Journal of Applied and Natural Science 5 (1): 10-16 (2013)

JANS

Phylogenetic analysis among Indian squill *Urginea indica* Kunth. Liliaceae

M. N. Shiva Kameshwari*, H. L. Geetha[1] and K. J. Tharasaraswathi

Department of Botany, Bangalore University, Jnanabharathi Campus, Bangalore - 560 056, INDIA
[1]Department of Biotechnology, Bangalore University, Jnanabharathi Campus, Bangalore - 560 056, INDIA
*Corresponding author. E-mail: shivamn2k7@yahoo.com

Received: January 22, 2011; Revised received:December 19, 2012;Accepted:January 13, 2013

Abstract: In the present study on *Urginea indica*, twelve different populations from southern part of India is considered. Fifteen parameters have been scored for each population to understand the relationship between different races. The main objective is to trace phylogeny in populations of *U. indica* to construct phylogenetic tree. The phylogenetic tree obtained is an unrooted tree. The parsimony tree describes that Shimoga and Chamundi hill populations have parallely evolved and forms the out group. Dopaegowdanapura population has given rise to Gopalaswamy betta, Gopalaswamy betta has given rise to Banganavadi and Banganavadi has given rise to one population which is missing in the tree and the missing population has given rise to Gorur on one hand and to Krishna Raja Sagar island and Gandhi Krishi Vighnayana Kendra on the other hand. Krishna Raja Sagar island and Gandhi Krishi Vighnayana Kendra shares a common clade. Gorur has given rise to Papanasini and Papanasini has given rise to Channamallipura, from Channamallipura another population has been evolved which is missing and the missing population has given rise to Basavanahalli and Ranganthittu which shares a Common clade. The Parsimony tree shows that these populations have evolved parallel. Dopaegowdanapura is the oldest from which all others are evolved. Ranganathittu and Basavanahalli form the youngest and latest. Therefore it is an unrooted tree with distance. Each population varied in their morphology and chromosome number and called as cytotypes. Difference in morphological character is mainly because of genetic characters. Habitat does not seem to play major role to mould morphological features.

Keywords: Parsimony, Phylogeny, Population, *Urginea indica*

INTRODUCTION

Urginea Steinhill is one of the extremely interesting polytypic genus with about 100 species (Airy Shaw, 1966) endemic to India, Africa and Mediterranean regions. It is represented in India by about nine species (Hemadri and Swahari, 1992). Among nine species *U. indica* is unique and has its own importance.*U. indica* (Fig.1. a-c) commonly called as Indian squill a geophyte fairly common in hilly regions and particularly abundant on the slopesof hilly places. Flowers appear on long or short scapes. It happens to be endemic to certain floristic regions of the world often occurring in remote and difficult terrains and not easily (Shiva Kameshwari, 1995).

Squill bulb has long been used as a source of medicine with biocidal applications. It is largely used as an anticancer agent, cardiac stimulant and expectorant and in treating edema, gout, dropsy, male sterility, dog bite, and in allergies. Due to these properties bulb has found its place in British and European pharmacopieas. Despite these properties, it is surprising that the genus has not attracted greater attention of the research workers in India.

A host of investigators have attempted to assess the role played by *U. indica* in morphological, cytological studies (Raghavan, 1935; Jha and Sen, 1983a,b; Yadav and Dixit, 1990; Shivakameshwari and Muniyamma, 1992, 1999, 2004). But attempt has not been made so far on evolution and phylogenetic relationship of this taxa. *U. indica* is a vegetatively propagated plant and variation among different populations gives lot of clue about formation of local populations (Shivakameshwari and Muniyamma, 2004). Knowledge about the existing genetic variations and the association between various plant characters and their heritability is a significant prerequisite for assessing the potentiality of plants to respond to plant improvement programmes.

Increasingly phylogenetic problems are being addressed using data from different source; morphology and molecules, DNA and protein, mitochondrial and nuclear genes, coding and noncoding sequences (Nylander *et al.*, 2004). Polymorphism or variation within species, is common in all kinds of data and is the major focus of research on microevolution. However, polymorphism is often ignored by those who study macroevolution: systematists and comparative evolutionary biologists. Polymorphism may have profound impact on phylogeny reconstruction, species delimitation, and studies of character evolution (John, 1999).

