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Ecotype diversity of numerical and structural chromosomes of *Drimia indica* (Roxb.) Jessop (Hyacinthaceae) as revealed from karyotype analysis

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ABSTRACT

Drimia indica (Roxb) Jessop commonly known as Indian squill, a perennial medicinal bulbous plant, is used in traditional medicine. Cytological studied for four ecotypes were analyzed from Odisha viz. ODi-16 (Khandapada), ODi-22 (Nayagarh), ODi-24 (Daspalla), ODi-26 (Odagaon) and found that diploid chromosome number 2n=20 with a anuploid number from Nayagrah (2n=16). Detailed karyotype analysis showed structural chromosome variations among the ecotypes and high number of secondary constricted chromosomes are available in comparatively higher altitude (178m) from Nayagarh area. Chromosome length varied from 196.36 µm in ODi-22 (Nayagrah) to 211.39 µm in ODi-26. Karyotypes showed more variations in Type A and Type B chromosomes as compared to Type C (Median constricted chromosomes) and Type D (Sub median constricted chromosomes). TF% varied from 31.37% to 34.28%. The occurrence of natural cytological abnormalities with sticky bridge formation, early separation, lagerds, DNA fragmentation in the ecotypes might be due to microevolution and stabilization of chromosomes for adaptation. Chromosome polymorphism could be an important parameter along with morphology and other DNA markers for phylogenetic analysis and species evolution.

1. Introduction

Indian squill, *Drimia indica* (Roxb) Jessop (*Syn. Urgenia indica* Kunth.) of the family Hyacinthaceae, is a perennial medicinal bulbous plant. In traditional medicine, bulbs of *D. indica* are having important therapeutic uses. This genus is extremely polytypic genus comprising of hundreds of species occurring in India, Africa and Mediterranean region (Ahmed *et al*., 2006). Nine species are commonly seen in India (Hemadri and Swahari, 1982) which contradicts with taxonomic revision by Deb and Dasgupta (1987), who recognized five species in India. It is reported to be used in chronic bronchitis and asthma, anthelmintic, cardio-tonic in heart insufficiency, deobstruent, digestive, expectorant, stomachic, diuretic (Blumenthal *et al*., 1998). It is also used in rheumatism, leprosy, skin diseases, internal pain and scabies (Kirtikar and Basu, 1988). Out of the phytochemicals, the glycosides, scillarin-A and scillarin-B have been reported in fresh squill (Prajapati *et al*., 2003). Other constituents found in squill include flavonoids, carbohydrates, antifungal glycoproteins, steroids, alkaloids, tannins, coumarins and saponins (Abbas *et al*., 2012; Siva Kameshwari *et al*., 2012, 2013; Bashir *et al*., 2013). Pharmacological evaluations revealed the presence of antibacterial, antifungal (Shenoy *et al*., 2006), laxative and spasmodic (Abbas *et al*., 2012), antioxidant, antiangiogenic and pro-apoptotic activities in *U. indica* (Deepak and Salimath, 2006). Since Indian squill bulbs have long been used as a source of natural product with pharmaceutical and biocidal applications, numerical and structural alteration of chromosomes play a major role in polyploidization and thus variation in phytochemical constituents.

The genus *Drimia* (*Syn. Urginea*), wild onion, has been studied for biosystematics (Shiva Kameshwari *et al*., 2012); cytogenetics (Raghavan, 1935; Sen, 1974; Jha and

