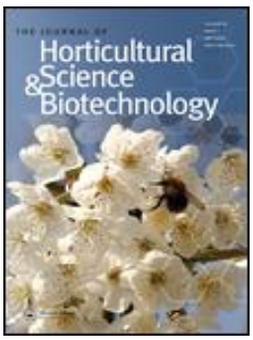

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Chromosome counts in wild and cultivated species of *Abelmoschus* Medikus. from the Indian sub-continent

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SUMMARY
Abelmoschus is regarded as a polyploid-polyspecies complex and a genus with variable genome organisation and resultant ambiguity in its speciation. A cytogenetic basis for taxonomy can be significant, due to the fact that many of the taxonomic units of *Abelmoschus* are heterogeneous in nature with deviant numbers of chromosomes. In the present investigation, 39 wild and cultivated accessions of *Abelmoschus* from India, belonging to three representative taxa, were used to record clear and authentic chromosome counts. From these data, the numbers of somatic chromosomes in *A. angulosus* var. *grandiflorus*, *A. moschatus* ssp. *moschatus*, and *A. moschatus* ssp. *tuberosus* are reported to be 2n = 66, 2n = 72, and 2n = 72, respectively. The present study also confirmed 2n = 130 to be the authentic number of somatic chromosomes in *A. esculentus*. Earlier conflicting reports are ruled out. Reports of the occurrence of cytotypes in *A. esculentus* are not acceptable, due to its wide area of cultivation.

The genus *Abelmoschus*, in the family Malvaceae, consists of both cultivated okra species and several wild or semi-wild species. These were originally included in the genus *Hibiscus* (section *Abelmoschus*) by Linnaeus (1753). Medikus (1787) raised this section to the rank of a distinct genus, based on their fruit characteristics. However, references to *Hibiscus* remained until *Abelmoschus* species were separated and described as a distinct genus by Hochreutiner (1924). The genus included 14 species, two of which (*A. moschatus* and *A. manihot*) were later recognised as "species complexes" (Charrier, 1984). These two species complexes are the most polymorphic and generally diverse (Hamon and Charrier, 1983). Van Borssum-Waalkes (1966) proposed a more restrictive classification, including six species divided into two groups, with three cultivated species (*A. esculentus*, *A. moschatus*, and *A. manihot*) in one group, while the other group had species that only occurred in a wild form (*A. crinitus*, *A. angulosus*, and *A. ficulneus*). Bates (1968) suggested three additional modifications, namely the inclusion of *A. tuberculatus* as *A. esculentus*; grouping all sub-species and varieties of *A. manihot* into one species; and *A. moschatus* spp. *tuberosus* becoming a new species called *A. rugosus*. Considering the van Borssum-Waalkes (1966) scheme as the starting point, an up-to-date classification was adopted at the International Okra Workshop, held at the National Bureau of Plant Genetic Resources (NBPGR) in 1990 (IBPGR, 1991). Eight species of *Abelmoschus* have been identified. Such variation in the number of distinctly identified species illustrates the complexity of the genus, which is evident with the discovery of a cultivated species, *A. caillei*, in Africa (Stevels, 1988).

The genus *Abelmoschus* is of Asiatic origin, but the exact ancestral home of cultivars of the common vegetable species, *A. esculentus*, is still in dispute. It is thought to have been domesticated in the region of Ethiopia (Vavilov, 1926) or in western Africa (Murdock, 1959; Joshi *et al*., 1974). A late nineteenth-century record shows that this species occurred in the wild, in the area of the white Nile, in Sudan (Singh *et al*., 1975). Joshi and Hardas (1976) suggested that the cultigen, *A. esculentus*, might originally have been present in Africa and Asia as a polyphyletic species. Van Borssum Waalkes (1966) considered Southeast Asia to be the centre of diversity for the genus *Abelmoschus*. An Indian origin for *A. esculentus* has also been advocated by Masters (1875), Zeven and Zhukovasky (1975), and Zeven and de Wet (1982), while Ethiopia and West Africa were also suggested as centres of origin by some workers (De Candolle, 1883; Vavilov, 1951; Chevalier, 1940; Murdock, 1959).

