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Unfolding chromosomal uniqueness of the Scilloid ornamental *Albuca virens* by application of EMA based Giemsa- DAPI staining

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Abstract. The cytogenetic features in the tribe Ornithogaleae of subfamily Scilloideae is a prerequisite for understanding genome evolution. Unfortunately, genomic or the foundational chromosomal features are neglected within majority of Ornithogaloids, incuding *Albuca*, one of the largest genus of the tribe. *Albuca virens* (Lindl.) J.C. Manning & Goldblatt is the only ornamental species of *Albuca* found to be exotic to India. Analysis of karyotype by EMA method followed by Giemsa and DAPI staining is the first step towards molecular cytogenetics attempted in this species and has currently brought significant resolution in chromosome morphology (especially NORs). The Indian population of *A. virens* with 2n=6 chromosomes, symmetric karyotype and heterophorphy of NORs provide excellent scope to navigate questions on dysploid origin of *Albuca*. The regular meiotic stages advocate genomic stability despite vegetative propagation and polysomaty in root cells. The comparative review of chromosomal evolution within *Albuca* has been discussed in relation to the Indian *A. virens* as a prototype.

Keywords: *Albuca virens*, EMA, DAPI, meiosis, dysploidy, NORs.

INTRODUCTION

The genus *Albuca* belongs to the tribe Ornithogaleae of Asparagaceae/Hyacinthaceae, subfamily Scilloideae, sensu APG III 2009; APG IV 2016) with more than 100 species distributed mainly in sub-Saharan Africa (Goldblatt and Manning 2011; Martinez- Azorin et al. 2011). Considering repeated taxonomic amendments and changes in species circumscription (Martinez-Azorin 2011, 2023; Manning 2020), chromosomal features are given special attention to resolve species boundaries within the tribes of Asparagaceae/Hyacinthaceae (Goldblatt and Manning 2011). It is evident from the recent compilation that cytogenetic investigation is so far confined to chromosome counts or karyotypes by conventional staining methods in less than 50% of accepted species belonging to tribes Hyacintheae, Urgineeae, Orni-

thogaleae and Oziroëeae (Nath et al. 2022). *Albuca virens* (Lindl.) J.C. Manning & Goldblatt of subgenus *Urophyllon* (Salisb.) J.C. Manning & Goldblatt (Manning et al. 2009a,b) has been found to occur as an ornamental exotic to India while some wild populations are reported from north eastern part (Bhattacharya et al. 2016). Within subgenus *Urophyllon* of *Albuca*, karyotype analysis by fluorochrome staining is reported in *Albuca bracteata* (Thunb.) J.C.Manning & Goldblatt (worked out as syn. *Ornithogalum longibracteatum* Jacq.), the sister species of *A. virens* (Pedrosa et al. 2001). Nomenclatural ambiguity in previous cytological reports is another reason for blurring analysis of chromosomal relationships in *Albuca*. *A. virens* has four subspecies of which *A. virens* ssp. *virens* is previously known and worked out as different species of *Ornithogalum* with varying chromosome counts *viz*. *Ornithogalum virens* Lindl. (2n=6*)*, *O. flavovirens* Baker (2n=10), *O. ecklonii* Schldl. (2n=10), *O. tenuifolium* Delaroche (2n=12, 10, 8, 16, 6, 4), *O. pretoriense* Baker (2n=12) or *O. inconspicuum* Baker (2n=20) (Goldblatt and Manning 2011). In our country also, very few traditional cytological studies have been conducted on *A. virens*, in the name of the syn. *Ornithogalum virens* (Ravindran 1977; Bhattacharya et al. 2016). Owing to the backdrops of conventional cytological studies, lucid karyotype features of *A. virens* is still missing.

Hence the aim of the present work is to obtain better cytogenetic analysis with enzymatic maceration and air drying (EMA) method (Kurata and Omura 1978; Fukui 1996) in *A. virens* over the traditional orcein staining approach. In spite of being a well-known alternate method for chromosome analysis (Fukui and Iijima 1991; Yamamoto *et al*. 2010, 2015; Nath et al. 2015; Jha et al. 2020; Jha 2021; Bhowmick and Jha 2022), EMA based approach has not been yet attempted in *Albuca virens*. Our adoption of EMA method enabled lucid karyotype interpretation, fluorescence staining and understanding meiotic chromosome behavior in this plant. The outcome of the present paper upgraded existing knowledge about chromosome configuration in this species and provides foundational data for taxonomic revisions, genome research and hybridization breeding of *A. virens* as a potential ornamental plant.

MATERIALS AND METHODS

Plant materials

Bulbs of *Albuca virens* were collected from local nurseries of Darjeeling district of West Bengal, India as ornamentals and were duly identified by Dr. Manoj M. Lekhak, Shivaji University, Kolhapur, India. The bulbs are potted, grown and maintained in the medicinal plant garden of Scottish Church College, Kolkata (Figs. 1a, b). Actively growing underground roots were used for chromosome analysis. Young flower buds from inflorescence (Fig. 1b) were picked to study meiotic chromosomes.

