



Karyomorphological and morphometric studies in *Urginea wightii*

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Abstract

Urginea is a bulbous perennial plant found in India, Africa, Mediterranean regions of the world. *Urginea* is represented in India by about nine species (*U. indica*, *U. coramandeliana*, *U. govindappae*, *U. nagarjuna*, *U. polyantha*, *U. raogibikii*, *U. razzi*, *U. wightii*, and *U. polyphylla*). We carried out Karyomorphological studies in five different *Urginea wightii* species collected from Gulbarga, Karnataka, India. Each plant of *Urginea* species varied in its morphology and ploidy. We noticed that in *U. wightii* of Gulbarga, there were triploid number of chromosomes which showed variation. Chromosome morphology in each plant is distinct. 'St' type of chromosome was a major component of the karyotype followed by 't' type with asymmetrical karyotype. Polysomaty is responsible for the origin of new genotypes (with different chromosomal configurations) through vegetative propagations. These cytotypes, morphotype and ecotypes can be used as tools to study diversification in *Urginea* species.

Keywords: *Urginea*, karyotype, triploid, polysomaty, sympatry speciation

Introduction

Urginea, belonging to the family Hyacinthaceae, is a perennial glabrous herb commonly known as 'Indian squill'. It is one of the fascinating polytypic genera with about 100 species (Airy Shaw, 1996) [1].

Due to its medicinal properties, the *Urginea* bulb has found its place in the British and European pharmacopeia and is unexplored from a cytotoxic perspective. Several studies have reported the medicinal properties of this species (Shivakameshwari *et al.*, 2011). In chronic bronchitis and asthma, *Urginea* is chiefly used; especially in the traditional indigenous system of medicine, *U. Indica* is reputed for several therapeutic benefits

The genus *Urginea* is known for a wide range of morphological and cytological variations (Shivakameshwari and Muniyamma, 1992) [3]. *U. Indica* is a perfect material for studying morphological variations; it has been recognized long back among the populations. This is probably because no field collection ever contains both floral vegetative & reproductive phase features together. Morphological variations such as the plant height, bulb diameter, leaf area, length of inflorescence etc., have been noted and reported in the plants collected from different localities in India. Each accession varies in its morphology and karyotype (Geetha *et al.*, 2018) [4]. At the population level, the vegetative characters showed significant uniform variations on dependable taxonomic names, and meantime and the reproductive characters. (Shivakameshwari, BijulLakshman *et al.*, 2012) [5].

A cytological examination of the diploid population of *Urginea indica* revealed the existence of chromosomal fragments in the somatic and meiotic cells. Variable number of fragments occurs in the same individual. Raghavan and Venkata Subban, 1940 [6]; Shivakameshwari and Muniyamma., 1999 [7] have made extensive studies and demonstrated the presence of natural polyploidy in the genus. Later Sen reported in this species tetraploids and B-chromosomes in one of the diploid populations. Karyotype

analysis has proved to be very effective for assessing taxonomic relationships in many cases. Comparative karyotype analysis of closely related species has been performed in many cases to explain patterns and directions of chromosomal evolution and to deduce the evolutionary role of karyotype changes (Cristina, F.D., 2005) [8]. In the present study, karyotype analysis is carried out to investigate karyotype data's effectiveness for assessing the phylogenetic relationship. The cytotypes are morphologically distinguishable phenotypic populations. RAPD analysis of *U. indica* reported a differential polymorphism of DNA, showing several polymorphic bands ranging from 2 to 15 with an average of 9.96 (per primer data) among 11 populations. (Narendranathan, *et al.*, 2014) [9].

A perusal of the literature shows that, different versions exist about the species; they show variations in chromosome number and morphology. Thus, the taxonomic identity of each of the species of *Urginea* is considered based on cytology and experimental interspecific hybridization (Dixit and Yadav, 1989) [10]. An attempt is made to inquire into the morphological variations that lead to the evolutionary divergence of *Urginea indica* Kunth. (Shivakameshwari *et al.*, 2010) [11]. This work may form a basis for future studies for assessing phylogenetic relationships in Hyacinthaceae with DNA-based molecular markers.

