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CYTOLOGICAL STUDIES ON SOME ENDEMIC TERRESTRIAL ORCHIDS

***T. Ramesh., K. Ganesh Kumari, P. Muruganantham, and J. Vanithamani**
Department of Botany, Srimad Andavan Arts and Science College, T.V.Kovil, Trichy-05. India
*Email: ramtv@andavancollege.ac.in
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ABSTRACT

Cytological studies on different species of Orchidaceae namely, *Arundina granifolia* Lindl, *Anoectochilus roxburghii* (wall) Lindl. *Brachycorthis obcordata* (Lindl), summarh, *Calanthe plantaginea* Lindl, *Calanthe purberula* Lindl, *Calanthe sylvatica* Lindl and *Calanthe tricarinata* Lindl. *Cypripedium cordigerum* Lindl and *Cypripedium elegans* Reichehb.f. *Nep*. Previous records of chromosome numbers has been made in *Anoectochilus roxburghii* (wall) Lindl, *Cypripedium cordigerum* Lindl. *Calanthe purberula* Lindl, *Cypripedium cordigerum* Lindl and *Cypripedium elegans Reichehb.f.* Among all the species studied correlation among the chromosome numbers are registered in the present investigation. This article emphasis the first report of chromosome number in 5 species of Orchids namely *Arundina granifolia* Lindl, 2n - 40, *Brachycorthis obcordata* (Lindl), 2n – 30, *Calanthe plantaginea* Lindl, 2n - 32. *Calanthe sylvatica* Lindl 2n = 40 and *Calanthe tricarinata* 2n = 28. Most of the species of *Arundina granifolia* Lindl, 2n - 40, *Brachycorthis obcordata* (Lindl), 2n – 30, *Calanthe plantaginea* Lindl, 2n - 32. *Calanthe sylvatica* Lindl 2n = 40 and *Arundina granifolia* Lindl, 2n =42. The origin of chromosome should be aneuploidy. The analysis of karyotype reveals that each and every species have distinct karyotype. The study of karyotype analyses shows that every species has a distinct karyotype which proved that both karyotype alterations and aneuploidy have important role in speciation.

***Keywords*:** Chromosome, aneuploidy, euploidy and orchids

Introduction

The Orchids are a group of extremely interesting plants which outnumber all the other plant groups in the plant kingdom. Numbering about 20,000 species, they exhibit pollination-related floral complexities, produce a large number of microscopic and poorly organized seeds, and require a fungal endophyte for their germination and growth in nature. The morphological, physiological and genetic peculiarities inherent in this group of plants have stimulated research to such a degree that Orchidology today is one of the most popular and dynamic branches of botany. Some of the orchids are medicinally important while certain others are used for the extraction of various chemical compounds. Previous cytogenetical studies of this family are fragmentary, as for as Tamil Nadu taxa are concerned. Orchids are valuable ornamental species mainly for their cut flower and multivarious forms of growth habits.

Materials and Methods

The plants for the present investigation were collected from Kolli hills. The species were identified and checked with the help of the Botanical Survey of India, Coimbatore and National Orchidarium and Experimental Garden, Southern Circle at Yercaud of Tamil Nadu. The Particulars regarding the species collected, wild or cultivated and the place of the collection are furnished in the following table (Table 1).

The root tips were collected and thoroughly washed in distilled water and they were pretreated in 0.002 M hydroxyquinoline at 4^{o}C for 3 hours. Then the root tips were thoroughly washed and fixed in 1:3 acetic alcohol for 3 hours and following from iron alum haematoxylin squash schedule described (Marimuthu and Subnramaniam, 1960). Squashes were made with 1 or 2 root tips per slide and sealed. Ten plates were considered for the karyotypic analysis in each species in the present investigation. The measurement of chromosomes was made with an ocular micrometer, the scale of which had been calibrated from a stage micrometer.

Results and Discussion

The present investigation first record of chromosome number has been made in species namely. *Arundina granifolia* 2n-40, *Brachycorthis obcordata* 2n – 30, *Calanthe plantaginea*, 2n-32. *Calanthe sylvatica* 2n = 40 and *Calanthe tricarinata* 2n = 28. The largest chromosome is found in *Arundina granifolia* 2n - 40, and the smallest chromosomes observed in *Calanthe tricarinata* 2n = 28. In all other species are chromosome size having large, medium and small-sized. Therefore, it is concluded that along with aneuploidy and euploidy, karyotype alterations of chromosomes also play important role in evolution.