Chromosome preparation

Pre-treatment of healthy underground secondary roots (0.5- 1 cm in length) was done in 0.002M hydroxyquinoline at 15 °C for 4 hours and then roots were fixed in freshly prepared 1:3 aceto-methanol solution. Mitotic frequency and chromosome morphology were firstly studied by conventional squashing method. Fixed roots were then subjected to enzymatic maceration and air drying method (Kurata and Omura 1978; Fukui 1996) after necessary standardization (Jha and Bhowmick 2021; Bhowmick and Jha 2022) for the present plant material. Roots were firstly digested in a cocktail of 1% cellulase (Onuzuka RS), 0.15% pectolyase (Y-23), 0.75% macerozyme (R-10) and 1mM EDTA (pH 4.2) for varying time durations (45-50 min) at 37 °C before maceration on clean glass slides in a drop of 1:3 aceto-methanol solution. Slides with macerated roots were air-dried and stained with 2% Giemsa solution (Giemsa azure eosine methylene blue solution Merck Germany: 1/15th phosphate buffer: distilled water :: 2:50:48) for 15 min. Metaphase plates were observed under 100X objectives of Zeiss Axioscope microscope with attached Axiocam 202 mono camera and Zen software for capturing photomicrographs.

Karyotype preparation and analysis

Minimally ten different metaphase plates from seven individual plants were selected for chromosome measurements [long arm length (l), short arm length (s), chromosome length (CL), total diploid chromatin length (TCL=$\sum_{i=1}^{2n} CL$)]. Chromosomes were categorized after Levan et al. (1964) on the basis of r value (l/s) and arranged in an order of decreasing length for constructing karyotypes and ideograms. Any trend of asymmetry in karyotype was determined after calculating values of twelve different indices. These include Stebbins asymmetry index (Stebbins 1971), total form percentage (TF%) (Huziwara 1962), intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) (Zarco 1986), coefficients of variation of chromosome length (CV_{CL}) (Paszko 2006), index of karyotype symmetry (Syi) (Greilhuber and Speta 1976), asymme-

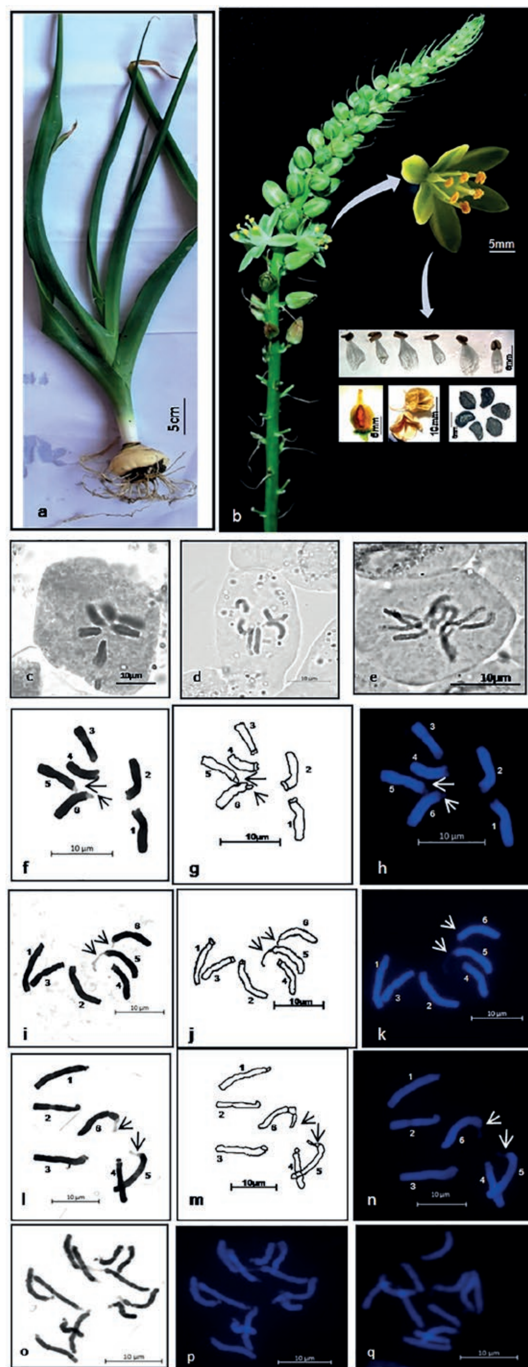

Figure 1. a. Whole plant. **b** Part of inflorescence with flowers, floral organs, fruit and seeds in insets. **c-e** Somatic metaphase chromosomes (2n=6) stained with orcein (**c, d**) and polysomatic cell photograph stained with Feulgen (2n=12) (**e**), note obscure chromosome morphology in conventional staining. **f-n** Somatic metaphase chromosomes (2n=6) represented from three individual plants: **f, i, l** Giemsa stained chromosomes with corresponding hand drawings (**g, j, m**) and subsequent DAPI stained plates (**h, k, n**). Arrows indicate satellite part of chromosomes in Giemsa plates that are correspondingly DAPI^{-ve}. **o-q** Somatic chromosomes showing polysomatic condition (2n= 12) stained with Giemsa (**o**), DAPI (**p-q**).