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Sen, 1983a, b; Subramanian, 1987; Dixit and Yadav, 1989; Yadav and Dixit, 1990) and DNA markers (Harini *et al*., 2008, Kawalkar 2010, Desai *et al*., 2012; Alluri *et al*., 2015). Ethno-medicinally bulbs of *D. indica* are reported to be antiulcerous, antinematodal, antitumorous, antiarthrities properties and also used to warts, abscesses, boils of skins, cardiac diseases, antidote to scorpion sting (Chittoor *et al*., 2012). The bulbs contain literally hundreds of phytocompounds that defend cells against free radical damage by blocking the development of heart diseases, cancer, rheumatism, edema, gout, asthma, dog bites, cut wound, infertility in man. Due to these medicinal properties of *D. indica* bulbs have been exploited commercially from natural habitats. As per IUCN criteria, threat status of *D. indica* is vulnerable for states like Chhattisgarh and Madhya Pradesh. Phylogenetic problems are being reported using morphology and cytology, DNA and protein, mitochondrial and nuclear genomes (Nylander *et al.,* 2004; Shiva Kameshwari, 2013).

Hence, it is necessary to initiate awareness, conservation and cultivation of Indian squill. Anthropogenic pressures such as habitat degradation are largely responsible for genetic depletion and loss of genetic diversity (Shiva Kameshwari *et al*., 2012). Therefore, genetic analysis of ecotypes is quite important for its active principle variation and there is urgent need for conservation and sustainable utilization of this economically important medicinal plant. The potential ecotype variation is very high in Odisha which is unexplored and that create interest for cytological study from various ecological area for karyotype and chromosomal analysis to ascertain the ploidy changes, if any, and possible role of genomic plasticity in adaptation of the plant and its implication in active principle accumulation.

2. Materials and methods

2.1. Cytological analysis

Four ecotypes of *Dremia indica* were collected from different parts of Odisha and maintained in green house of Department of Botany, Utkal University, Bhubaneswar (Table 1). Actively growing root tips (1.5-2 mm) were pre-treated in half saturated para dichlorobenzene (pDB) and aesculin mixture (1:1) for 4 h at 18^{o}C in refrigerator and then fixed in 1:3 acetic acid:ethanol overnight at room temperature. Fixed roots were treated in 45% glacial acetic acid for 15 min. Root tips were stained in 2% aceto-orcein followed by cold hydrolysis with 5N HCl at 4^{o}C for 5 min. Chromosome squash were made using 45% glacial acetic acid and were observed under Olympus BX-53 microscope. Digital microphotographs were captured in Micro Publisher 5.0 RTV camera using QCapture Pro 7 (Canada) software for karyotype analysis.

Total chromosome length was estimated by adding the length of all chromosomes in the karyotype by applying formula ðr^{2}h, where 'r' is the radius and 'h' is the length of the chromosome respectively. Analysis of the chromosome type was conducted according to Levan *et al*. (1964), and that of the karyotype in accordance with the classification standard of Stebbins (1971) modified by Das and Mallick (1993). Form percentage (F %) of individual chromosome was calculated.

3. Results and discussion

Chromosome numbers of all the four cultivars showed 2n = *2x* = 20 except ODi-22 collected from Nayagarh with 2n=16 chromosomes. The size variation of chromosomes within the karyotype was obtained in all the ecotypes ranged from small to large size. All the somatic chromosomes are classified as Type A with comparatively large chromosomes having nearly median (nm) primary and nearly median or sub-median (nsm) secondary constrictions; Type B with medium sized chromosomes having nearly sub-median (NSM) primary constriction and nearly sub terminal (nst) secondary constriction; Type C with medium size chromosome having nearly median primary constriction (nm) and Type D with small to medium size chromosomes having nearly sub-median (nsm) primary constriction (Table 1). Although all the ecotypes showed numerical variation in diploid somatic chromosome number with four Types of chromosome, the numerical differences of different Types of chromosomes were recorded which was revealed by karyotype formulae of all the genotypes (Figs. 1 – 4, 1a-4a) showing definite differences in their chromosome structure recorded as follows;

Ecotype ODi-16 (Khandapara)

The karyotype formula of ODi-16 was assigned to be $1A_{nm,nsm}+1A_{nm,nst}+2B'_{nsm,nst}+1C_{nm}+5D_{nsm}$ with 2n = 20 chromosomes (Figs. 1,1a). The total chromosome length was found to be 176.72 µm with a total form percentage of 33.35%.