ISSN : 0974-9411 (Print), 2231-5209 (Online)

In view of the above and commercial importance of the species as well as indication of morphological and cytological races already observed in this species, a detailed analysis of twelve populations have been carried out for phenotypic and genotypic coefficient of variation. Such an investigation would not only indicate the principle feature of evolution within the species but may also lead to exploitation of certain distinct genotypes for commercial purposes.

MATERIALS AND METHODS

Twelve different cytotypes of *U. indica* employed in the present investigation (Tables 1 and 2) Morphological studies (Dixit and Yadav, 1989) were made from fresh materials collected from various localities and were grown under uniform environmental conditions. Voucher specimens have been deposited in the herbarium of Botany Department.

Karyological studies were made following the methods employed by Jha and Sen, 1983a. The symmetry and asymmetry were determined according to the system described by Stebbins (1958).

Phylogenetic studies were made and a dendrogram was constructed by employing the parsimony and neighbour joining method of Saitou and Nei (1987). The data for morphological variations fed in mesquit and analyzed in paup program (software's).

RESULTS

The twelve cytotypes of *U. indica* revealed several interesting features. Fifteen morphological and reproductive characters were scored for each population. The quantitative characters showed many differences (Tables 1 and 2).The most distinguishable characters of each cytotype have been discussed.

Cytotype 1: It is diploid showing 2n = 20 chromosomes. Largest bulb of 26 cms in diameter and lengthy roots noticed in this population. It produces more number of flowers.

Cytotype 2: It is aneuploid with 2n = 32 chromosomes. Highest number of 'B' – chromosomes noticed in this population. Drooping flowers with lengthy pedicel.

Cytotype 3: The somatic cells revealed 2n = 34 chromosomes an aneuploid. Longest pedicel of 3.7 cms with dropping flowers is characteristic.

Cytotype 4: Aneuploid showing 2n = 38 chromosomes with only two chromosomes showing secondary constrictions. Leaf index is lowest in this population.

Cytotype 5: This population from K.R.S. Island is another aneuploid with 36 chromosomes. Highest plant height and lengthy inflorescence with only 3 leaves and 3 flowers is characteristic.

Cytotype 6: Tetraploid showing 40 chromosomes. Bulbs and leaf shape vary within population.

Cytotype 7: Again another tetraploid with 2n = 40 chromosomes. Active principles isolated in this population is unique.

Cytotype 8: Another aneuploid showing 2n = 46 chromosomes. Leaf index is highest in this population with shortest inflorescence noticed in this cytotype.

Cytotype 9: This population collected from Gorur is a tetraploid showing 2n = 40 chromosomes.

Cytotype 10: An aneuploid with smallest bulbs showing 2n = 38 chromosomes with highest number of secondary constrictions.

Cytotype 11: Aneuploid with 2n = 24 chromosomes. Highest number of leaves noticed in this population.

Cytotype 12: 2n = 28 chromosomes an aneuploid collected from G.K.V.K. Campus, Bangalore.

Morphological and cytological characters were used for constructing a tree using parsimony method. Parsimony tree observed in the present investigation revealed an unrooted tree (Figs. 2 and 3).

The analysis of the tree based on fifteen parameters suggests that Chamundi hills and Shimoga evolved parallely and stands as an out group from the other population. Dopaegowdana Pura is the oldest and has given rise to Gopalaswamy Betta. This has given rise to Banganavadi. Banganavadi gave rise to another population which is missing, the missing population might have given to Gorur on one hand and Gandhi Krishi Vighnayana Kendra and Krishna Raja Sagar Island on other. Krishna Raja Sagar island and Gandhi Krishi Vighnayana Kendra shares a Common clade. Gorur has given rise to Papanasini and Papanasini has given rise to Channamallipura. From Channamallipura another population has been evolved which is missing and the missing population might have given rise to Basavanahalli and Ranganathittu which shares a common clade and this two can be considered as youngest and latest.

