in having equal numbers of nearly median and nearly submedian types of chromosomes. *A. procumbens* has 2 pairs of A-type and 2 pairs of C-type in contrast to 4 pairs of 'C'-type found in *A. longifolius*. The karyotype of *A. procumbens* therefore, appear slightly more asymmetrical than *A. longifolius*.

A. tetragonolobus and *A. monilifer* are closely comparable in having similar types and equal numbers of satellited and secondarily constricted chromosomes.

Table 2. Comparison of the somatic chromosomes of investigated taxa of *Alysicarpus*

Taxa	Somatic number (2n)	Number of chromosomes with stated types of centromere			Number of Secondarily constricted and satellited chromosomes		Mean length (in μ)	T. F. %	Ratio of longest to shortest pair of chromosomes (L/S)
		Nearly median	Nearly submedian		Secondary constrictions	Satellited			
<i>Alysicarpus longifolius</i>	16	8	8		2	—	2.51	38.02	1.50
<i>Alysicarpus procumbens</i>	16	8	8		2	—	2.44	36.75	1.76
<i>Alysicarpus tetragonolobus</i>	16	4	12		2	2	3.00	34.91	1.43
<i>Alysicarpus monilifer</i>	16	2	14		2	2	2.94	35.68	1.55
<i>Alysicarpus ovalifolius</i>	16	4	12		2	2	1.96	35.62	1.80
<i>Alysicarpus vaginalis</i>	16	2	14		2	2	1.94	34.90	1.65

The complement of *A. monilifer* has only 1 pair of nearly median type in contrast to 2 pairs (C-type) found in *A. tetragonolobus*. As the L/S (Table 2) in *A. monilifer* is 1.55 in contrast to 1.43 in *A. tetragonolobus*, the karyotype of the preceeding species is considered more asymmetrical.

2 species viz. *A. ovalifolius* and *A. vaginalis* share the common features like the presence of long ($2-4\mu$) and short (less than 2μ) types of chromosomes, a pair of satellited and a pair of secondarily constricted chromosomes. As the number of nearly submedian types are more in *A. vaginalis* than in *A. ovalifolius*, the karyotype of *A. vaginalis* is considered more advanced. However, TF% and L/S (Table 2) of *A. ovalifolius* are 35.62 and 1.80 respectively in contrast to 34.90 and 1.65 of *A. vaginalis*. The karyotype of *A. vaginalis* though an advanced one, exhibits less asymmetrical nature than that of *A. ovalifolius*.

The meiotic behaviour of chromosomes in all the species is fairly regular showing 8 distinct bivalents at diakinesis and metaphase I. However, the occurrence of cytomixis and micronuclei in some PMCs were observed in all the species (Figs. 7r-s). A few noteworthy features like interbivalent connections, a tendency towards associations of bivalents, non-synchronisation and unequal distribution of chromosomes, though not common were recorded in some species. Interbivalent connections leading to secondary associations have been reported earlier by Thomas and Ravell (1946) in *Cicer* and Majumdar (1953) in *Monochoria hastaefolia* Presl. Significance of these in the presently investigated species cannot be ascertained at this juncture. Further work on the other species of the genus will be published in due course.

Summary

6 species of the genus *Alysicarpus* are investigated. The detailed karyotypic analysis revealed the presence of 1 pair of secondarily constricted chromosomes (D type) in all the species justifying their inclusion in one genus. Though karyotypes exhibit gross similarity, they differ from each other in minor details. The regularity of meiotic behaviour is noticed, in all the taxa. However, some abnormalities are also recorded. The basic number $x=8$ for the genus is supported by this work.

Acknowledgement

The authors are grateful to Professor C. H. Pathak, and Dr. S. D. Sabnis, Department of Botany, The M. S. University of Baroda, Baroda, for their constant help during the course of the work.

References

- Adhikary, A. K. 1974. Precise determination of centromeric locations. *Cytologia* **39**: 11-16.
- Bir, S. S. and Sidhu, S. 1967. Cytological observations on the north Indian members of family Leguminosae. *Nucleus* **10**: 47-63.

- Baquar and Warsi, 1968. IOPB Chromosome number reports. *Taxon* **17**: 17: 91-104.
- Bir, S. S. and Kumari, S. 1973. IOPB Chromosome number reports. *Taxon* **22** (2/3): 285-291.
- Bhatt, R. P. 1974. Studies on the flora in north Gujarat III. *Cytology*. *Nucleus* **17** (1): 33-39.
- Cooke, T. 1903. *Flora of the Presidency of Bombay*, Vol. I, (Repr. Edition), Calcutta, 1958.
- Frahm-Leliveld, J. A. 1957. Observations cytoologiques sur quelques légumineuses tropicales et sub tropicales. *Rev. Cytol. et Biol. Veg.* **8**: 273-287.
- Gadella, T. W. J. and Kliphuis, E. 1964. Chromosome numbers of some flowering plants collected from Surinam. *Acta. Bot. Neerland.* **13**: 432-434.
- Majumdar, A. 1953. An investigation on the cytology of *Monochoria hastaeifolia* Presl. *Caryologia* **5**: 306-312.
- Miege, J. 1960. Quartrima liste de nombres chromosomiques d'plantes d' Afrique occidentale. *Cytol. et Biol. Veg.* **21**: 373-384.
- Mitra, K. and N. Datta, 1967. IOPB Chromosome number reports. *Taxon* **16** (5): 445-461.
- Mehra, P. N. and Dhawan, H. 1971. IOPB Chromosome number reports. *Taxon* **20**: 785-797.
- Sareen, T. S. and Singh, P. D. 1975. IOPB Chromosome number reports. *Taxon* **24** (4): 501-516.
- Thomas, P. T. and Revell, S. H. 1964. Secondary association and heterochromatic attraction I. *Cicer arietinum*. *Ann. Bot., N. S.* **10**: 159-164.
- Tjio, J. H. and Levan, A. 1950. The use of oxyquinoline in chromosome analysis. *Ann. Estae. Expt. Aula. Dei.* **2** : 21-64.
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