Ecotype ODi-22 (Nayagarh)

The karyotype formula of ODi-22 was assigned to be $2A_{nm,\ nsm}+2B_{nsm,nsm}+1B'_{nsm,nst}+1C_{nm}+2D_{sm}$ with 2n = 16 chromosomes (Figs. 2, 2a). The total chromosome length was found to be 163.64 µm with a total form percentage of 34.28%.

Ecotype ODi-24 (Daspalla)

The karyotype formula of ODi-24 was assigned to be $2A_{nm,\ nsm}+1B_{nsm,\ nsm}+1C_{nm}+6D_{nsm}$ with 2n = 20 chromosomes (Figs. 3, 3a,). The total chromosome length, volume, TF%

Table 1. Detailed karyotype analysis of the four ecotypes of *D. indica* with different chromosomal parameters.

Ecotype	Place of collection	Latitude/longitude/ altitude	Somatic chromosome number (2n=2x)	Karyotype formula	NSC+	Total chromosome length (µm±SE)	Total F%
ODi-16	Khandapada	20.26°N, 85.17°E, 65m	20	$1A_{nm,nsm}+1A'_{nm,nst}+2B'_{nsm,nst}+1C_{nm}+5D_{nsm}$	4	176.72±2.80	33.35
ODi-22	Nayagarh	20.12°N, 85.10°E, 178m	16	$2A_{nm,nsm}+2B_{nsm,nsm}+1B'_{nsm,nst}+1C_{nm}+2D_{nsm}$	5	163.64±3.43	34.28
ODi-24	Daspalla	20.33°N, 84.85°E, 122m	20	$2A_{nm,nsm}+1B_{nsm,nsm}+1C_{nm}+6D_{nsm}$	3	196.36±3.55	32.74
ODi-26	Odagaon	20.02°N, 84.98°E, 32m	20	$3A_{nm,nsm}+1B_{nsm,nsm}+1C_{nm}+5D_{nsm}$	4	211.39±4.58	31.37

+ NSC = Number of secondary constricted chromosome

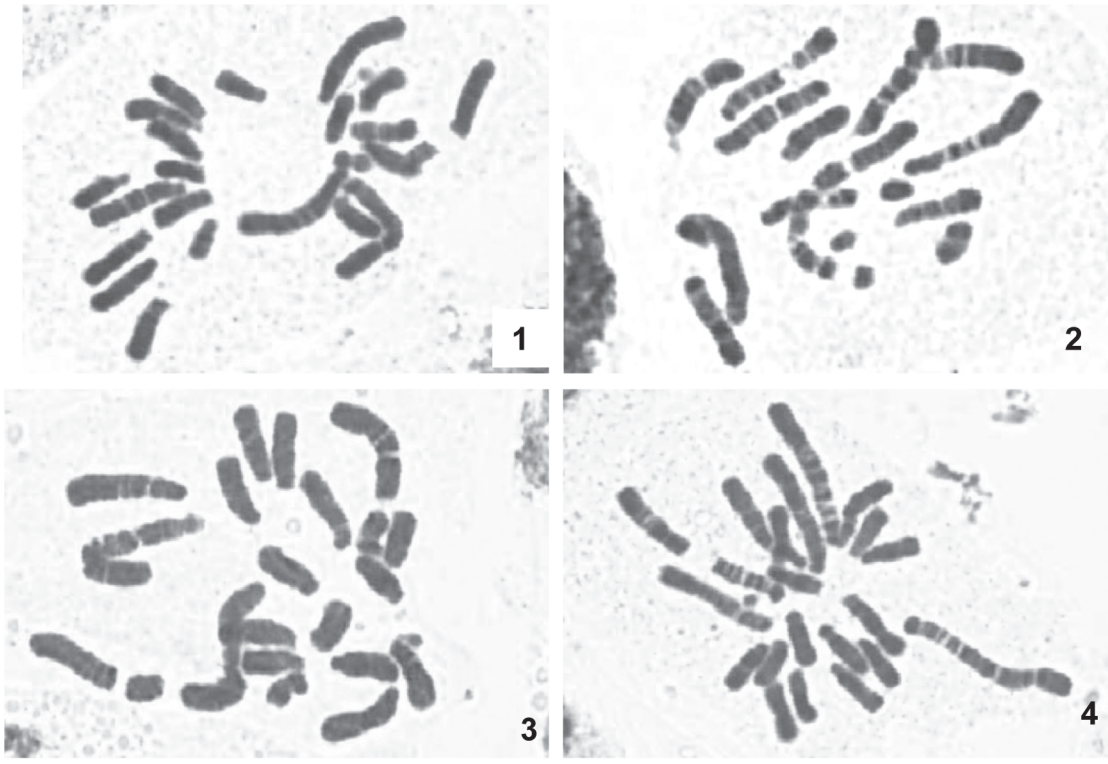
and INV were found to be 196.36µm and 32.74 % respectively.