Vredebregt (1990) observed that the rich genotypic diversity of *A. ficulneus* and *A. tuberculatus* in India may be an argument for an Asian origin of *A. esculentus*, since both species have probably contributed to the natural evolution of *A. esculentus*. Existing taxonomic classifications of the genus *Abelmoschus* at the species level are unsatisfactory. Detailed cytogenetic

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observations on Asian okra, and related species, are likely to provide more examples of the existence of amphidiploids in the genus (Siemonsma, 1982a).

There are significant variations in chromosome numbers and ploidy levels among the different species reported for the genus *Abelmoschus*. The lowest number recorded was 2n = 56 for *A. angulosus* (Ford, 1938), whereas the highest chromosome number reported was close to 2n = 200 for *A. caillei* (syn. *A. manihot* var. *caillei*; Singh and Bhatnagar, 1975; Siemonsma, 1982a, b). Various numbers of somatic chromosomes in *A. esculentus* L. (Moench) have been reported by different authors. The most frequently observed number was 2n = 130, although Datta and Naug (1968) suggested that 2n = 72, 108, 120, 132, or 144 were also possible as a regular series of polyploids based on a haploid with n = 12.

Darlington and Wylie (1955) proposed a basic chromosome number of ≥ 10 for the genus *Hibiscus*. Six of the chromosome numbers appeared to agree with the numbers reported in the genus *Abelmoschus* (x = 9, 11, 12, 17, 19, and 39), while two new ones (x = 18 and 29) were added (Charrier, 1984).

The cytogenetic complexity of *Abelmoschus* spp. is challenging and requires verification, especially among wild Indian accessions. Therefore, we have undertaken chromosome counts for 39 accessions belonging to three species of *Abelmoschus* [*A. angulosus* var. *grandiflorus* (a wild species from higher altitudes), *A. moschatus* (*A. moschatus* ssp. *moschatus* and *A. moschatus* ssp. *tuberosus*; a semi-wild species of the Indian plain), and *A. esculentus* (a widely cultivated species)].

MATERIALS AND METHODS

Plant material and cultivation

The germplasm used in the present investigation was supplied by the National Bureau of Plant Genetic Resources (NBPGR) under a collaborative research project funded by the World Bank through a consortium research programme of the Indian Council of Agricultural Research (ICAR) through the National Agricultural Innovative Project (NAIP).

Seed treatment

Seeds were rinsed in 70% (v/v) ethanol for a few seconds, then washed several times in distilled water. An incision was made on the hard seed coat on the opposite side of the raphe, to enhance germination. Root tips (approx. 0.5 – 1.0 cm-long) were excised from the growing seed, pre-treated with 2.0 mM 8-hydroxyquinoline for 3 h at room temperature, then fixed in Carnoy's Fluid [a 1:3 (v/v) mixture of glacial acetic acid:ethanol] for 24 h and stored in 70% (v/v) ethanol at 4ºC in a refrigerator.

For slide preparation, root tips were hydrolysed in 1.0 M HCl for 10 min at 60ºC, then stained in Leuco-Basic Fuschin stain (Singh, 2003) for 45 min in the dark. Root tips were squashed on a clean glass slide in 1% (v/v) acetocarmine to spread the chromosomes. Photomicrographs were taken using a trinocular research microscope (Motic BA 400; Motic, Asia Pacific Unit, Causerway Bay, Hong Kong) with a Moticam 2300 photomicrographic attachment. At least 15 clear preparations of the chromosome complement of each accession were analysed for chromosome counts. To avoid bias in chromosome counts, each preparation was analysed by more than one researcher.

RESULTS

In the present investigation, 39 accessions belonging to three species of *Abelmoschus* (wild or cultivated) were evaluated with regard to variation in their mitotic chromosome counts. The somatic chromosome counts observed, probably for the first time in the cases of *A. angulosus* var. *grandiflorus*, *A. moschatus* ssp. *moschatus*, and *A. moschatus* ssp. *tuberosus*, are summarised in Table I.