try index of karyotype (AsK%) (Arano 1963), degree of karyotype asymmetry (A) (Watanabe *et al*. 1999), mean centromeric index (X_{CI}) (Seijo and Fernández 2003), dispersion index (DI) (Lavania and Srivastava 1992), asymmetry index (AI) (Paszko 2006) and mean centromeric asymmetry (M_{CA}) (Peruzzi and Eroğlu 2013). Considering the absence of the allied *Albuca* species in India, chromosome morphometric data have been borrowed from a published report involving *Ornithogalum comosum* Sadler, *O. montanum* Cirillo, *O. pyrenaicum* L. and *O. sigmoideum* Freyn & Sint. (Öztürk et al. 2014) to compare the asymmetry indices and comment on trend of karyotype evolution in *Albuca virens*. Since only chromosome lengths and centromeric indices were provided in the published paper (Öztürk et al. 2014), only A2, CV_{CL}, X_{CI} and AI values could be estimated for the reference taxa.

Fluorochrome staining of somatic metaphase chromosomes

The Giemsa stained slides were marked under the fluorescent microscope Zeiss Axioscop5 followed by destaining in 70% methanol for 45min. Fluorochorme staining with DAPI (4',6-diamidino-2-phenylindole) was performed following Schweizer (1976) after required standardization and modifications (Bhowmick and Jha 2021; Jha and Bhowmick 2021). Slides were incubated in McIlvaine buffer (0.1M citric acid, 0.2M Na_2HPO_4, pH 7.0) for 30 min and stained with 0.1mg/ml DAPI solution for 25-30 min in dark. Excess stain from slides was washed off in McIlvaine buffer followed by blow drying and mounting in non-fluorescent glycerol. Observation of DAPI stained metaphase plates was carried out under the Zeiss Axioscop2 with UV filter cassette. Fluorescent chromosome images were captured with the attached Axiocam 202 mono camera and Zen software.

Meiotic chromosome preparation following DAPI staining

Young flower buds of approximately 0.5-0.8cm length were collected at around 10a.m. Initial screening of the meiotic stages were conducted by staining PMCs (pollen mother cells) in 2% acetocarmine solution. Due to dense cytoplasmic content in the PMCs, EMA- DAPI staining (Bhowmick and Jha 2015) was conducted with minor modifications. The anthers were isolated from flower buds and then digested in enzyme cocktail (same as mentioned for somatic chromosome preparation) for 2-4mins at 37°C. Macerated anthers were pipetted out in clean glass slides and P.M.C.s were spread in 1:3 v/v acetic acid- ethanol solution and air dried. The slides

were kept in McIlvaine buffer (0.1M citric acid, 0.2M Na_2HPO_4, pH 7.0) for 10min. Slides were then stained with 0.1mg/ml DAPI solution for 20min in dark. Excess stain was washed off in Mc Ilvaine's buffer and slides were mounted in non-fluorescent glycerol. Slides were observed and images of meiotic stages were captured under UV filter cassette of Zeiss Axioscope 5 fluorescence microscope with attached Axiocam 202 mono camera and Zen software.

RESULTS AND DISCUSSIONS

Somatic chromosome morphology and karyotype

In the conventional method, cytoplasmic density was difficult to overcome and visualization of chromosome morphology was problematic (Fig. 1c-e). Application of EMA method enabled elimination of cytoplasmic background and rendered chromosome spreading in one plane with clear morphology (Fig. 1f-q) which was not possible in previous reports due to limitations of conventional method (Ravindran 1977; Bhattacharya et al. 2016). There are six chromosomes in the diploid complement (2n=6) (Fig. 1c, d, f-n), with few records of 2n=12 chromosomes in some cells (4-8%), corresponding with previous reports (Bhattacharya et al. 2016) (Fig. 1e, o-q). Present study shows that the chromosomes are telocentric in nature with one satellited pair of chromosomes. The chromosomes range in size from 6.88 to 10.83µm (Table 1), with an average TCL (total length of diploid complement) of 53.20±14.58. The modal karyotype of *A. virens* is 4t+2T.sat (Fig. 2a-c), showing no signs of distinct difference of short or long chromosomes and hence Indian population of *A. virens* does not show bimodal nature of karyotype (Table 1). However, every pair has at least a little difference in chromosome length between the homologues (Table 1). The largest chromosomes are the ones with a terminal satellite (Table 1). Centromeric position in this pair cannot be determined as they are terminally located, only the satellite part is visible in metaphase plates (Fig. 1f-n, 2a-c). Again, the size of the satellited region varies between the homologues (Fig. 1f-n).