2n = 10 being the basic number in a majority of populations found growing in forest canopy are tetraploids. The populations growing in island and in foot hills show increased aneuploidy (34, 36, 38). The populations growing in open field and hilly regions show decreased aneuploidy (24, 28) tetraploid flowers bloom in the forenoon while majority of aneuploids show night blooming.

Tetraploids show uniformity in their secondary constriction (6) and in the number of Bchromosome (1) while aneuploids vary in the no of secondary constrictions (2-14) and in the no of B-chromosomes (1-10).

Systematic position of Urgenia genus: AntoineLaurent de Jussieu (1789) erected the family Liliaceae in his genera Plantarum after the plant *Lillium Candidum* L., Lily being extolled in poetry as an emblem of whiteness and purity. Liliaceae is one of the largest families of flowering plants

Table 1. Population of *U. indica* collected from various localities.

S. No.	Taxon	Collection number	Place of collection	Habitat	Blooming time
1	*U. indica*	S and M 802	Kuvempu University (Shimoga)	Open field	Forenoon
2	*U. indica*	S and M 803	Basavanhalli (Mysore)	Crop field	Night
3	*U. indica*	S and M 804	Ranganathittu Island (mandya)	River island	Night
4	*U. indica*	S and M 805	Chamundi Hills (Mysore)	Foot hills	Night
5	*U. indica*	S and M 806	K.R.S. Island (Mandya)	River island	Forenoon
6	*U. indica*	S and M 807	Gopalaswamy Betta (Chamarajanagara)	Foot hills	Forenoon
7	*U. indica*	S and M 808	Dopae Gowdanapura (Chamarajanagara)	Forest canopy	Forenoon
8	*U. indica*	S and M 809	Channamalipura (Chamarajanagara)	Forest canopy	Night
9	*U. indica*	S and M 814	Gorur (Hassan)	Forest canopy	Forenoon
10	*U. indica*	S and M 815	Banganavadi (Mandya)	Forest canopy	Forenoon
11	*U. indica*	S and M 824	Papanasini (Andra Pradesh)	Hilly region	Afternoon
12	*U. indica*	S and M 829	G.K.V.K. Campus (Bangalore)	Open field	Forenoon

comprising 250 genera and 3,700 species (Willis, 1973) constituting a heterogenous assemblage. The genus *U. steinhill* belongs to division-Monocotyledon Class-Liliopsida, Order-Liliales, Tribe-Scilleae-Family-Lilaceae. Urginea was erected by Steinhill (1834) and named it after an Arabian tribe Ben urgin. Steinhill recognized, 7 species under this genus and further distinguished them on the basis of their leaves, bulb scales and scape Lindley (1836) placed this genus under the tribe scilleae. (Airy Shaw, 1966) recognished 100 species occurring in Mediterranean regions. In India the genus is represented by about 9 species. Deb and Dasgupta (1987) in a taxonomic revision of the genus *Urginea* recognized only 5 species. Among 5 species of Urginea, *U. indica* Kunth. is highly polymorphic and has been discussed in the present study.

DISCUSSION

The interpopulation variation pattern found in *U. indica* is very complicated and difficult to elucidate. The morphological complexity is accompanied by a high degree of cytological variation. The variation range of a given character can be small in one population while being considerably wider in another. *U. indica* seems to be a very good material for the study of phenotypic plasticity and phylogeny. Fifteen different parameters have been scored from each population. An analysis of variation in morphology cytology and distribution of different populations of *U. indica* studied during the present investigation has revealed intriguing facts. Of the fifteen parameters that have been analysed, the vegetative character show great variations which could be used as dependable taxonomic characters at the population level. On the other hand, the reproductive characters have shown less variation and are almost uniform.

The largest bulb noticed in population (1) and smallest in population (10). Height of the plant is highest in population (5) and lowest in population (7). Length of root is longest in population (1) and least length in population (8) and (10). The number of leaves is more in population (11) and only three leaves in population (5). Leaf index is highest in population (8) and lowest in population (4). Longest inflorescence noticed in population (5) and shortest in population (8). No of flowers is more in population (5), (1) and (11) and less no in population (15). Pedicel length deviates from other population is longest in population (3) and shortest in other few population. Length of flower is more population (8). While the length of stamen, gynoecium and fruit length are almost similar and show less variations. The populations vary in one or the other character and stands apart in their morphology. While in reproductive character show lesser variations. Interesting feature which was noticed was peculiar blooming of flower. There were

Table 2. Morphological variations in populations of *U. indica* Kunth.