A. angulosus *var.* grandiflorus *Thw.*

Six accessions belonging to *A. angulosus* var. *grandiflorus* were evaluated for their chromosome counts. A stable somatic chromosome number of 2n = 66 was observed in all six accessions investigated (Figure 1A–C). Ford (1938) reported a chromosome number of 2n = 56 for this species No deviant chromosome number counts were observed in any of the root tip cells of the six accessions studied.

A. moschatus *Medik.*

Variable chromosome counts were recorded in 13 accessions of *A. moschatus*, *A. moschatus* ssp. *moschatus*, and *A. moschatus* ssp. *tuberosus*. A single accession of *A. moschatus* (IC-316073) had a somatic chromosome count of 2n = 72 (Figure 1D), with no variation in any of the cells examined. Similarly, 11 accessions of *A. moschatus* spp. *moschatus* were also evaluated. Of these, nine had a stable chromosome count of 2n = 72 (Figure 1 E, F; Figure 2A–B), and a chromosome count of 2n = 68 (Figure 2D, E) was observed in the other two accessions. No deviations from these chromosome numbers were observed in all the root tip cells studied. However, a single observation of 2n = 144 (Figure 2F) was recorded, and may represent a polyploid accession. One accession belonging to *A. moschatus* ssp. *tuberosus* had 2n = 72 (Figure 2C), with no aneusomaty in any of the cells examined. Somatic chromosome counts of 2n = 72 or 2n = 68 for *A. moschatus* ssp. *moschatus* and 2n = 72 for *A. moschatus* ssp. *tuberosus* are reported here for the first time.

A. esculentus *(L.) Moench.*

Chromosome counts in 20 accessions of *A. esculentus* revealed a stable karyotype of 2n = 130 (Figure 3A–D). This value agrees with previous studies on *A. esculentus* by Skovsted (1935), Joshi and Hardas (1953; 1976), Gadwal *et al.* (1968), Joshi *et al.* (1974), and Singh and Bhatnagar (1975). A few aneusomatic cells, with chromosome counts of 2n = 200 and 2n = 230 (Figure 3E, F) were recorded in accession IC-117235.

DISCUSSION

Taxonomic differentiation in the genus *Abelmoschus* is interesting since many of the taxonomic units are heterogeneous in nature and exhibit deviant chromosome numbers. Chromosome counts for *A. angulosus* var. *grandiflorus* (2n = 66), *A. moschatus* (2n =

TABLE I
Numbers of somatic chromosomes in this study and previously recorded numbers for three Abelmoschus *species*

Taxon	Accession No.‡	Diploid (2n)	Previous value (Reference)
A. angulosus var. *grandiflorus* Thw.	IC-260043	66	–
	IC-213314	66	
	IC-203863	66	
	IC-203824	66	
	IC-203833	66	
	IC-470751	66	
A. moschatus Medik.	EC 316073	72	2n = 72 (Skovsted 1935; 1941; Gadwal *et al*., 1968; Joshi *et al*., 1974)
A. moschatus ssp. *tuberosus* (Span.) Borss.	IC-470750	72	
A. moschatus ssp. *moschatus* Medik.	IC-469584	72	
	IC-333520	68	
	IC-212557	72	
	IC-140970-A	72	
	EC-329390	72	
	EC-316077	72	
	IC-141065	72	
	IC-141056	72	
	IC-140986	72	
	IC-140985	68	
	IC-470731	72	
A. esculentus (L.) Moench.	NIC-8183	130	2n = 66 (Ford, 1938)
	IC-128027	130	2n = 72 (Teshima,1933; Ugale *et al*., 1976; Kamalova, 1977)
	NIC-9025	130	2n = 108 (Datta and Naug, 1968)
	NIC-9301	130	2n = 118 (Tischler, 1931)
	NIC-9317	130	2n = 120 (Tischler,1931; Purewal and Randhawa, 1947;
	IC-128043	130	Datta and Naug, 1968)
	NIC-9349	130	2n = 122 (Tischler, 1931)
	NIC-9354	130	2n = 124 (Kuwada,1957a; 1966)
	NIC-9495	130	2n 126-134 (Chizaki, 1934)
	IC-128031	130	2n = 130 (Skovsted,1935; Joshi and Hardas, 1953; Joshi and
	IC-117235	130	Hardas, 1976; Gadwal *et al*., 1968, Joshi *et al*.,1974;
	IC-117285	130	Singh and Bhatnagar, 1975)
	IC-282294	130	2n = 131-143 (Siemonsma, 1982a; 1982)
	NMB-2924	130	2n = 132 (Medwedewa,1936; Roy and Jha, 1958)
	NMB-2933	130	2n = 132 (Breslavetz *et al*., 1934; Ford, 1938)
	TCR-1410	130	2n = 144 (Datta and Naug, 1968)
	TCR-1444	130	
	IC-205657	130	
	SUA/1	130	
	SUA/14	130	