In the previous works, 2n=6 was consistently associated with *O. virens* and 2n=4, 6, 12, 16, 26 was reported in the African *A. virens* (worked out as *Ornithogalum tenuifolium*) (Goldblatt and Manning 2011). However, *Ornithogalum tenuifolium* was included later in *Stellarioides* (Castiglione and Cremonini 2012). Other counts (2n=8, 10 and 12) were reported in *O. setifolium*, *O. eck-*

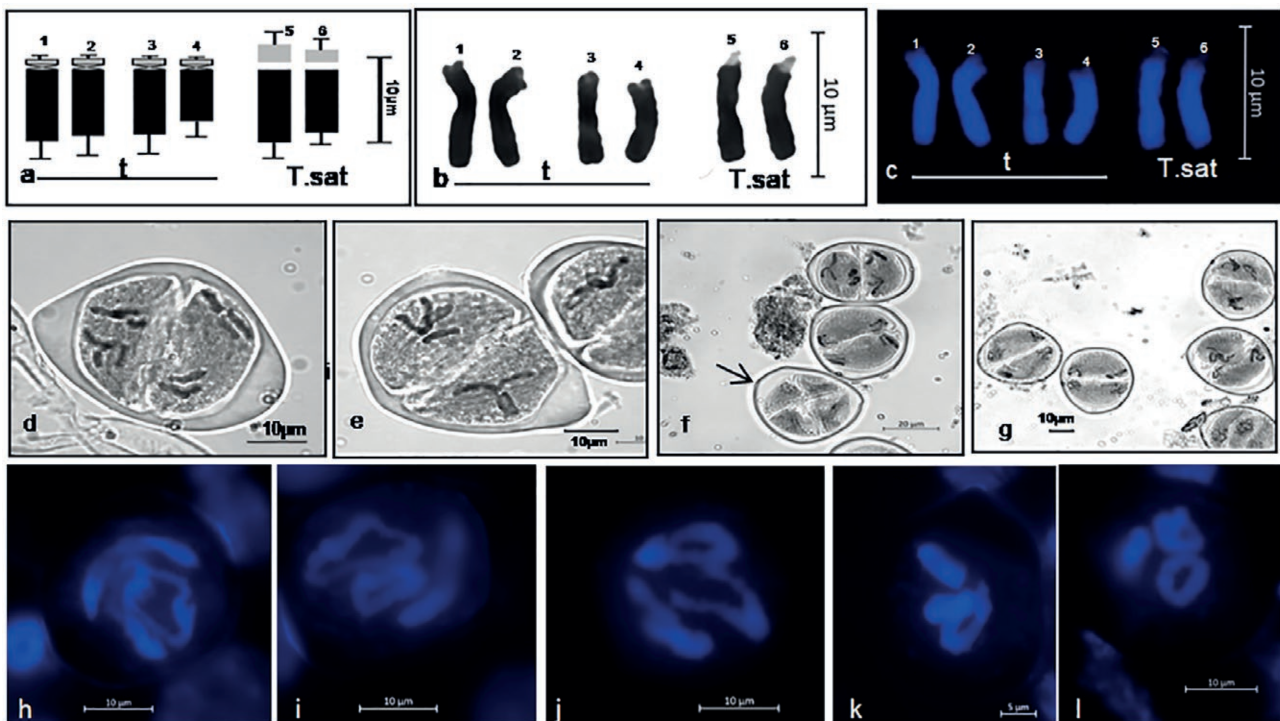

Figure 2. a-c Idiogram and Giemsa (**b**)- DAPI (**c**) karyotype. **d-g** Carmine stained meiotic chromosomes from PMCs at metaphase II (**e**) with three chromosomes in each cell of diad, anaphase II showing regular segregation (**d, f, g**), telophase II indicated with arrow (**f**), showing no signs of abnormality. **h-l** DAPI stained meiotic chromosomes from PMCs at diakinesis (**h-j**) and metaphase I (**k, l**) showing 3 bivalents.

Table 1. Chromosome morphometric features of *Albuca virens.*

Chromosome no.	Long arm (l) (μm)	Short arm (s) (μm)	Sat (μm)	Chromosome length (μm)	r=l/s	Type	RL
1	8.5 ± 2.18	0.68± 0.08	-	9.18±2.34	12.25 ± 2.51	t	17.37 ± 0.75
2	7.87± 2.66	0.788± 0.28	-	8.65± 2.80	10.52 ±4.36	t	15.99± 1.18
3	7.718± 2.66	0.75± 0.11	-	8.47± 2.63	10.57±4.44	t	15.91± 2.67
4	6.15± 1.90	0.73± 0.18	-	6.88± 1.98	8.67±2.98	t	12.92± 0.73
5	8.65±2.27	-	2.18± 1.14	10.83± 3.14	-	T. sat	20.35± 1.67
6	7.48±1.52	-	1.8±1.30	9.28± 2.61	-	T. sat	17.43± 0.45

The data are shown as average values± standard deviation from ten different metaphase plates chosen from seven individual plants. Sat: satellite part of chromosome, r: arm ration (Levan et al. 1964), RL: relative length calculated as: Chromosome length/ Total Chromatin Length*100.