Materials and Methods

Morphological studies were made from the plant material collected from Gulbarga and were grown under uniform environmental conditions in the Department of Botany, Bangalore University. Morphological parameters such as height, length, diameter of the bulb, number of leaves per plant, inflorescence length, number of flowers per plant, length of flower, length in pedicel, length of stamen, length of gynoecium, length of fruit, inflorescence, vegetative, reproductive phase size, length, breadth of a leaf, root length, were analyzed in the 5 plants of *U. wightii*.

Karyological studies were performed from the collected root tips of the plant. Root tips were excised from a potted plant and pretreated with 0.1% of colchicine and fixed in acetic alcohol (1:3) for 24 hrs. and stored in 70% alcohol. The roots were hydrolyzed in 1N HCl for 5-10 minutes at 60°C. Root tips were squashed and stained in 4% iron hematoxylin and propionic acid. The chromosome numbers of all the plants were recorded and accuracy of the measurement examined using Ideokar tool. Karyotyping procedures were performed as reported in earlier studies (Levan *et al.*, 1964).

Results and Discussion

The cytological variations obtained in the present study provided a basis for recognizing them as distinct cytotypes (Figs. 2–6). The five cytotypes of *Urginea wightii* investigated revealed triploid in all the plants. The karyotypes were asymmetrical in all the accessions, with sub telocentric being the major component of the karyotype followed by sub metacentric, terminal point, and finally telocentric chromosomes (Table 2). These accessions are phenotypically distinct types.

Chromosome number was analyzed using karyotype. Each bulb was analyzed; and according to the analysis, each root showed a varied number of chromosome numbers. The high frequency of triploid cytotypes is most common. The chromosomal variations were observed in the cells of the same root tip. Such variations within the same tissue are termed chromosome mosaics. Similar cases of Polysomaty have been reported in growing shoot apex by Sharma (1956) [13].

The main objective of this study is to identify the ploidy level of the *Urginea wightii* and to compare their morphological characteristics with plants collected from same place with the same chromosome number or different cytotypes. Each plant varies in their morphology (Table 1) and karyotype (Table 2). The total length of chromatin material at metaphase of each karyotype differs from the others. All the plants were brought from their original habitat and grown under uniform environmental conditions; still the morphological differences were retained.

The plants with longer chromatins are supposed to be primitive, whereas plants with shorter chromatins were treated as advanced (Stebbins 1971). Thus, the present study revealed that amongst the five plants of *U. wightii*, the following observations were found:

Plant no. 2 Gulbarga (i) is the most advanced and Plant no.3 Gulbarga (ii) is the most primitive. There is a close homology observed between Plants no. 3 Gulbarga (ii) and 4 (ii). In Pl no.1 Gulbarga (i) $2n=36$, sub telocentric being the major component is followed by submetacentric and telocentric with total chromosomal length 326.52 μm .

In Pl no. 5 Gulbarga (ii) $2n=30$, sub telocentric being the major component is followed by telocentric with total chromosomal length of 215.27 μm .

In Pl no.2 Gulbarga (i): $2n=32$, metacentric being the major component is followed by sub telocentric and submetacentric with total chromosomal length of 710.10 μm .

Gulbarga (ii): $2n=30$, sub metacentric being the major component is followed by sub telocentric and telocentric with total chromosomal length of 156.96 μm .

In Pl no.3 Gulbarga (i): $2n=30$, metacentric being the major is followed by submetacentric and sub telocentric with total chromosomal length 206.51 μm .

Gulbarga (ii): $2n=32$, sub telocentric being the major is component followed by telocentric and submetacentric with total chromosomal length 103.39 μm .

In Pl no. 4 Gulbarga (i): $2n=34$, submetacentric and sub telocentric being the major component followed by metacentric with total chromosomal length 149.96 μm .

Gulbarga (ii): $2n=32$, submetacentric being the major component followed by sub telocentric and telocentric with total chromosomal length 103.82 μm .

