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CYTOLOGICAL INVESTIGATIONS IN THREE SPECIES OF *CONVOLVULUS* LINN. FROM PAKISTAN

VIQAR ANJUM SAEED, S. AFAQ HUSAIN AND S. SHAHID HUSAIN

PCSIR Laboratories Complex, Karachi-75280, Pakistan

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Convolvulus arvensis Linn., *C. glomeratus* Choisy and *C. prostratus* Fors, were cytologically investigated (n= 25 for *C. arvensis* and n= 14 for *C. glomeratus* with normal meiotic behaviour were found). Two chromosomal forms with n=18 and 20 were recorded for *C. prostratus*. Anomalous behaviour during microsporogenesis was observed with univalents, trivalents and quadrivalents in 53% of pollen mother cells (PMCs). Complete bivalent formation was recorded in 47% of PMCs scored.

Key words: *Convolvulus*, Anomalous microsporogenesis, Polyploidy, Diploidization.

Introduction

Convolvulus Linn. is a large genus comprising of 250 species, out of which 21 occur in Pakistan [1]. *Convolvulus arvensis* Linn.,*C. glomeratus* Choisy and *C. prostratus* Forsk, commonly grow as weeds in the region of study. These species show considerable morphological variations in shape, size, pubescence of leaf, colour and size of flower etc. [1], *Convolvulus prostratus* is the most variable among them and apparently forms a complex. Previously two species *C. microphyllus* Sieb. ex spreng and *C. pluricaulis* Choisy have been identified from this region by many workers [2–5]. Later Austin *et al.* [1] working on *Convolvulaceae* (for Flora of West Pakistan) did not identify the above two species as separate taxonomic units, rather put them under *C. prostratus* Forsk, probably due to continuity in variability of characters. The present cytological studies were initiated to determine if these variations are reflected in chromosonal behaviour during microsporogenesis. Variable meiotic and somatic counts have been reported by many workers [6–12]. However, there has been only one report of *C. glomeratus* [5].*Convolvulus prostratus* as such has not been studied cytologically but has been studied as *C. microphyllous* Seib. ex spreng and *C. pluricaulis* Choisy with variable chromosome counts [6–8,11–13].

Materials and Methods

Suitable young buds of *C. arvensis, C. glomeratus and C. prostratus* were fixed in carnoys solution (Chloroform: ethenol: acetic acid 6:3:1) for 24 hrs and stored in 70% alcohol for meiotic studies. Young anthers were teased out of a bud and squashed in 2% aceto carmine solution, after gentle heating pressure was applied to the cover slip so that the chromosomes are well spread and are observable in one plane. Normal and abnormal PMCs were scored under the microscope. Camera lucida drawings were made at approximately 2000 x. Pollen fertility was studied by staining pollen grains with acetocarmine and recording stained rounded and unstained shrunken pollen grains.

Identification of plants was made according to Austin *et al.* [1]. The specimens for cytological investigations were collected from four localities *viz.* Memon Goth, Nazimabad, Karachi University Campus and Damloti. Voucher specimens are deposited in PCSIR Herbarium.

Observations. During cytological studies of three species of *Convolvulus* two species *C. arvensis* and *C. glomeratus* displayed normal meiotic behavior. In *C. arvensis*, 25 bivalents were recorded at metaphase-I and anaphase-I (Figs. 1,2) with 98% pollen fertility. *Convolvulus glomeratus* also exhibited normal meiosis with 14 bivalents at meta-I and ana-I (Figs. 3,4), in *C. prostratus* two chromosomal forms with n=18 and n=20 have been recorded (Figs. 5–8). Contrary to *C. arvensis* and *C. glomeratus*, both the chromosomal forms in *C. prostratus* showed anomalous behaviour. Univalents, trivalents and quadrivalents were found in 53% PMCs at metaphase-I (Figs. 9–12). Due to the presence of univalents and multivalents, bridges and laggards at anaphase-I were also noted (Figs. 13,14). These irregularities were carried over to the second meiotic division as chromosomes were found lying away from metaphase plate (Fig. 15). Bridges and laggards were also recorded at 2nd meiotic division (Figs. 16,17). Meiotic irregularities in n=20 (52%) and n=18 (54%) forms were observed to be 53%. Similarly normal PMCs with complete bivalent formation was 47% in both n=20 (48%) and n=18 (46%) forms (Table 2). In both the forms 49.5% of pollen grains scored were fertile.

It has also been noted that comparatively chromosomes were small sized in *C. arvensis*, medium sized in *C. glomeratus* and large sized in *C. prostratus*.