Sl. No	Taxon	Height of plant in cm	Length of root in cm	Circum-ference of bulb in cm	No. of leaves / plant	Leaf index in cm	Inflor-escence length in cm	No. of flowers / plant	Pedicel length in cm	Flower length in cm	Stamen length in cm	Gynoce civn length in cm	Fruit length in cm	No. of chromo some	No. of B-chromo some	No. of secondary constrictions
1	*U. indica*	13	14	26	8	17.6	6	22	0.4	0.8	0.5	0.7	0.7	20	--	4
2	*U. indica*	53.5	10	14	5	6.2	50	12	3.0	1.2	0.6	0.9	1.2	32	10	6
3	*U. indica*	39	11.5	16	4	9.8	34.2	14	3.7	1.0	0.6	0.7	1.0	34	2	4
4	*U. indica*	15	9.5	17	6	5.4	10	14	0.3	0.6	0.3	0.6	0.7	38	3	2
5	*U. indica*	63	11	12	3	8.9	52	3	2.5	0.8	0.8	0.7	0.8	36	1	8
6	*U. indica*	41	12	18	6	59.8	30	8	2.0	0.8	0.5	0.7	0.8	40	--	6
7	*U. indica*	33	8.5	18	8	44.0	25	12	0.5	0.9	0.4	0.9	0.8	40	1	6
8	*U. indica*	38	8	14.5	6	67.6	3.5	14	3.0	1.5	0.8	0.7	1.3	46	2	10
9	*U. indica*	49	10	13	5	31.5	36	6	0.3	1.3	0.7	0.6	1.2	40	1	6
10	*U. indica*	48	8	10	5	35	31	8	1.0	0.8	0.8	0.7	0.8	38	1	14
11	*U. indica*	58	10	15	13	30	42	22	0.6	1.2	0.8	0.7	1.2	24	--	4
12	*U. indica*	58	10	12	4	22.4	50	12	0.6	0.6	0.8	0.6	0.6	28	--	12

(a)

(b) **(c)**

Fig. 1. ***a.*** *Urginea indica - Vegetative phase,* ***b.*** *Reproductive phase,* ***c.*** *Fully bloomed flower*

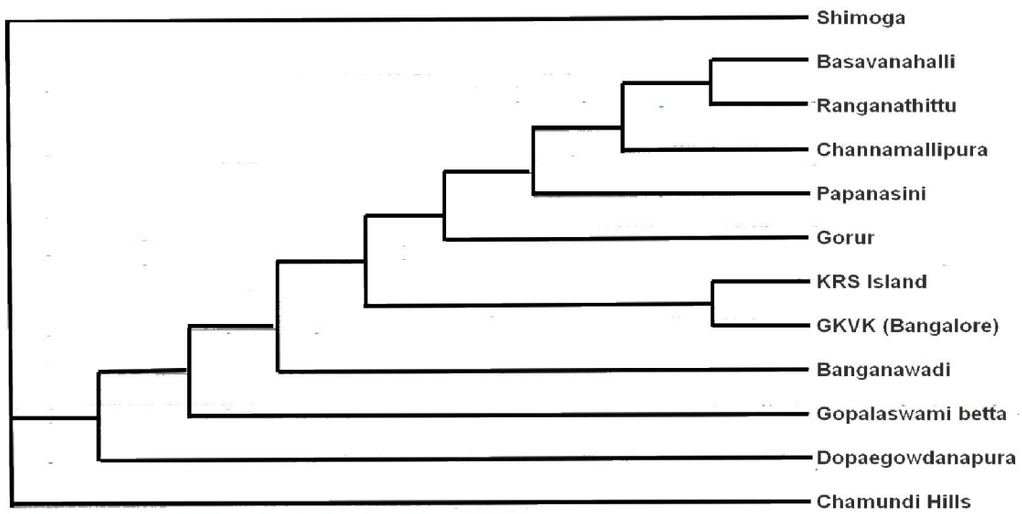

Fig.2. *Parsimony tree-unrooted tree showing the position of different populations.*