In Pl no. 5 Gulbarga (i): $2n=32$, sub telocentric being the major component followed by submetacentric with total chromosomal length 152.25 μm .

Gulbarga (ii): $2n=30$, sub telocentric being the major component followed by submetacentric and metacentric with total chromosomal length 215.27 μm .

Thus, the total chromosomal length and karyotype formula revealed significant difference within the diploid plants. Screening all the plants of *U. wightii* is necessary to corroborate the results mentioned above. The total chromosomal length varies in all the plants studied. Numerical evolution by polyploidy and structural changes leading to intra karyotypic size differences of chromosomes and shifting of centromeres from median to sub median and sub telocentric have been concomitantly operating in the genus (Shivakameshwari *et al.*, 2012) [15].

Urginea species is known for phenotypic plasticity. Such population differences along with 90% uniformity within a population clearly shows plasticity. It may be due to cytological and environmental conditions (Shivakameshwari *et al.*, 2010) [11] and also, due to the genotypic and phenotypic dynamics variations in *Urginea* species. The five plants collected from Gulbarga have been treated as different cytotypes of *U. wightii*.

Morphologically, based on the length of inflorescence, *U. wightii* (8-10cm), all the plants were grown in the greenhouse under uniform environmental conditions. The morphological and cytological complexity of the genus *Urginea* made the taxonomic identity of different species and their accessions in India difficult and is considered as a secondary center of origin (Yadav and Dixit 1990) [15]. Cytological differences are of common occurrence in *Urginea* species. These cytologically different accessions are referred to as cytological races or cytotypes (Lekhak *et al.*, 2017) [16]. The primary ancestral chromosome number of *Urginea* was proposed as $X=10$ by (Goldblatt *et al.*, 2012) [17].

The karyotype of diploid and tetraploid of *D. wightii* are of highly asymmetrical nature (Neetin *et al.*, 2012) [18]. Polyploids are no doubt adaptive nature and habitat in separate ecological conditions than their diploid relatives (Neetin *et al.*, 2012) [18]. Polyploidy represents an important evolutionary phenomenon in angiosperms, and is considered to be a significant sympatric speciation mode (Neetin *et al.*, 2012) [18].

The recognition of different karyotypes that correspond to different morphological forms is noteworthy. In situ hybridization and banding are needed to study the cause of diversity in the *Urginea*. Detailed karyotype analysis in same plants of *U. wightii* in the present study were helpful to ascertain the differences within the species and assign them as different cytotypes at intraspecific levels. Differences in absolute chromosome size reflect different amounts of gene duplication either in a tandem fashion or through polytene multiplication of chromonemata.

The total chromosomal length and karyotype formula revealed significant difference within the diploid plants. Screening all the plants of *U. wightii* is necessary to verify the results mentioned above. The total chromosomal length varies in all the plants studied. The karyotypes done were characterized by heterogenous chromosomes with subterminal chromosomes being the major component. According to Desai *et al.* (2012) [18], the karyotype studies of *Indian drimia* species revealed polyploidy, making their identification more difficult. Flowering and blooming times also varied wightii complexes, which might have played an important role in speciation and evolution of the species. In wightii complex, perforated exine ornamentation was observed. Thus, the conventional karyotypic studies plus pollen and flower phenology proved to be of great use in understanding the evolutionary pattern and relationship among wightii complex (Geetha *et al.*, 2016) [21]. Some variant nuclei with increase or decrease in chromosome

number have also been recorded. Limited seed dispersal is an important determination of the spatial of gene flow and genetic differentiation within species, which in turn can be key precursors to speciation (Thomas J. Givnish 2010) [22]. Sympatric speciation proceeds without spatial isolation and with divergence occurring under conditions of random mating (Richard *et al.*, 2008) [23].

In the present study, sympatric type of speciation has been noticed with different chromosome numbers in the same plant. Polysomaty has been noticed. Polysomaty is responsible for the origin of new genotypes (with different chromosomal configurations) through vegetative propagations (Sen 1973) [24].