‡IC, indigenous collection; EC, exotic collection.

72), *A. moschatus* ssp. *moschatus* (2n = 72 and 68), *A. moschatus* ssp. *tuberosus* (2n = 72), and *A. esculentus* (2n = 130) resolved them into Group I and Group II, but not into Group III, according to the scheme proposed by Charrier (1984). Although the ploidy levels recorded in these taxa were as expected, the occurrence of 2n = 66 did not support the earlier chromosome count of 2n = 56 for *A. angulosus* (Ford, 1938). Thus, we are proposing a new somatic chromosome number of 2n = 66 for *A. angulosus* var. *grandiflorus*, which may be regarded as a cytotype or variety most commonly reported from the coastal plains and midlands up to an altitude of 800 m (Sivarajan and Pradeep, 1996). *A. angulosus* var. *grandiflorus* also deserves a distinct status, based not only on its cytological distinction (2n = 66), but also for its unique growth habit, namely the absence of stiff, bristly indumentums, membranous involucellar bracts, and bright yellow pendant flowers (Sivarajan and Pradeep, 1996).

A somatic chromosome number of 2n = 72 has been reported for *A. moschatus* (Skovsted, 1935; Gadwal *et al*., 1968; Joshi *et al*., 1974). Despite the occurrence of two distinct sub-species (viz. *A. moschatus* ssp. *moschatus* and *A. moschatus* spp. *tuberosus*), no efforts to check on the chromosome counts in these taxa have been reported until now. Thus, our observation of 2n = 72 in both taxa can be regarded as a first report. Deviant chromosome numbers (2n = 68) in two accessions of *A. moschatus* spp. *moschatus* (IC-33520 and IC-40985) did not have any apparent bearing on the chromosome number of *A. moschatus* (i.e., 2n = 72).

Chromosome counts in *A. esculentus*, the commonly grown "vegetable okra", have been reported to show the presence of 2n = 66 to 2n = 144 chromosomes. In contrast, we have recorded only 2n = 130, consistently in all root tip cells of all 20 accessions of *A. esculentus* studied here. However, a few cells showed 2n = 200 and 2n = 230, which may be regarded as exceptions and of no significance to the chromosome counts being reported here. Thus, the feasibility of cytotype differentiation is a common feature in the genus *Abelmoschus*.

In conclusion, somatic chromosome numbers in *A. angulosus* var. *grandiflorus*, *A. moschatus* ssp. *moschatus*, and *A. moschatus* ssp. *tuberosus* have been reported for the first time, and we confirm 2n = 130 as the authentic number of somatic chromosomes for *A. esculentus*.

This work was carried out in the Department of Biotechnology and Bioinformatics, North-Eastern Hill University, Shillong, Meghalaya, India. The authors are grateful to the National Bureau of Plant Genetic Resources (NBPGR), New Delhi and to Thrissur (Kerala) for providing the germplasm, the World Bank-Indian Council of Agriculture Research (ICAR) through the National Agricultural Innovation Project (NAIP) for grant support (Component 4/ C: 2070). Thanks are also due to all members of Plant Biotechnology Laboratory for their encouragement and help.