Ecotype ODi-26 (Odagaon)

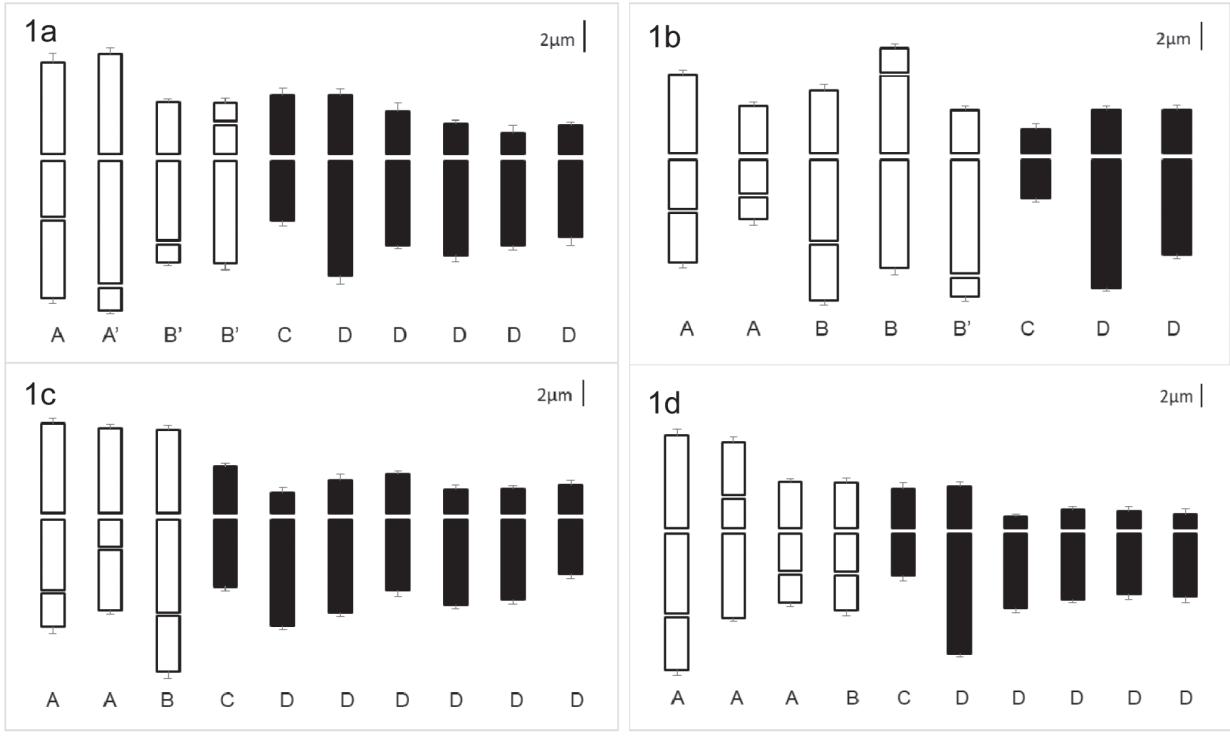
The karyotype formula of ODi-26 was assigned to be $3A_{nm,nsm}+10_{nsm,\ nsm}+1C_{nm}+5D_{nsm}$ with 2n = 20 chromosomes (Figs. 4, 4a). The total chromosome length was found to be 211.39 µm with a total form percentage of 31.37 %. The ideogram of the genomic constitutes varied significantly as depicted in Figs. (1a-4a).