Discussion

Three species namely *C. arvensis*, *C. glomeratus* and *C. prostratus* are reported to vary morphologically in shape, and pubescence of leaf, peduncle length, size and colour of flower. Similarly a lot of variation in chromosome numbers have been reported for *C. arvensis* and *C. prostratus*; n=12, 24

Fig. 1. Metaphase-I with 25 IIs.

Fig. 2. Anaphase I with 25 chromosomes at each pole.

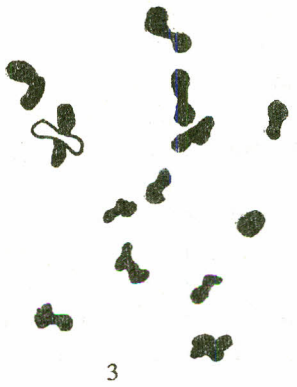

Fig. 3. Metaphase-I with 14 IIs.

Fig. 4. Anaphase- I, 14 chromosomes at each pole.

Fig. 5. Diakinesis with 18 IIs.

Fig. 6. Diakinesis with 18 IIs.

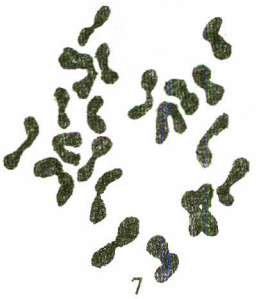

Fig. 7. Metaphase-I with 20 IIs.

Fig. 8. Diakinesis with 20 IIs.

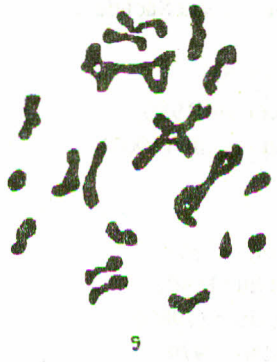

Fig. 9. Metaphase I with 4-I, 10-II, and 2- IV. (n=18).

Fig. 10. 2-Is, 9-IIs, 4-IVs (n=18).

Fig. 11. 3-Is, 9-IIs, 1 III, 4-IVs (n=20).

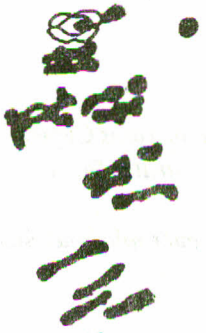

Fig. 12. 5-I, 10-IIs, 1 -III, 3 IVs (n=20).

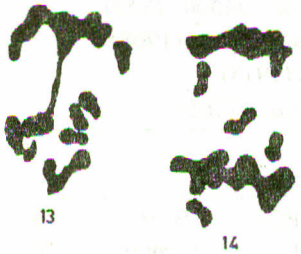

Fig. 13. Anaphase-I with a bridge.

Fig. 14. Anaphase-I with 4 laggards.

Fig. 15. Metaphase II with chromosomes lying away from the plate.

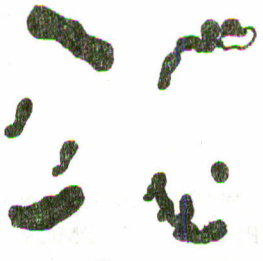

Fig. 16. Anaphase-II with laggards.

Fig. 17. Telophase II with laggards.

Figs. 1,2: Microsporogenesis in *C. arvensis.*, Figs. 3, 4 : Microsporogenesis in *C. glomeratus.*, Figs. 5-14: Microsporogenesis in *C. prostratus*

and 25 were reported for the former and n=9,10,12,18 and 20 for the later species Table 1 [6-13].

Chromosomal variability, however, has not been found in *C. glomeratus*; only n=14 individuals have been recorded previously [5] as well as by the present authors. Though *C. glomeratus* individuals also exibit some polymorphism [1], it is also noteworthy that in the region of study, *C. arvensis* and *C. glomeratus* are represented by single gametic numbers i.e. 25 and 14 respectively [6] (Table 1). Despite morphological variation, *C. arvensis* and *C. glomeratus* display a normal course of microsporogenesis resulting in normal tetrads (Table 1 and 2, Figs 1–4).

Contrary to the two above mentioned species *C. prostratus is* represented by two gametic numbers n=18 and 20 in the region under investigation and it also displays considerable meiotic abnormalities resulting in 49.5% of abnormal pollen, (Table 2) It, therefore, seems difficult to correlate chromosomal behaviour to the polymorphic nature of three species. Darlington has reported only two basic numbers 10 and 11 for the genus *Convolvulus*, [14], but the reports of n=9,12,14,18,20,24 and 25 (Table 1) in the species under discussion suggest revision of basic number including 9,12 and 14 as well. Presence of n=12 and 24 forms in *C. arvensis* indicate that diploid and tetraploid forms are present in this

TABLE 1. CHROMOSOME NUMBER IN *CONVOLVULUS* SPP. FROM PRESENT AND PREVIOUS INVESTIGATIONS.