lonii, *O. flavovirens* and *O. pretoriense*, respectively, all of these species being now identified as *Albuca virens* (Goldblatt and Manning 2011). Again, one population of *A. virens* from southern Mozambique shows 2n=4 (Stedje 1989), with one long metacentric pair and one medium sized sub telocentric pair. So far, the ancestral base number of x=10 is suggested for *Albuca*, with many evidences for derived base numbers x=9 (for subgenera *Albuca*, *Monarchos*, *Osmyne*) and x=6 (subgen. *Urophyllon*) (Goldblatt and Manning 2011). Later, 2n=6 was suggested to be a primitive cytotype that may have potentially led to origin of 2n=4 by chromosome translocation and fusion (Castiglione and Cremonini 2012). The other phylogenetically sister species with 2n= 18, 54 (*A. bracteata*, syn *O. caudatum*, *O. longibracteatum*), are suggested to have x=9 (Goldblatt and Manning 2011). Extensive cytological interpretations led Goldblatt and Manning (2011) conclude dysploidy to be a characteristic of subgenus *Urophyllon*, with particularly extreme intraspecific dysploidy notable in *Albuca virens*. Previously, bimodal karyotype has been reported in diploid populations of *A. virens*, like its sister species *Albuca volubilis* from Madagascar (Goldblatt and Manning 2011). The species of subgenus *Albuca* consistently show bimodal karyotypes (Knudtzon and Stedje 1986; Jong 1991; Stedje 1996; Johnson 1999). Although, the present population *of A. virens* in India does not have bimodal karyotype, differences in chromosome length between the homologues may be expressions of ongoing intraspecific rearrangements, as reported for cytotypes with 2n=6 (Castiglione and Cremonini 2012). Considering the difference in the size of satellites in the present study, heteromorphy in the size of the satellite NORs is reported in sections of subgenus *Albuca* (Jong 1991) and hence can be treated as a conserved phenomenon in *A. virens* of subgenus *Urophyllon*.

Knowledge about the symmetric/asymmetric nature of karyotype is a fundamental requirement to gain substantial concept of evolution in any group (Liang and Chen 2015). The inter-chromosomal asymmetry indices depict heterogeneity in chromosome sizes while intra-chromosomal asymmetry depends on relative centromere position (Paszko 2006). Unfortunately, detailed chromosome measurement data of *Albuca* species is missing in literature (Ravindran 1977; Knudtzon and Stedje 1986; Jong 1991; Stedje 1996; Johnson 1999; Pedrosa 2001). Therefore, the present karyomorphometric analysis has been conducted in comparison with some species of *Ornithogalum* by retrieving a few chromosome morphometric records from published data (Öztürk et al. 2014) (Table 2). According to the values determined, *A. virens* shows symmetric nature of karyotype, considering decreased values for A2, CV_{CL}, X_{CI} and AI (Table 3). Symmetric nature of karyotype is justified also according to Stebbins' index (2A) (Table 3). The chromosomes do not have discrete difference in size or centromeric position, in contrast to the bimodal karyotypes of other *Albuca* species and the *Ornithogalum* taxa referred in the present analysis (Table 3). The present detail of karyotype asymmetry parameters can be used as a reference to the allied species of *Albuca* in future studies.

Confirmation of nucleolar chromosomes following DAPI staining

The same Giemsa stained chromosome plates were subjected to DAPI staining. Chromosomes were brightly stained with DAPI except at the short arm which was relatively faintly stained (Fig. 1f-n). Comparing the same metaphase plate stained with Giemsa and then with DAPI, satellite part of the two chromosomes was found to be the $DAPI^{-ve}$ NORs (Fig. 1h, k, n). Thus, DAPI staining supported confirmation of NORs in the somatic metaphase chromosomes. $DAPI^{-ve}$ NORs are a common

Table 2. Chromosome morphometric data from *Ornithogalum* species retrieved from published work (Öztürk et al. 2014).

Ornithogalum comosum:			*Ornithogalum montanum:*			*Ornithogalum pyrenaicum :*			*Ornithogalum sigmoideum:*		
Chromosome Pair no.	ChL (A) (μm)	CI (A)	Chromosome Pair no.	ChL (A) (μm)	CI (A)	Chromosome Pair no.	ChL (A) (μm)	CI (A)	Chromosome Pair no.	ChL (A) (μm)	CI (A)
1	7.84	19.87	1	4.64	36.45	1	10.5	43.75	1	7.8	32.63
2	6.58	27.35	2	4.2	30.39	2	7.28	30.12	2	6.64	27.2
3	6.05	22.72	3	3.68	39.92	3	7.05	21.71	3	6.51	24.31
4	5.86	24.81	4	3.31	33.34	4	6.78	27.06	4	6.22	41.08
5	5.55	35.36	5	3	20.48	5	6.28	27.46	5	5.7	46.28
6	5.04	23.65	6	2.88	22.52	6	5.96	22.75	6	5.2	26.16
7	4.46	22.6	7	1.84	34.98	7	4.46	44.26	7	4.2	31.71
8	4.35	24.02				8	4.39	40.85			
9	4.12	27.12				9	3.48	28.16			
10	3.08	45.39				10	2.91	36.74			
						11	2.68	37.15			
						12	1.6	40.86			

ChL: chromosome length, CI: centromere index, A: average.