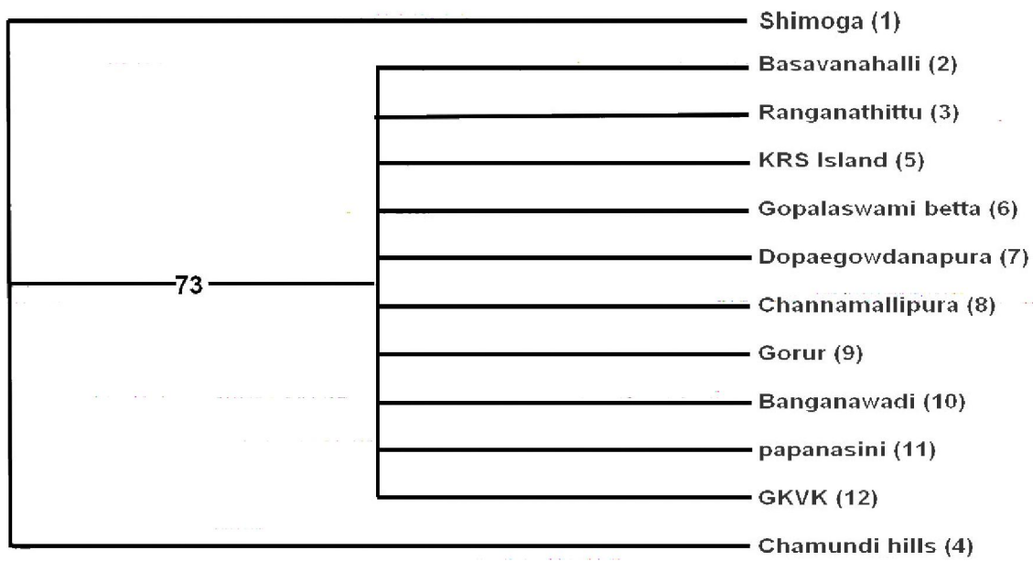

Fig. 3. *Showing boot strap value*

three different periods of blooming forenoon blooming noticed in population 1, 5, 6, 7, 9, 10 and 12. Afternoon (3 O'clock) blooming in population 11 and night blooms in population 2, 3, 4 and 8. Reproductive isolation through flowering might have played an important role in speciation and evolution of the species. Tetraploids show forenoon blooming and aneuploids show night blooming. Cytological studies revealed the presence of diploid, aneuploid and tetraploid populations, the aneuploids 2n = 24, 32, 34, 36, 38, 46 are new records for the species. The presence of 'B' chromosomes and secondary constrictions also plays a role in delimiting the populations. Highest number of B – chromosomes noticed in population (2) collected from Basavanahalli and secondary constrictions are more in population (10) collected from Banganavadi. Tetraploids shows only 1B-chromosome 6 secondary constrictions. While aneuploids vary in their no of secondary constriction (2-4) and in the no. of B-chromosome (1-10).

The present cytotypes are morphologically distinguishable phenotypic populations. The occurrence of these forms cannot be attributed merely to phenotypic plasticity. It may be due to environmental conditions and genetic factors. Accumulation of particular genetic characters to adopt to particular environment must have lead to bring variation in cytological and morphological character.

In fact no two populations were alike in their morphological characters, such population difference along with 90% uniformity within a population clearly indicate that each population represents the distinct morphocytotypes such difference amongst different populations might have also lead to evolution of races. These morphological variations along with cytological variations and similarities are considered responsible for designating them as cytotypes. Thus 12 cytomorphotypes have been recognized. Such type of investigations on variations would not only indicate the principle feature of evolution within species but may also lead to exploitation of certain distinct genotypes for commercial purposes. Parsimony tree is unable to show the root therefore it is concluded that the tree is unrooted. Several population is found missing which forms a connecting link from tetraploid 2n = 40 to diploid 2n = 20. in between these two stands the aneuploid populations. To construct a concrete phylogenetic tree to *U.indica* and trace its phylogeny the missing populations has to be incorporated to form a link between diploids and tetraploids. The parsimony tree described in the present study indicate that the tetraploids have undergone reduction in chromosome number and have given rise to diploids.