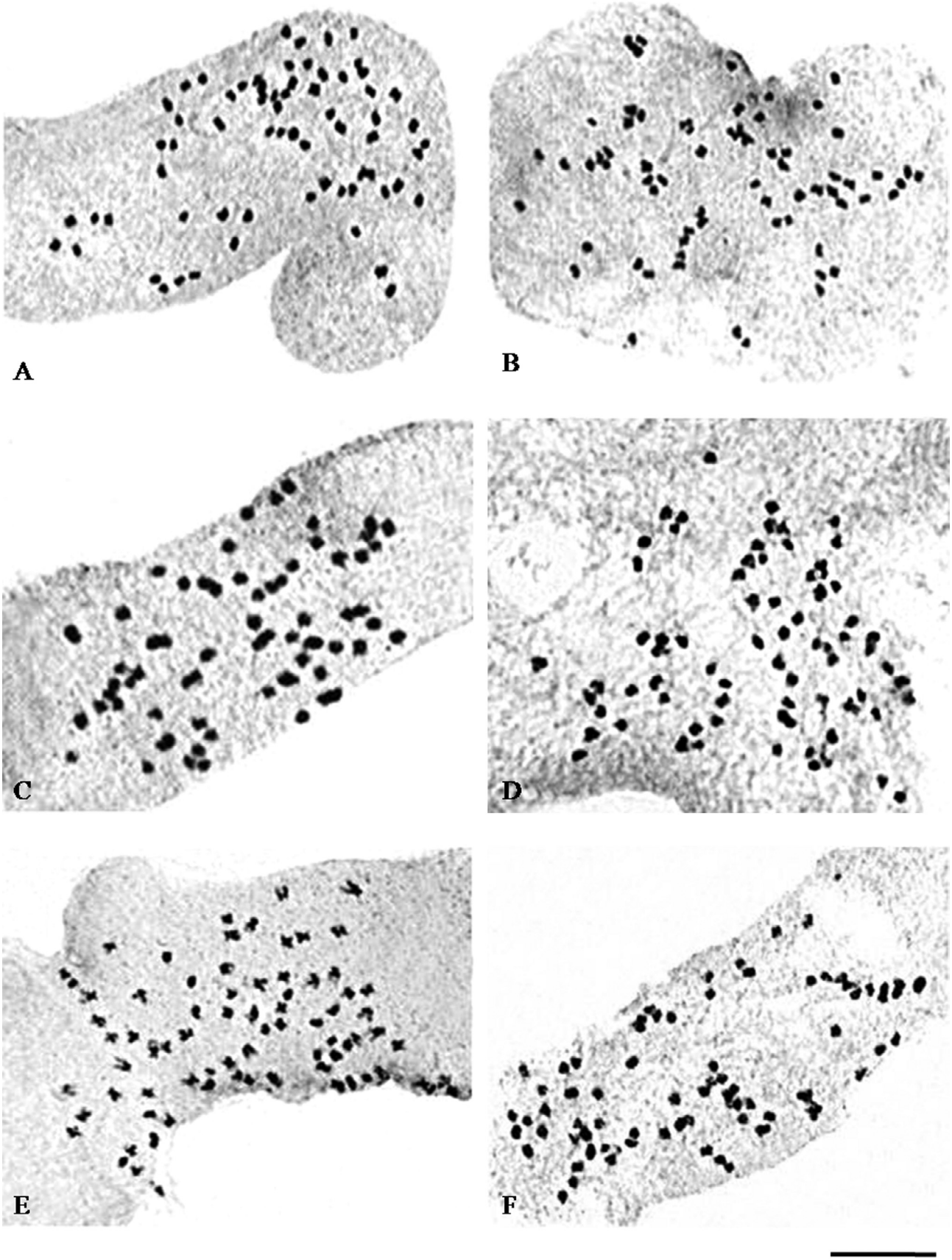

FIG. 1
Somatic chromosome counts for *Abelmoschus* spp. Panels A–C, *A. angulosus* var. *grandiflorus* (2n = 66); Panel D, *A. moschatus* (2n = 72); Panel E, F, *A. moschatus* ssp. *moschatus* (2n = 72). Scale bar = 5 μm.