Table 3. Karyotype Symmetry/Asymmetry values in *Albuca virens* estimated with quantitative and qualitative indices in comparison with published data from *Ornithogalum* species (Öztürk et al. 2014).

	Quantitative parameters											Qualitaive parameter
	Inter-Chromosomal Symmetry/Asymmetry		Intra-Chromosomal Symmetry/Asymmetry							Inter- & Intra- (Combined) Chromosomal Asymmetry		Stebbins asymmetry index
	A2	CV_{CL}	Syi	TF%	Ask%	A1	A	X_{CI}	M_{CA}	DI	AI	
Albuca virens	0.14	14.48	6.38	5.55	86.98	0.06	0.74	1.50	74.66	8.31	2.04	2A
*Ornithogalum comosum**	0.25	25.98	-	-	-	-	-	2.72	-	-	7.24	-
*O. montanum**	0.27	27.45	-	-	-	-	-	4.45	-	-	6.36	-
*O. pyrenaicum**	0.47	47.32	-	-	-	-	-	2.78	-	-	11.52	-
*O. sigmoideum**	0.19	19.02	-	-	-	-	-	4.98	-	-	4.73	-

A2: Interchromosomal asymmetry index (Romero-Zarco 1986); $CV_{CL:}$ Coefficient of variation of chromosome length (Paszko 2006); Syi: Index of karyotype symmetry (Greilhuber and Spelta 1976); TF%: Total form percentage (Huziwara 1962); AsK%: Asymmetry index of karyotype (Arano 1963); A1: Intrachromosomal asymmetry index (Romero-Zarco 1986) ; A: Degree of karyotype asymmetry (Watanabe et al 1999); X_{CI} : Mean centromeric index (Seijo et al. 2003) ; $M_{CA:}$ Mean centromeric asymmetry (Peruzzi and Eroglu 2013) ; DI: The dispersion index (Lavania and Srivastava 1992); AI: Asymmetry index (Paszko 2006); Stebbins asymmetry index A-C,1-4 (Stebbins 1971);* data derived from previously published average chromosome length and average centromeric index of four *Ornithogalum* species (Öztürk et al. 2014).

feature of plant chromosomes since nucleolar regions are usually enriched with GC- heterochromatin and thus take up dense stain after application of GC- specific fluorochrome namely chromomycin A3 (Schweizer 1976; Guerra et al. 2000). *Albuca virens* also conforms to the usual negative banding pattern of DAPI at NORs. Previously, $DAPI^{+ve}$ signals on centromeric and intercalary regions and $CMA^{+ve}/DAPI^{-ve}$ signals on nucleolar regions are reported in the phylogenetically close *A. bracteata* (Pedrosa et al. 2001). Therefore, distribution of non-nucleolar AT-rich heterochromatin (depicted by $DAPI^{+ve}$ bands, Bhowmick and Jha 2015, 2019, 2021) may follow differential distribution among the species of subgenus *Urophyllon*, to be confirmed after analysis of DAPI banding pattern in *A. volubilis* and related species in question (Goldblatt and Manning 2011). Till then, $DAPI^{-ve}$ NORs remain to be a conserved chromosomal landmark so far in the subgen. *Urophyllon* of *Albuca*. In future, chromo-

mycin A^{3+ve} banding or FISH with nucleolar rDNA probe in *A. virens* and related species is needed to complement our observation. Additionally, somatic paring is reported in *A. bracteata* around the AT rich intercalary heterochromatin which was also claimed long back in *A. virens* (Ravindran 1977). However, in the present population, neither AT rich intercalary $DAPI^{+ve}$ bands nor such association trend was noted.

Meiotic chromosome study

Different stages of meiosis were obtained following carmine staining, showing regular chromosome segregation patterns (Fig. 2d-g). After EMA method followed by DAPI staining, clearly visible bivalents were observed in diakinesis and metaphase I of PMCs (Fig. 2h-l). The haploid number was confirmed as n=3. The absence of any earlier gametic count reports, haploid number of *A. virens* was questionable, especially considering the occurrence of polysomaty (Bhattacharya et al. 2016, present study). Presently, regular chromosome paring (Fig. 2h-l) and segregation (Fig. 2d-g) is confirmed in *Albuca virens*, in line with successful fruit and seed set in spite of frequent vegetative propagation. This information is a pre-requisite for infra- or inter-specific crosses for floriculture purposes as in the *Ornithogalum* species (Griesbach et al. 1990, 1993).