Thus, Polysomaty involves the occurrence of structurally and numerically altered chromosome complements in the somatic cells along with the normal chromosome complements. Similar studies have been made by Sumitha Sen 1973 [24] in some members of Liliales.

Table 1: Morphological variations in accessions of *Urginea Wightii*

Sl. no	Parameters	Plant no1	Plant no2	Plant no 3	Plant no 4	Plant no 5
1	Height of the plant in cm	28cm	20.5cm	16.4cm	23.5cm	23cm
2	Length of the root in cm	15cm	11cm	18cm	7cm	11cm
3	The diameter of the bulb in cm	14cm	16cm	18.4cm	20cm	17.5cm
4	No. of leaves per plant	3	5	4	5	4
5	Inflorescence Length in cm	11.5cm	11.9cm	20cm	31cm	10.5cm
6	No of flowers per plant	12	13	6	5	11
7	Length of flower in Cm	5	4	3	5	6
8	Length of pedicel in cm	3	3.7	4	4	4
9	Length of stamen in cm	0.6	0.6	0.6	0.8	0.8
10	Length of the gynoecium in cm	0.9	1	0.2	1.2	1
11	Length of fruit in cm	1.2	1	1	1.2	1
12	Blooming Time	Night	Night	Night	Night	Night
13	Inflorescence	Hysteranthous	Hysteranthous	Hysteranthous	Hysteranthous	Hysteranthous
14	Vegetative phase	June–Dec	June–Dec	June–Dec	June–Dec	June–Dec
15	Reproductive phase	March–April	March–April	March–April	March–April	March–April

Table 2: Karyometric data in *Urginea wightii*

Plant no	Place of Collection	Chromosome number	ploidy	Karyotype formula	Total chromosome Length in μm	Karyotype
1	Gulbarga (i)	2n=36	Triploid	Sm4st12t2	326.523	Asymmetrical
	Gulbarga(ii)	2n=30	Triploid	St11t4	215.271	Asymmetrical
2	Gulbarga(i)	2n=32	Triploid	St4sm2 m10	710.105	Asymmetrical
	Gulbarga(ii)	2n=30	Triploid	Sm9st4t2	156.962	Asymmetrical
3	Gulbarga(i)	2n=30	Triploid	Sm5st4m6	206.51	Asymmetrical
	Gulbarga(ii)	2n=32	Triploid	Sm3st9t4	103.395	Asymmetrical
4	Gulbarga(i)	2n=34	Triploid	St7sm7m3	149.962	Asymmetrical
	Gulbarga(ii)	2n=32	Triploid	St7sm8t1	103.823	Asymmetrical
5	Gulbarga(i)	2n=32	Triploid	St12sm4	152.25	Asymmetrical
	Gulbarga(ii)	2n=30	Triploid	St7sm6m2	215.271	Asymmetrical



