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Karyotypic Study in *Urginea indica* Kunth Collected from Bundu, Jharkhand

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Abstract: The morphologically similar or uniform group of plants having same chromosome number is reported to have separate genetic system. Therefore, to understand the evolutionary development and genetic relationship of the morphologically distinguished varieties of plants, chromosome study is an absolute device. Similarly, stomatal variations among the species are of taxonomic significance. Hence, in the present research work, *Urginea indica* Kunth from Bundu, Jharkhand, were collected to carry out its detailed karyotype as well as the stomatal analysis. It was observed to be diploid with $2n = 20$ chromosomes, showing the asymmetrical karyotype. The stomata were of anomocytic type, being comparatively larger in size.

Key words: *Urginea indica* Kunth, Karyotype, asymmetry, diploids, stomatal index, anomocytic stomata.

I. INTRODUCTION

Urginea indica Kunth is a bulbous herb of Liliaceae family having a magical potential to heal several human ailments. It has long been used extensively as a source of medicine. It is commonly known with the names Indian squill and van pyaz or jungli dungli. *Urginea indica* Kunth exists in several distinct morphological forms and identification of such plants on the basis of cytotaxonomical and epidermal features from remote areas would be of great use in preparing the database.

Therefore, the main highlights of this research were to analyze (i) the detail karyomorphology and stomatal characters of the cytotype of *Urginea indica* Kunth collected from Bundu, Jharkhand.

II. RESEARCH METHODOLOGY

Bulbs of *Urginea indica* Kunth were collected from Bundu, Jharkhand and were brought into cultivation in experimental gardens. For the karyotypic analysis, primary roots were cut off and pretreated, fixed (with ethanol-acetic acid 3:1 for 24 hours) and preserved (in 70% alcohol). Preserved root tips were hydrolyzed in 1N HCL for five to ten minutes depending upon the thickness of the roots. Washed and hydrolyzed root tips were then stained with 2 per cent acetocarmine and slides were prepared by squash technique.

Well spread metaphase plates were selected to measure the length and width of long arms and short arms of chromosomes for karyotype analysis. The plates were then photographed in digital SLR Nikon camera. Visible chromosomes at metaphase plates were scored under 450x magnifications. Types of chromosome were identified and classified according to Abraham and Prasad (1983)^[1].

Data obtained were analyzed statistically using the various formulae. Idiogram was prepared from the statistically analyzed data. For stomatal studies, the leaves of the selected cytotype were taken. The epidermal peels of the leaves were obtained using a sharp, pointed forceps from both dorsal and ventral surfaces^[2]. The epidermal peels were thoroughly washed with water, stained with aqueous safranin and mounted in a drop of glycerin on a glass slide. The slides were examined with the compound microscope (450x magnification) and the nature of distribution and dimensions of stomata were studied. The studied features included: length and width of stomata and also the stomatal index (SI) which was given as:

$$SI = \frac{\text{Number of stomata per unit area}}{\text{Number of Epiermal cells per unit area} + \text{Number of Stomata per unit area}} \times 100$$

Well spread metaphase plates and stomata were photographed in digital SLR camera.

III. RESULTS AND DISCUSSION

The statistical data of Karyotype and stomatal characters are presented in tables (table 1 to 3; fig. 1 to 4). Karyotype analysis including relative length of individual chromosomes and the ratio of long arm and short arm of the observed cytotype of *U. indica* Kunth are demonstrated in table 1, in which the chromosome numbers were named from I to X in accordance with their length. The cytotype of *U. indica* Kunth under investigation was reported diploid with $2n = 20$ chromosomes, showing normal mitotic division

in all the examined cells. Neither secondary constriction nor satellites were observed. Although diploids of *Urginea indica* Kunth often shows the presence of satellites and secondary constrictions but their absence in the investigated cytotype showed that the genic constitution of such cytotype may have the adaptive value not requiring to be supplemented by satellites and secondary constrictions^[3]. The absence of satellites might be due to the deletion of satellites and subsequent loss during evolution^[4].

However, significant variations were observed with respect to chromosome length which was noticed to be comparatively longer ranging from very long to short (table 1; fig. 2).

This shows the asymmetrical tendency of the observed cytotype as asymmetrical karyotypes possess many chromosomes with sub terminal centromeres or great differences in size between the largest and the smallest chromosomes or both.

The species and varieties having greater number of sub-median and telocentric chromosomes should be treated more evolved than those where there are lesser number of sub metacentric and telocentric chromosomes^[5].