Name of species	Present count	Previous count		Authority
	n	n	2n	
C. arvensis Linn.	25	–	50	Wolcott (1937). Hagerup (1941). Heiser, Whitaker (1948) Garajoba (1959) in Federov (1974)
				Bir and Sidhu, (1980).
		25		Baquar and Husain (1967)
				Bir and Sidhu, (1979)
				Sidhu (1979).
		24		Khoshoo and Usha Sachdeva (1961)
				Bir *et al.* (1978)
		12		Bir and Neelum (1980)
C. glomeratus Choisy	14	14	–	Baquar and Husain (1967)
C. prostratus Forsk.	18			
	20			
= *C. microphyllous* Sieb ex spreng	–	18	–	Baquar *et al.* (1965)
				Bir and Sidhu (1980)
		12	–	Bir and Neelum (1980)
		9	–	Bir and Sidhu (1979)
				Bir (1979)
=*C. pluricaulis* Choisy	–	10	–	Singh, (1951) in Federov (1974)
		9,18		Malick and Tandon (1959)
		9	–	Malick and Grover (1968)
				Bir *et al.* (1978)
		20		Baquar *et al.* (1965)

TABLE 2. CHROMOSOME ASSOCIATION AT METAPHASE-I AND POLLEN GRAIN FERTILITY.

Species	PMC's with complete-II formation	PMC's with I-IV	PMC's with 2-IV	PMC's with 3-IV	PMC's with 4-IV	Fertile PG/ Total PG scored	% of normal PMC's	% of fertility
C. arvensis	104	–	–	–	–	245/250	100	98.0
C. glomeratus	106	–	–	–	–	280/300	100	96.6
C. prostratus								
n = 20	88	43	34	18	2	198/400	48	49.5
n = 18	84	47	36	14	1	247/450	46	49.5

species, the n=25 individuals probably seem to be aneuploid of tetraploid. The present study shows that populations of *C. arvensis* in this region only consist of aneuploids with n=25. Formation of univalents and multivalents, therefore, should be expected, but no such irregularities were observed during microsporogenesis either in tatraploids [10] or in aneuploids (present study).

Previously two varieties, namely one with large and the other with small leaves were described [2]. But Austin *et al.* [1] have attributed this morphological difference to environment. *Convolvulus glomeratus* is represented only by diploid from in this region, [6 and present study] exhibiting normal chromosomal behaviour during sporogenesis (Figs 3,4). Contrary to the above two species *C. prostratus* displays anomalous behaviour with univalents, trivalents, quadrivalents besides bivalents and resulting in certain percentage of abortive pollen (Table 2, Figs 9–12).

Previously *C. microphyllus* and *C. pluricaulis* were treated as seperate taxonomic units. But Austin and Gazanfar [1] have treated these as synonymous to *C. prostratus* and described it as a species much variable in pubescence, size, shape of leaf and length of penduncle as well as flower size. *C. prostratus* is represented by three diploids i.e. n=9,10 and 12 and two tetraploids n=18 and 20 (Table 1) [6–8,11–13]. In the region of study it is represented by tetraploids only with the chromosomal form n=18 and 20 [6 and present study].

Contrary to the tetraploid forms n=24 and 25 of *C. arvensis* [5,10], n=18 and 20 forms of *C. prostratus* display anomalous behaviour during sporogenesis. One to four quadrivalents have been recorded in both the chromosomal forms (n=18 and 20, Table 2).

There may be many possible explanations for normal bivalent formation in *C. arvensis* and multivalent formation in *C. prostratus*. A correlation has been demonstrated between chromosome length and frequency of quadrivalent formation in polyploids [16–19]. In *C.arvensis*, all chromosomes are small, which may not facilitate multivalent formation, while larger chromosome are present in *C. prostratus* which may help in multivalent formation. Secondly, the genetic control mechanism that inhibits multivalent formation and regulates diploid like behaviour is known in many polyploids. Kimber [19], Waines [20] and Singh *et al.* [21] have attributed the diploid like behaviour in tetraploids to natural selection for a gene or genes that control regular meiosis. It may, therefore, be assumed that tetraploid form of *C. arvensis* has attained complete diploidized system, whereas *C. prostratus* is partially diploidized or rather has a weak diploidized system. Based on the above information it is apparent that there exists a confusion as to taxonomy and gametic number of *C. prostratus*. This complex, therefore, needs a comprehensive study.

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