Fig 1: Vegetative and reproductive bulbs of *U. wightii*

Karyotypic variation in *Urginea wightii*. hyacinthaceae

Fig 2: Plant number 1

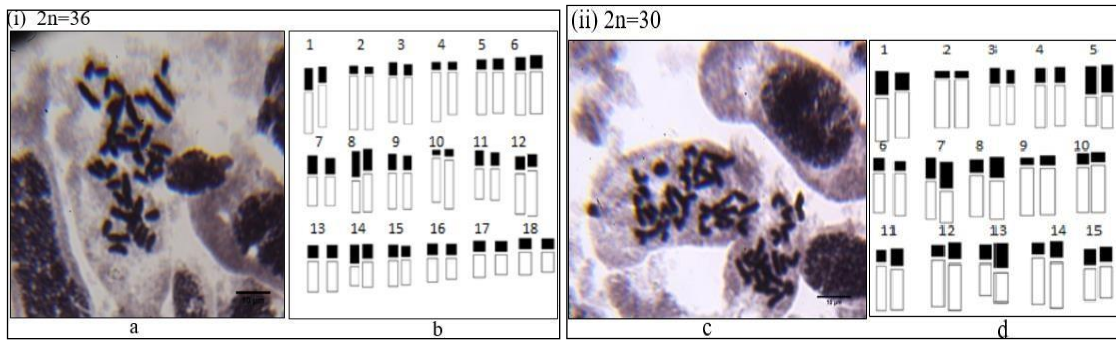


Fig 2: a-d. Somatic metaphase chromosomes and ideograms of *U. wightii*. (i) Somatic metaphase chromosomes of Gulbarga plant no 1(a) showing $2n=36$. (b) Ideogram of Gulbarga plant $2n=36$. (ii) Somatic metaphase chromosomes of plant no 1(c) showing $2n=30$. (d) Ideogram of Gulbarga plant $2n=30$. Scale bar= $10\mu\text{m}$.

Fig 3: Plant number 2

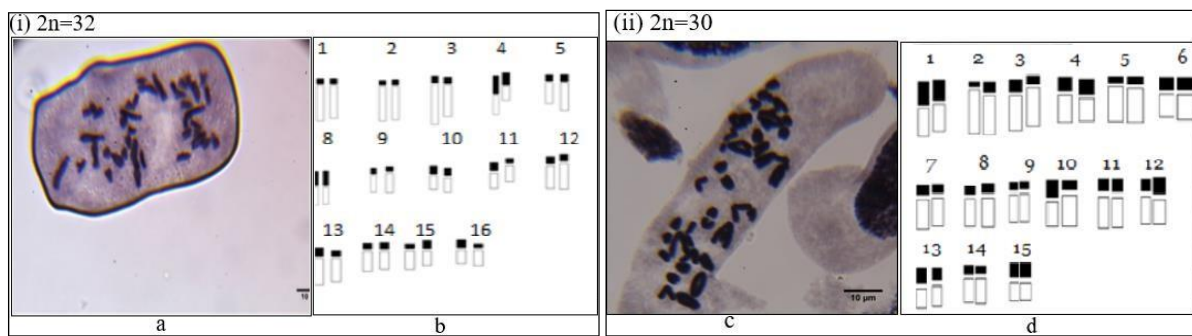


Fig 3: a-d. Somatic metaphase chromosomes and ideograms of *U. wightii*. (i) Somatic metaphase chromosomes of Gulbarga plant no 2(a) showing $2n=32$. (b) Ideogram of Gulbarga plant $2n=36$. (ii) Somatic metaphase chromosomes of plant no 2(c) showing $2n=30$. (d) Ideogram of Gulbarga plant $2n=30$. Scale bar= $10\mu\text{m}$.

Fig 4: Plant number 3

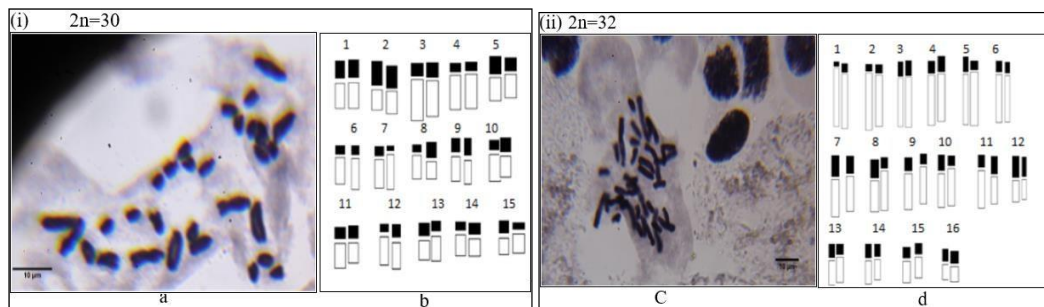


Fig 4: a-d. Somatic metaphase chromosomes and Ideogram of *U. wightii*. (i) Somatic metaphase chromosomes of Gulbarga plant no 4(a) showing $2n=30$. (b) Ideogram of Gulbarga plant $2n=36$. (ii) Somatic metaphase chromosomes of plant no 4(c) showing $2n=30$. (d) Ideogram of Gulbarga plant $2n=32$. Scale bar= $10\mu\text{m}$.