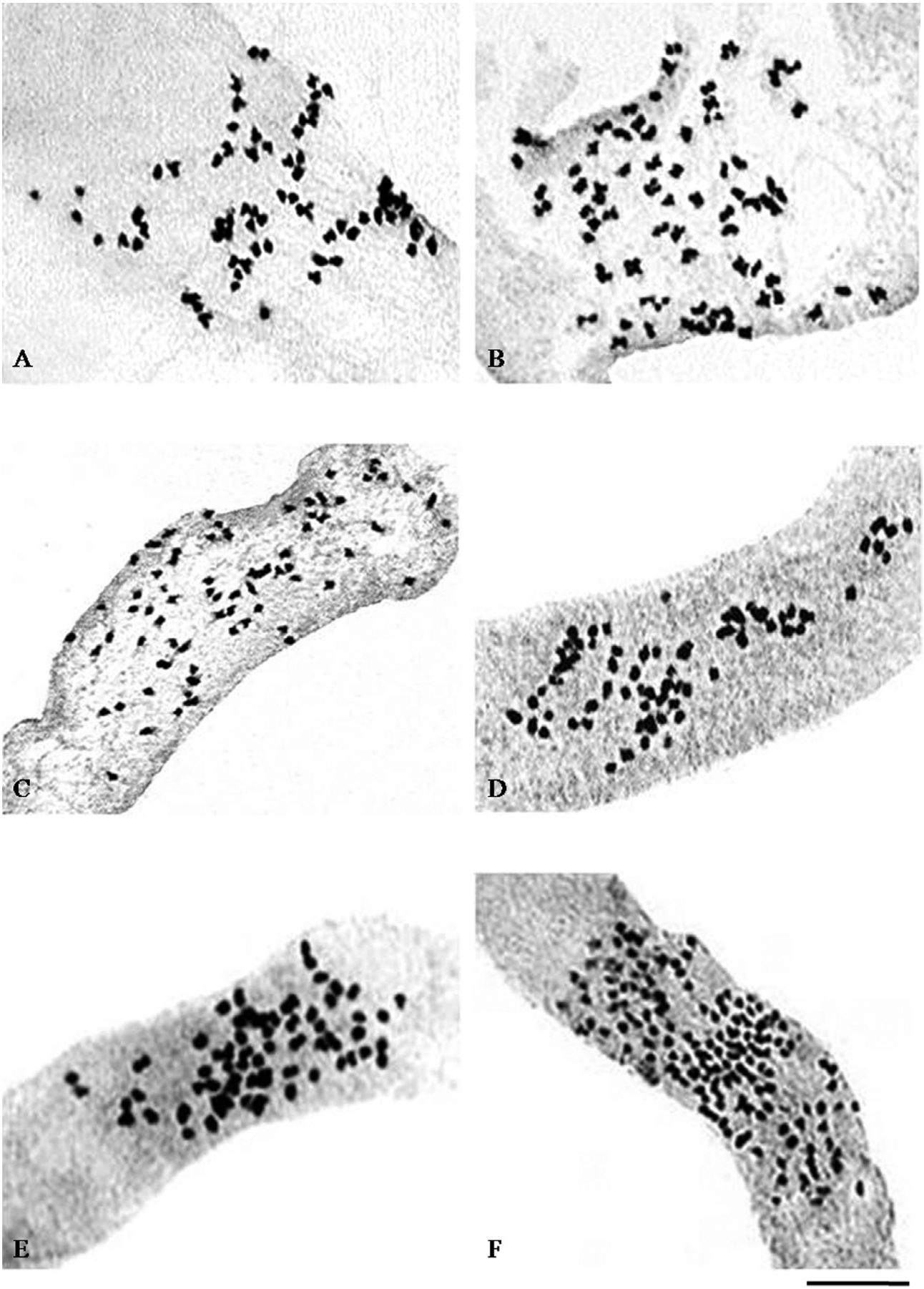

Fig. 2
Somatic chromosome counts in *Abelmoschus* spp. Panels A, B, *A. moschatus* ssp. *moschatus* (2n = 72); Panel C, *A. moschatus* ssp. *tuberosus* (2n = 72); Panel D, E, *A. moschatus* ssp. *moschatus* (2n = 68); Panel F, *A. moschatus* ssp. *moschatus* (2n = 144). Scale bar = 5 µm.