A deviant number of the chromosome has been observed in the species namely *Calanthe sylvatica* against the previous reports of chromosome numbers. In the *Habenaria*

viridiflora there are 2n=22 chromosomes as observed in the present investigation but the earlier reports show 2n = 44 chromosomes (Jorapur and Kulkarani, 1980) and 2n = 84 chromosomes (Foja Singh, 1983). The members of the Tamilnadu Orchidaceae studied show variation of somatic chromosome numbers from 2n = 10 to 41.

The present investigation are categorized by following tables –

Table - 1. Place of collection

Table - 2. Present and previous report of chromosome

Table - 3. Karyomorphological features of chromosome

Based on the length, the chromosomes are classified into long, medium and short sized. Under each of these groups, the following chromosomes have been recognized. Histogram has been drawn with the help of the absolute chromosome lengths of the various species studied.

Chromosome types:

Type S = Chromosome with a sub-median or median primary and secondary construction and satellite on the long arm or short arm.

Type J = Chromosome with a sub-median construction.

Type V = Chromosome with a median construction

Type I = Chromosome with a sub-terminal construction

Chromosome Size

1. Long chromosomes (more than 5.0 µm)
2. Medium-sized chromosomes (3.0 to 4.9 µm)
3. Short chromosomes (0.1 to 2.9 µm)

More than 5 µm size chromosomes are

1. Sub median primary construction and subterminal construction
2. Chromosome with the sub-median centromere and subterminal secondary construction.
3. Chromosome with median primary construction and subterminal secondary construction.

Medium (3.0 to 4.9 µm) size chromosomes are

1. Chromosome with median primary construction and subterminal secondary construction.
2. Chromosome with median construction.

Short (0.1 to 2.9 µm) size chromosomes are

1. Chromosome with sub-median construction.
2. Chromosome with median construction.

Group first:

1. Same genus and different species

Calanthe sylvatica Lindl, *Calanthe purberula* Lindl *Calanthe plantaginea* Lindl and *Calanthe tricarinata*Lindl, *Cypripedium elegans Reichehb.f. Nep, Cypripedium cordigerum*Lindl

Group second:

1. Different species

Brachycorthis obcordata (Lindl), summarh, *Arundina granifolia* Lindl, *Anoectochilus roxburghii* (wall) Lindl

Group third

1. New record of chromosome

Arundina granifolia Lindl, *Brachycorthis obcordata* (Lindl), summarh, *Calanthe plantaginea* Lindl, *Calanthe tricarinata* Lindl, *Calanthe purberula* Lindl, *Calanthe sylvatica* Lindl

Cytological studies on the family Orchidaceae received much attention during the past two decades. Mitotic studies have been made in various species of Orchidaceae by eminent cytologists from various parts of the world and these studies have attracted the attention of taxonomists and biosystematics uniformly.

Vatsala (1964) described the cytology and evolution of Orchidaceae with special references to orchids of South India. Jorapur and Hedge (1974) described the chromosome morphology of *Calanthe tricarinata* Lindl Jorapur and Kulkarni (1980) reported detailed karyological studies in a few members of Orchidaceae. The somatic chromosome numbers of various species of *Vanda* were reported by Harmsen (1943), Sulabha, Pathak (1982), Sulabha Pathak and Jorapur (1983). In recent years Mehra and Viji (1972) reported the diploid chromosome numbers of H. plantaginea (2n= 126). In 1882, cytoembrological works have been made in Epidendreae and Vander by Divakar (1987). Jorapur and Kulkarni (1979) reported the diploid chromosome numbers of the species of Eria. Stenar (1937) reported the diploid chromosome number of the species of Malaxis Kamemoto and Randolph (1949), Kamemoto (1950) and Blumenschein (1960) reported the somatic chromosome numbers of the species of *Epidendrum.*

The species having 2n = 20 chromosomes namely, *Cypripedium cordigerum* and *Cypripedium elegans* diploids as revealed by the present investigation. In the same way, the species having 30 somatic chromosomes namely *Calanthe purberula* Lindl 2n-40, *Vanda cristata* 2n–40 and *Anoectochilus roxburghii* 2n-30. may be considered as triploids. Similarly, the species having 40 somatic chromosomes namely Coelogyneovalis, Eriareticosa and Spathoglottisplicata may be considered as tetraploids. The diploid, triploid and tetraploid species are examples of euploids when we consider n = 10 chromosomes as the primary basic chromosome number of this family. Therefore *Arundina granifolia* Lindl, 2n-40, and *Brachycorthis obcordata* (Lindl), 2n–30 all these species may be considered as aneuploids.