The chromosome structure and genomic length variation as evident from karyotype and idiogram (1a-4a) confirms the structural alteration of chromosomes and stabilization of cytotypes in ecotypic level. However, heteromorphicity in respect of centromeric position indicated the occurrence of a deletion at the short arm of the respective chromosome was noticed in *Allium* species (Mahbub *et al.,* 2014) having distinct CMA- and DAPI-banding patterns to localize intensity and percentage of GC- and AT-rich repeats. Differences in chromosome length might be due to differential condensation and spiralization of the chromosome arms and species-specific compaction of DNA threads along with nucleosomes (Das and Mallick, 1989) with altered non-histone proteins (Chattopadhyay and Sharma, 1990). The alteration in the TF% might be due to chromosomal alteration due to break and reunion of the chromosome arms in early stages of evolution in the genome. Duplication of chromosomes or translocation between the chromosomes with or without secondary constrictions at a very early stage of evolution might be the reason there is a structural alteration of the chromosome morphology as well as the variation of secondary constricted chromosomes in the above ecotypes (Das and Das, 1994; Rai *et a*., 1997; Ghosh *et al.,* 2013; Das *et al.,* 2015). High TF% in all the ecotypes indicates the alteration of chromosome structure in the genome. These factors indicate greater genome stability conferring resistance to the cultivars against biotic or abiotic environmental stresses which is a characteristic feature of ecotypes that need to confirm in future by fluorescent *in situ* hybridization (FISH) or genomic *in situ* hybridization (GISH) as shown (Doležel *et al.,* 2004, Jeridi *et al.,* 2011, Nath *et al*., 2015).