But in the present investigation, the chromosomes constituted nearly median and nearly sub-median (-) groups with the karyotype formula: 3nm+7nsm. Therefore, the Total Chromatin Length (TCL), Total Form Percentage (TF%), Gradient Index (G.I.), Symmetry Index (S.I.) and Disparity Index (D.I.) of *Urginea indica* Kunth were also calculated (table 2) to find out the karyotypic type of the cytotype. And based on these factors, it was observed to possess asymmetrical karyotype.

Urginea indica Kunth is reported to show significant variation with respect to its morphology. So, to identify the potential cytotypes of this species, cytological analysis along with stomatal study can be of significant importance. Anatomical studies in plants had long been used extensively for the identification of different species and to define the taxonomic status of the species. It also helps to expand the diagnostic features of taxon.

This in turn, helps to confirm the identity of the plant species with respect to the external characters and the internal features. Among the vast anatomical features, leaf surface character is used as secondary or supporting characters in biosystematics studies^[6]. Leaf anatomy including the morphology of stomata has great importance in taxonomy^[7].

The study of stomata is considered as a desirable tool in taxonomic research as the genera and even families show great constancy for their possession of stomatal complex. There still remains a considerable variability among different species. Thus, stomatal studies help to understand true evolutionary relationship of monocotyledons^[8].

It is also reported to show phylogenetic relationships within plants^[9,10]. Furthermore, the stomatal studies (size, shape as well as number of stomata) allow detecting some genetic processes involved in speciation process and adaptation within the species to the environmental conditions^[11].

Therefore, this research work presents the stomatal characters of the observed cytotype of *Urginea indica* Kunth. Stomatal index and stomatal size were calculated (table 3; fig. 1-4) at dorsal and ventral surfaces of the leaves in apex, middle and base portions. The stomata were observed to be present on both dorsal and ventral surfaces of leaf in all the cytotypes, but were more abundant on ventral surface of leaves.

Significant variations in the Stomatal Index were observed. Guard cells were not found to be surrounded by subsidiary cells and the stomata were reported to be of anomocytic type.

This corresponds to the earlier findings as the stomata without subsidiary cells are particularly confined to the members of Liliaceae family. This type of stomata is reported to be derived independently from the primitive types containing numerous parastomatal cells and is considered most advanced evolutionary^[12,13]. The types and features of stomata are often considered helpful in defining and identifying taxa. This finding is similar to the earlier findings.

Moreover, the size of stomata was also observed which was noticed to be larger. Stomatal size is considered to be positively correlated with genome size across wide range of major taxa. Stomata size predicts genome size within angiosperms^[14].

It has been reported that the larger size of stomata is correlated with the higher level of ploidy in plants and slower response to dehydration^[15]. It also increases the transpiration rate which increases the photo-synthesis in turn plant grows as faster rate in wet season and invariably increase the production^[16].

Thus, the present finding showed the leaf epidermal anatomy of selected cytotype of *Urginea indica* Kunth which are influenced by environmental conditions. These findings would be helpful to understand the taxonomic and systematic value of stomata type and distribution patterns^[17].

Table-1 : Cytotaxonomical data of *Urginea indica* Kunth

| Cytotype | Chrom. number | Arm length | | Chrom. Length (μ) | Arm ratio | R. L. (μ) | F% | TCI | Classification |
|-----------------------------|---------------|--------------------|---------------------|-------------------------|-----------|-----------------|--------|--------|----------------|
| | | Long arm (μ) | Short arm (μ) | | | | | | |
| <i>Urginea indica</i> Kunth | I | 8.11 | 4.41 | 12.52 | 1.838 | 100 | 35.235 | 14.063 | nsm(-) |
| | II | 7.77 | 4.62 | 12.39 | 1.682 | 98.993 | 37.288 | 13.922 | nsm(-) |
| | III | 6.76 | 4.20 | 10.96 | 1.610 | 87.584 | 38.314 | 12.317 | nm |
| | IV | 6.68 | 4.24 | 10.92 | 1.574 | 87.248 | 38.846 | 12.270 | nm |
| | V | 6.17 | 3.02 | 9.20 | 2.042 | 73.490 | 32.877 | 10.335 | nsm(-) |
| | VI | 5.67 | 2.86 | 8.53 | 1.985 | 68.121 | 33.498 | 9.580 | nsm(-) |
| | VII | 4.99 | 2.56 | 7.56 | 1.951 | 60.403 | 33.889 | 8.495 | nsm(-) |
| | VIII | 4.12 | 2.73 | 6.85 | 1.508 | 54.698 | 39.877 | 7.692 | nm |
| | IX | 3.65 | 2.10 | 5.75 | 1.740 | 45.973 | 36.496 | 6.465 | nsm(-) |
| | X | 2.77 | 1.55 | 4.33 | 1.784 | 34.564 | 35.922 | 4.861 | nsm(-) |