Fig 5: Plant number 4

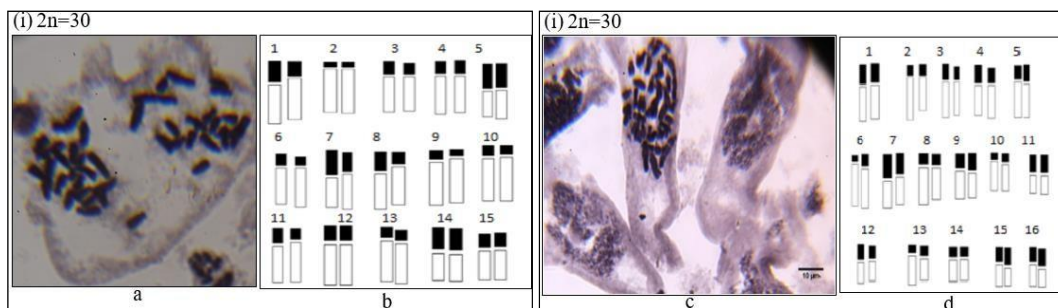


Fig 5: a-d. Somatic metaphase chromosomes and Ideogram of *U. wightii*. (i) Somatic metaphase chromosomes of Gulbarga plant no 4(a) showing $2n=30$. (b) Ideogram of Gulbarga plant $2n=36$. (ii) Somatic metaphase chromosomes of plant no 4(c) showing $2n=30$. (d) Ideogram of Gulbarga plant $2n=32$. Scale bar= $10\mu\text{m}$.

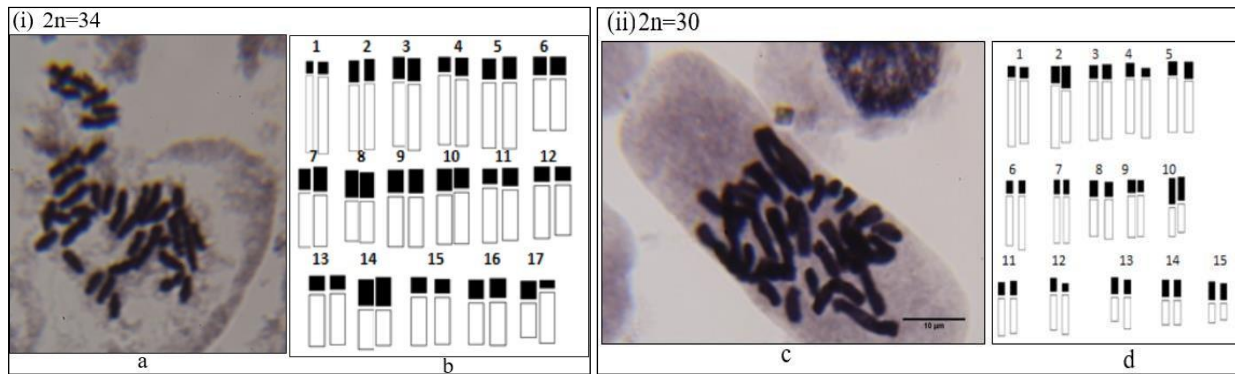
Fig 6: Plant no 5

Fig 6: a-d. Somatic metaphase chromosomes and Ideogram of *U. wightii*. (i) Somatic metaphase chromosomes of Gulbarga plant no 5(a) showing $2n=30$. (b) Ideogram of Gulbarga plant $2n=36$. (ii) Somatic metaphase chromosomes of plant no 5(c) showing $2n=30$. (d) Ideogram of Gulbarga plant $2n=32$. Scale bar= $10\mu\text{m}$.

Conclusion

The cytological and morphological variations observed in these five plants of *U. wightii* have shown that each plant has distinct cytotypes and morphotypes, indicating the inherent phenotypic and genotypic dynamics.

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