The phylogenetic tree has been constructed using mesquite and paup programs reveals several interesting features (Figs. 2 and 3). The parsimony tree describes that tree is an unrooted tree and 12 different populations have been evolved parallely. The horizontal line seen in the parsimony tree shows the genetic distance between populations of *U. indica* and the time taken for variations. The two out groups such as Chamundi hills and Shimoga is removed and tree is constructed, even then the parsimony tree is unable to show the root therefore it is concluded that the tree is unrooted.

ACKNOWLEDGEMENTS

Author is grateful to CSRI, New Delhi, for financial assistance and to Bangalore University, Bangalore.

REFERENCES

Airy Shaw, H.K. (1966). Diagnoses of new families name etc for the seventh edition of Willis Dictionary. *Kew Bull.* 18 : 249-273.

Deb, D.B. and Dasgupta, S. (1987). On the identity of three

new spicies of *Urginea* (Liliaceae). *J. Bombay Nat.Hist.Soc.*, 84: 409-412.

Dixit, G. B. and Yadav, S.R. (1989). Cytotaxonomical and genetical studies in *Urginea* species from India. *Cytologia,* 54 : 715-721

Hemadri, K. R. and Swahari, S. (1992). *Urginea nagarajunae* Hemadri et Swahari a new species of Liliaceae from India (a new plant discovery). *Ancient Sci Life*., 2: 105-110

Jha, S. and Sen, S. (1983a). Chromosomes study of diploid Indian squill. *Cytologia,* 41: 79-86.

Jha, S. and Sen, S. (1983b). Chromosomes of polyploid Indian squill. *Cytologia,* 48: 407-418.

John J. Wiens (1999). Polymorphism in systematics and coparative biology. *Annu. Rev. Ecl. Syst.,* 30: 327-362.

Jussieu, Antoine Laurent de (1789). *Genera Plantarum, secundum ordines naturales disposita juxta methodum in Horto Regio Parisiensi exaratam*. Paris. OCLC 315729. http://books.google.com/books?id=nikkCxpBLm8C. Retrieved 2007-02-19.

Lindley, J. (1836). Natural system of Botany. pp.353-354.

Nylander, J. A. A., Ronquist, F., Huelsenbeck, J.P., and Nieves-Aldrey (2004). Bayesian phylogentic analysis of combined data. *Syst Biol*., 53 (1): 47-67.

Raghavan, T. S. (1935). Observation on the somatic chromosomes of *Urginea indica* Kunth. *J Indian Bot Soc.*, 151-158.

Saitou, N. and Nei, M. (1987). The neighbour joining method, A new method of reconstructing phylogentic tree. *Mol Biol Evol*., 4: 406-425.

Shiva Kameshwari, M N. (1995). Biosystematic studies on some members of Lillaceae. *Ph.D Thesis* Mysore University, Mysore.

Shiva Kameshwari, M. N. and Muniyamma, M. (1992). Cytomorphological studies in *Urginea indica* Kunth Liliaceae. *J. Mysore Uni*., 32: 518-522.

Shiva Kameshwari, M. N. and Muniyamma, M. (1999). Karyomorphological studies in *Urginea polyphylla* Hook.f Liliaceae. *J. Swamy Bot. Cl.,* 16: 91-93.

Shiva Kameshwari, M. N. and Muniyamma, M. (2004). Karyomorphological studies in populations of *Urginea indica* Kunth. *Beitr. Biole pflanzen*, 73: 377-394.

Steinhill (1834). Materiaux pour server a la flore de Barbarie. III. Article note sur le genere Urgines nouvellement forme dans la families des Liliaceaes. *Annls Sci. Nat. Bot.,* 2: 1:322

Stebbins, G. L. (1958). Longevity habitat and release of genetic variability in the higher plants cold spring harb. *Symp Quant Biol.,* 23: 365-377.

Willis, J. C. (1973). A dictionary of the flowering plants and ferns 7th edn. Cambridge University Press Cambridge, U.K

Yadav, S. R. and Dixit, G.B. (1990). Cytotaxonomical studies in Indian *Urginea* steinhill species. *Cytotlogia*, 55: 293-300.