R.L. = Relative Length

F% = Form Percentage

T.C.I. = Total Chromatin Index

nm = Nearly Median

nsm(-) = Nearly Sub median (Centromere away from the terminal point)

Table 2: Data related to karyotype of *Urginea indica* Kunth

| TCL | Total form percentage (TF%) | Gradient index (GI) | Symmetry index (SI) | Disparity index (DI) |
|--------|-----------------------------|---------------------|---------------------|----------------------|
| 88.998 | 36.291 | 34.564 | 56.963 | 48.628 |

Table-3: Stomatal index, length and width of dorsal and ventral leaf surfaces of the three cytotypes of *Urginea indica* Kunth

| SURFACE OF LEAVES | Apex Portion of Leaf | | | Middle Portion of Leaf | | | Base Portion Of Leaf | | |
|-------------------|--------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
| | Stomatal Index | Length (μ) | Width (μ) | Stomatal Index | Length (μ) | Width (μ) | Stomatal Index | Length (μ) | Width (μ) |
| DORSAL SURFACE | 10.830 \pm 1.278 | 21.1 \pm 0.430 | 15.6 \pm 0.545 | 14.280 \pm 1.352 | 20.7 \pm 0.685 | 15.9 \pm 0.513 | 9.246 \pm 1.629 | 22.7 \pm 0.397 | 15.8 \pm 0.556 |
| VENTRAL SURFACE | 11.766 \pm 0.086 | 19.7 \pm 0.505 | 16.0 \pm 0.342 | 12.849 \pm 1.812 | 22.9 \pm 0.549 | 15.9 \pm 0.832 | 14.892 \pm 1.004 | 23.2 \pm 0.480 | 17.2 \pm 0.500 |

Fig.1-4: Photograph of *Urginea indica* Kunth with photomicrographs showing Chromosomes at Metaphase Plate and stomata



Fig. 1: Bulb of *Urginea indica* Kunth

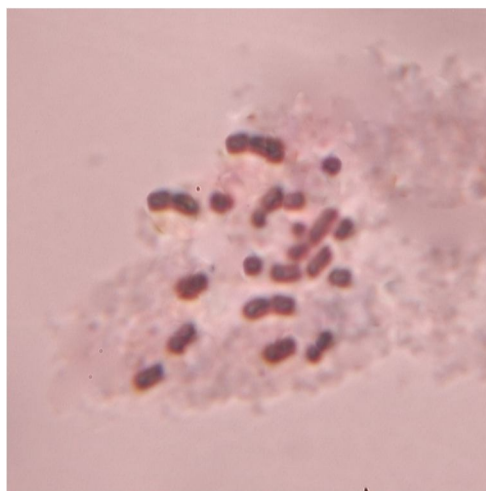


Fig.2: Chromosomes at metaphase stage



| NEARLY MEDIAN | NEARLY SUBMEDIAN (-) |
|---|--|
|  |  |

Fig. 3: Idiogram of mitotic chromosomes

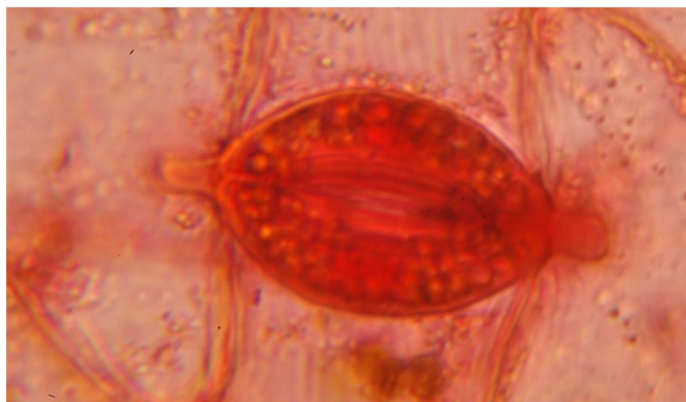


Fig.4: Photomicrograph of Stomata

IV. CONCLUSION

The present research showed the asymmetrical karyotype of the selected cytotype of *Urginea indica* Kunth with diploid ($2n=20$) set of chromosomes. The stomata were observed to be of anomocytic type. Thus, the present research justified the earlier findings.

IV. ACKNOWLEDGMENT

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