Conclusion

The chromosome analyses of different species of Orchidaceae indicate that mostly asymmetrical karyotypes of the chromosome have been observed. Therefore, it is concluded that along with aneuploidy and euploidy, karyotype alterations of chromosomes also play important role in evolution. The introduction of natural orchid character based on cytology would strongly support the success of orchids plant breeding. However, research on natural orchid plant cytology is very rarely done. Orchids are a diploid number of chromosomes, one pair of chromosomes consists of two sets of homologous chromosomes. Therefore, variations in the number of chromosome sets (ploidy) in the bark of plants included in the group euploidy, which state that the number of chromosomes is observed from a living creature is a multiple of the number of chromosomes

essentially. Differences chromosome generally describes the genetic and protein differences in the content of an individual. The main variations that can be observed that the absolute size or length, morphology, the relative size and number of chromosomes. Individuals within a species have the same chromosome number but different species in a single genus have different numbers of chromosomes. The shape, size and number of chromosomes of each species are always fixed, so it can be used for the purpose taxonomy, knowing diversity, kinship and the evolution although in certain circumstances also occur in variation

Root-tip mitosis revealed 2n = 32 chromosomes at metaphase The somatic complement is bimodal and comprises 6 large chromosomes measuring 5.4-4.3 p.m. and 26 small chromosomes 2.48-0.99 p.m. Total chromatin length is 72.8 p.m. The karyotype is asymmetrical with 20m+ 2sm+ +6st+4t chromosomes. The 2nd pair was found to be heteromorphic. The largest pair possessed a secondary constriction in the short arm and thus is nucleolar organizer.

Table 1 : Place of collection and species

Sl.No.	Name of the taxa	Epiphyte or terrestrial	Wilds (or) cultivated	Place collection
1.	*Arundina granifolia* Lindl	Terrestrial	Wild	Kolli hills
2.	*Anoectochilus roxburghii* (wall) Lindl	Terrestrial	Wild	Kolli hills
3.	*Brachycorthis obcordata* (Lindl), summarh	Terrestrial	Wild	Kolli hills
4.	*Calanthe plantaginea* Lindl	Terrestrial	Wild	Kolli hills
5.	*Calanthe purberula* Lindl	Terrestrial	Wild	Kolli hills
6.	*Calanthe sylvatica* Lindl	Terrestrial	Wild	Kolli hills
7.	*Calanthetricarinata* Lindl	Terrestrial	Wild	Kolli hills
8.	*Cypripedium cordigerum* Lindl	Terrestrial	Wild	Kolli hills
9.	*Cypripedium elegans Reichehb.f. Nep*	Terrestrial	Wild	Kolli hills

Table 2: Chromosome number of the species investigated

S.No.	Name of the taxa	Present study	Previous report
1.	*Arundina granifolia* Lindl	2n = 40	New record
2.	*Anoectochilus roxburghii* (wall) Lindl	2n = 30	N =15 Vij and shekhar 1985
3.	*Brachycortis obcordata* (Lindl), summarh	2n =30	New record
4.	*Calanthe plantaginea* Lindl	2n = 32	New record
5.	*Calanthe purberula* Lindl	2n = 40	Mehra and kashyap 1984b
6.	*Calanthe sylvatica* Lindl	2n = 40	New record
7.	*Calanthe tricarinata* Lindl	2n = 28	New record
8	*Cypripedium cordigerum* Lindl	2n = 20	VijandGupta 1975. Mehra and kashyap, 1978 and 1984
9	*Cypripedium elegans* Reicheh b.f.	2n = 20	Roy and Sharma 1972

Table 3: Summarized karyomorphological features.

S. No.	Name of the taxa	2n	S	J	V	I	Total chromosome length in µm	Absolute chromosome length in µm	Average chromosome length µm	Relative chromosome length in µm
1.	*Arundina granifolia* Lindl	40	15	7	6	12	165.7	82.3	4.1425	2
2.	*Anoectochilus roxburghii* (wall) Lindl	30	2	8	9	11	107.4	53.7	3.58	2
3.	*Brachycorthis obcordata* (Lindl), summarh	30	-	12	13	05	101.0	50.5	3.36	2
4.	*Calanthe plantaginea* Lindl	32	2	16	12	10	129.2	64.05	3.225	2
5.	*Calanthe purberula* Lindl	40	6	8	22	4	187.2	93.6	4.68	2
6.	*Calanthe sylvatica* Lindl	40	12	16	8	4	162.8	81.4	4.07	2
7.	*Calanthe tricarinata* Lindl	28	6	12	10	-	94.4	47.2	3.37	2
8.	*Cypripedium cordigerum* Lindl	20	2	6	6	6	63.6	31.8	3.18	2
9.	*Cypripedium elegans* Reichehb.f. Nep	20	2	8	4	6	60.6	30.3	3.03	