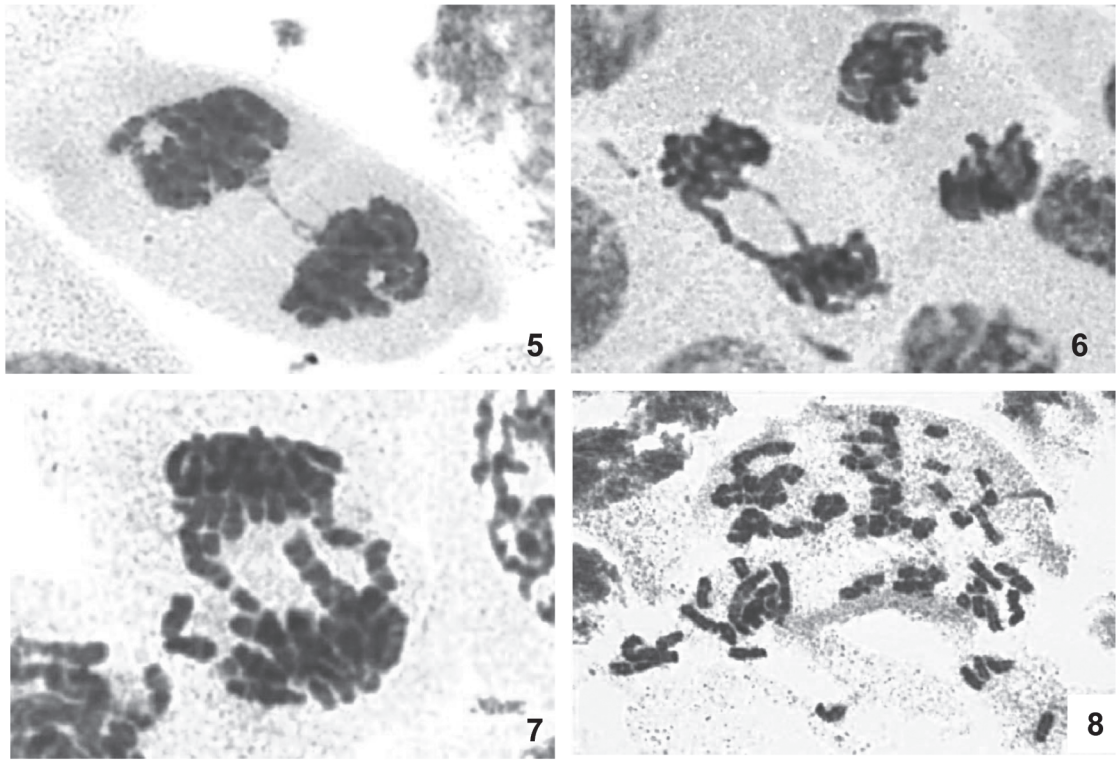
Chromosome polymorphism within species is often ignored by systematic botanist and comparative evolutionary biologists. However, the cytocypic variation are found in this study might be due to macroevolution within the species. The occurrence natural cytological abnormalities with sticky bridge formation (Fig. 5), early separation (Fig. 6), lagerds (Fig. 7), DNA fragmentation (Fig. 8) in some of the ecotypes might be due to microevolution and stabilization of chromosomes of the species in a specific condition for adaptation. Polymorphism may have profound impact on phylogeny reconstruction, species delimitation, and studies of character evolution as suggested by John (1999). The interpopulation variation pattern found in *D. indica* is very complicated and difficult to conclude. The morphological complexity is accompanied by a high degree of cytological variation has been reported earlier (Jha and Sen, 1983a, b, Shiva Kameshwari *et al*., 2013, Nath, 2015). *D. indica* seems to have high genomic and phenotypic plasticity and phylogeny. Of the four studied ecotypes the vegetative character could not show great variations but anuploids number 2n=16 was obtained in ecotypes from Nayagrah (ODi-22). On the other hand, the reproductive characters have shown less variation and are almost uniform. Cytological studies recorded the presence of diploid and aneuploid populations (Figs. 1-4) 2n = 16 was new records



Figs. 1-4. Metaphase plates of four ecotypes of *D. indica* of Odisha; (1) Khandapada, (2) Nayagarh (3) Daspalla (4) Odagaon. Magnification bar = 5 µm.



Figs. 1a-4a. Idiogram of four ecotypes of *D. indica* of the corresponding metaphase plates.



Figs. 5-8. Chromosome aberration are found along with root tips cells (5) Sticky bridge (6) Early separation of chromosome (7) Lagard chromosomes (8) Chromosome fragmentation.

for the species. These morphological variations along with cytological variations and similarities are considered responsible for designating them as cytotypes. But there may be a chance of variation in secondary metabolites which need to be studied. Such type of investigations on variations would not only indicate the principal feature of evolution within species but may also lead to exploitation of certain distinct genotypes for commercial purposes. Several populations are recorded with ploidy and anuploidy with connecting link from tetraploid 2n = 40 to diploid 2n = 20 (Shiva Kameshwari *et al*., 2010, 2013).

4. Conclusion

Cytological studied for four ecotypes of *Drimia indica*, commonly known as Indian squill, a perennial medicinal bulbous plant, ODi-16 (Khandapada), ODi-22 (Nayagarh), ODi-24 (Daspalla), ODi-26 (Odagaon) were under taken. Diploid chromosome number 2n=20 was found in all ecotypes except a anuploid 2n=16 from Nayagrah. Detailed karyotype analysis showed structural chromosome variations among the ecotypes and high number of secondary constricted chromosomes. TF% also varied from from sub-median to nearly median type. The occurrence of natural cytological abnormalities might lead to cytotypes during microevolution.

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