CONCLUSION

The karyotype of *Albuca virens* shows distinguishable features like i) diploid number 2n=6, ii) variation in length of chromosomes between homologues and iii) variation in the size of NORs. With the knowledge of dysploid reduction and several rearrangements among the chromosomes, the Indian *A. virens* certainly stands as one unique population of *Albuca*, like the one found by Stedje (1989) from Mozambique with 2n=4. It is questionable whether occurrence of polysomatic numbers in the present plants represents evidences in support of an unstable chromosomal background or a usual feature associated with bulbous ornamentals enjoying abundant vegetative propagating (Sharma and Sharma 1956). Considering the literatures in support of dysploidy and chromosome evolution from 2n=6 (Castiglione and Cremonini 2012), present study adds to the significance of this species as a prototype to analyze chromosome evolution with the help of modern cytogenetic methods. In course of the conventional mitotic chromosome analysis, it was felt that the chromosomes remain in different planes, rendering analysis of morphology difficult, as we also encounter inadequate karyotype information in earlier report of *A. virens* (Bhattacharya et al. 2016). Successful preparation of EMA-based Giemsa- DAPI stained slides and DAPI staining of PMCs open the path for application of molecular cytogenetics like florescence *in situ* hybridization or Ag-NOR staining. Therefore the present dataset along with the technical standardization part, would be an asset for molecular cytogenetic assessment of evolution of the understudied *Albuca virens* in relation to its allied species.

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REFERENCES

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot J Linn Soc. 161: 105–121.

Angiosperm Phylogeny Group. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot J Linn Soc. 181: 1-20.

Arano H. 1963. Cytological studies in subfamily Carduoideae (Compositae) of Japan IX. Bot Mag Tokyo. 76: 32.

Bhattacharya S, Ghosh B, Choudhury M. 2016. A Simple Reliable Protocol for Cytogentically Stable Mass Propagation of *Ornithogalum virens* Lindl. Plant Tissue Cult Biotech. 26: 1-14.

Bhowmick BK, Jha S. 2022. A critical review on cytogenetics of Cucurbitaceae with updates on Indian taxa. Comp Cytogenet. 16: 93-126.

Bhowmick BK, Jha S. 2015. Differential heterochromatin distribution, flow cytometric genome size and meiotic behavior of chromosomes in three Cucurbitaceae species. Sci Hortic. 193: 322-329.
Bhowmick BK, Jha S. 2019. Differences in karyotype and fluorochrome banding patterns among variations of *Trichosanthes cucumerina* with different fruit size. Cytologia. 84: 237-245.
Bhowmick BK, Jha S. 2021. A comparative account of fluorescent banding pattern in the karyotypes of two Indian *Luffa* species. Cytologia. 86: 35-39.
Castiglione MR, Cremonini R. 2012. A fascinating island: 2n= 4. Plant Biosyst. 146: 711-726.
Fukui K. 1996. Plant chromosomes at mitosis. In: Fukui K, Nakayama S, editors. Plant chromosomes. Laboratory methods. Boca Raton, Tokyo: CRC Press, p. 1-18.
Fukui K, Iijima K. 1991. Somatic chromosome map of rice by imaging methods. Theor Appl Genet. 81: 589-596.
Greilhuber J, Speta F. 1976. C-banded karyotypes in the Scilla hohenackeri group, S.persica and Puschkinia (Liliaceae). Plant Syst Evol. 126: 149–188.
Griesbach RJ, Meyer F, Koopowitz H. 1990. Interspecific hybridization of *Ornithogalum*. Hort Science. 25: 1142d-1142.
Griesbach RJ, Meyer F, Koopowitz H. 1993. Creation of new flower colors in *Ornithogalum* via interspecific hybridization. J Am Soc Hortic Sci. 118: 409-414.
Goldblatt P, Manning JC. 2011. A review of chromosome cytology in Hyacinthaceae subfamily Ornithogaloideae (*Albuca*, *Dipcadi*, *Ornithogalum* and *Pseudogaltonia*) in sub-Saharan Africa. South Afr J Bot. 77: 581-591.
Guerra M, Santos KGBD, E Silva AEB, Ehrendorfer F. 2000. Heterochromatin banding patterns in Rutaceae-Aurantioideae—a case of parallel chromosomal evolution. Am J Bot. 87(5): 735–747.
Huziwara Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of *Aster*. Am J Bot. 49: 116–119.
Jha TB. 2021. Karyotype diversity in cultivated and wild Indian rice through EMA-based chromosome analysis. J Genet. 100: 81.
Jha TB, Saha PS, Jha S. 2020. A comparative karyo-morphometric analysis of Indian landraces of *Sesamum indicum* using EMA-giemsa and fluorochrome banding. Caryologia. 73: 81-88.
Jha TB, Bhowmick BK. 2021. Conservation of floral, fruit and chromosomal diversity: a review on diploid and polyploid *Capsicum annuum* complex in India. Mol Biol. 48: 5587-5605.
Johnson MA. 1999. Two new chromosome numbers in an unusual *Albuca* (Hyacinthaceae) from Saudi Arabia. Kew Bulletin. 437-444.
Jong K. 1991. Cytological observations in *Albuca*, including a survey of polymorphic variation in thesat-chromosome pair. Edinb J Bot. 48: 107–128.
Knudtzon SH, Stedje B. 1986. Taxonomy and cytology of the genus *Albuca* (Hyacinthaceae) in East Africa. Nord J Bot. 6: 773-786.
Kurata N, Omura T. 1978. Karyotype analysis in rice. 1. A new method for identifying all chromosome pairs. Japan J Genet. 53:251–255.
Lavania UC, Srivastava S. 1992. A simple parameter of dispersion index that serves as an adjunct to karyotype asymmetry. J Biosci. 17: 179–182.
Liang G, Chen H. 2015. Scaling chromosomes for an evolutionary karyotype: a chromosomal trade off between size and number across woody species. PLoS One. 10: e0144669.
Manning JC. 2020. Systematics of *Ledebouria* sect. *Resnova* (Hyacinthaceae: Scilloideae: Massonieae), with a new subtribal classification of Massonieae. S Afr J Bot. 133: 98-110.
Manning JC, Goldblatt P, Williamson G. 2009a. Hyacinthaceae. *Pseudogaltonia liliiflora* (Ornithogaloideae), a new species from the Richtersveld, Northern Cape. Bothalia. 39: 229–231.
Manning JC, Forest F, Fay MF, Devey S, Goldblatt P. 2009b. A molecular phylogeny and a revised classification of Ornithogaloideae (Hyacinthaceae) based on an analysis of four plastid DNA regions. Taxon. 58: 77–107.
Martinez-Azorin M, Crespo MB, Juan A, Fay MF. 2011. Molecular phylogenetics of subfamily Ornithogaloideae (Hyacinthaceae) based on nuclear and plastid DNA regions, including a new taxonomic arrangement. Ann Bot. 107: 1-37.
Martínez-Azorín M, Crespo MB, Alonso-Vargas MÁ, et al. 2023. Molecular phylogenetics of subfamily Urgineoideae (Hyacinthaceae): Toward a coherent generic circumscription informed by molecular, morphological, and distributional data. J Syst Evol. 61: 42-63.
Nath S, Jha TB, Mallick SK, Jha S. 2015. Karyological relationships in Indian species of *Drimia* based on fluorescent chromosome banding and nuclear DNA amount. Protoplasma. 252: 283-299.
Nath S, Sarkar S, Patil SD, et al. 2022. Cytogenetic Diversity in Scilloideae (Asparagaceae): a comprehensive recollection and exploration of karyo-evolutionary trends. Bot Rev. 1-43.
Öztürk D, Koyuncu O, Koray Yaylacı Ö, Özgişi K, Sezer O, Tokur S. 2014. Karyological studies on the four