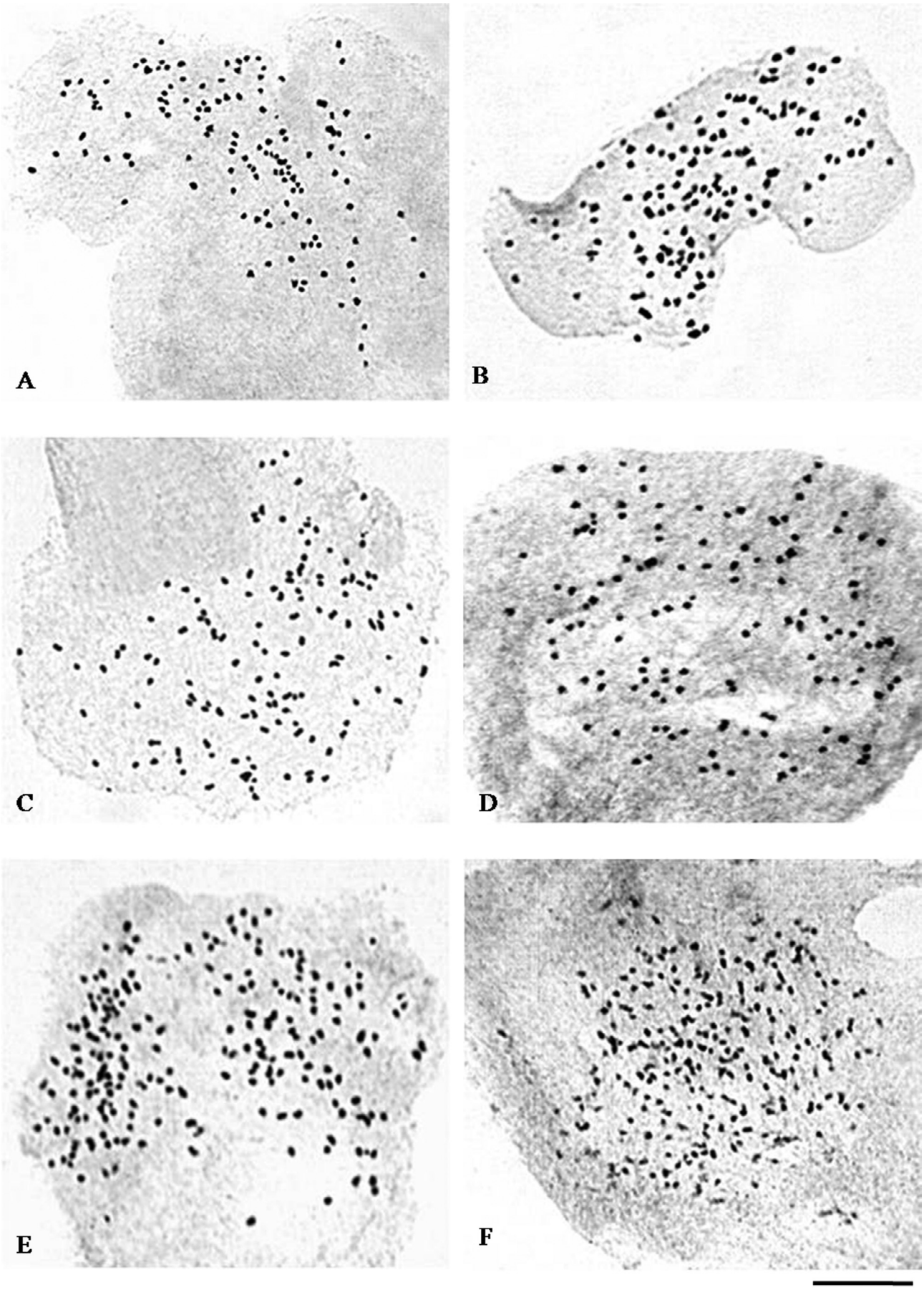

FIG. 3
Somatic chromosome counts in *Abelmoschus* spp. Panels A–D, *A. esculentus* (2n = 130), Panel E, *A. esculentus* (IC-117235; 2n = 200); Panel F, *A. esculentus* (IC-117235; 2n = 230). Scale bar = 5 μm.

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