Ornithogalum L. (Asparagaceae) taxa from Eskişehir (Central Anatolia, Turkey). Caryologia. 67: 79-85.

Paszko B. 2006. A critical review and a new proposal of karyotype asymmetry indices. Plant Syst Evol. 258: 39-48.

Pedrosa A, Jantsch MF, Moscone EA, Ambros PF, Schweizer D. 2001. Characterisation of pericentromeric and sticky intercalary heterochromatin in *Ornithogalum longibracteatum* (Hyacinthaceae). Chromosoma. 110: 203–213.

Peruzzi L, Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp Cytogenet. 7: 1–9.

Ravindran PN. 1977. Homologous Association in Somatic Cells of *Ornithogalum virens*. Cytologia. 42: 1-4.

Schweizer D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma. 58: 307–324.

Seijo JG, Fernández A. 2003. Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). Am J Bot. 90: 980–987.

Sharma AK, Sharma A. 1956. Fixity in chromosome number of plants. Nature. 177: 335-336.

Stebbins GL. 1971. Chromosomal changes, genetic recombination and speciation. In: Barrington EJW, Willis AJ, editors. Chromosomal Evolution in Higher Plants. Edward Arnold Publishers Pvt. Ltd., London. p. 72–123.

Stedje B. 1989. Chromosome evolution within the *Ornithogalum tenuifolium* complex (Hyacinthaceae), with species emphasis on the evolution of bimodal karyotypes. Plant Syst Evol. 166: 79–89.

Stedje B. 1996. Karyotypes of some species of Hyacinthaceae from Ethiopia and Kenya. Nord J Bot. 16: 121–126.

Watanabe K, Yahara T, Denda T, Kosuge K. 1999. Chromosomal evolution in the genus Brachyscome (Asteraceae, Astereae): statistical tests regarding correlation between

changes in karyotype and habit using phylogenetic information. J Plant Res. 112: 145–161.

Yamamoto M, Takada N, Hirabayashi T, Kubo T, Tominaga S. 2010. Fluorescent staining analysis of chromosomes in pear (*Pyrus* spp.). J Jpn Soc Hortic Sci. 79: 23-26.

Yamamoto M, Terakami S, Yamamoto T. 2015. Enzymatic maceration/air-drying method for chromosome observations in the young leaf of pear (*Pyrus* spp.). Chromosome Sci. 18: 29-32.

Zarco CR. 1986. A new method for estimating karyotype asymmetry. Taxon. 35: 526–530.