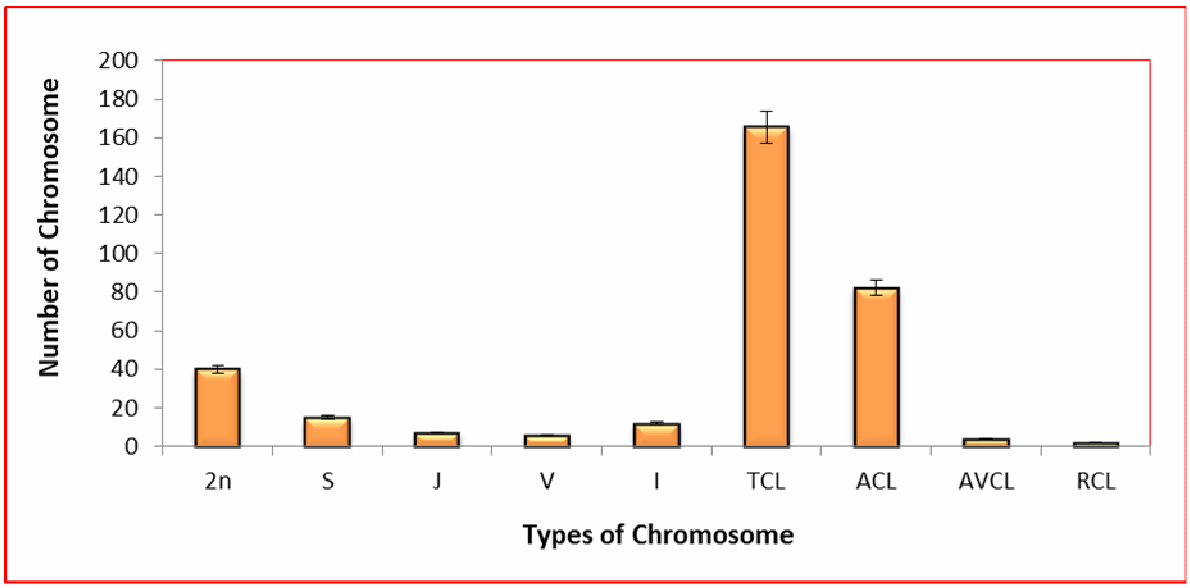

Fig. 1 : Number of chromosomes in *Arundina granifolia Lindl* on Kolli hills

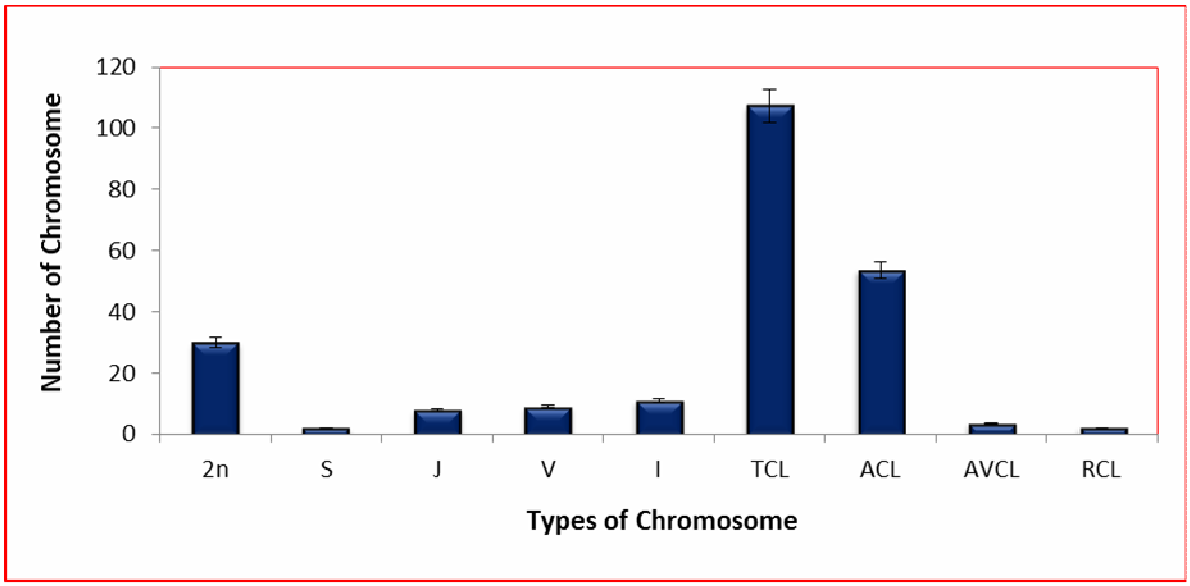

Fig. 2 : Number of chromosomes in *Anoectochilus roxburghii* (wall) Lindl on Kolli hills

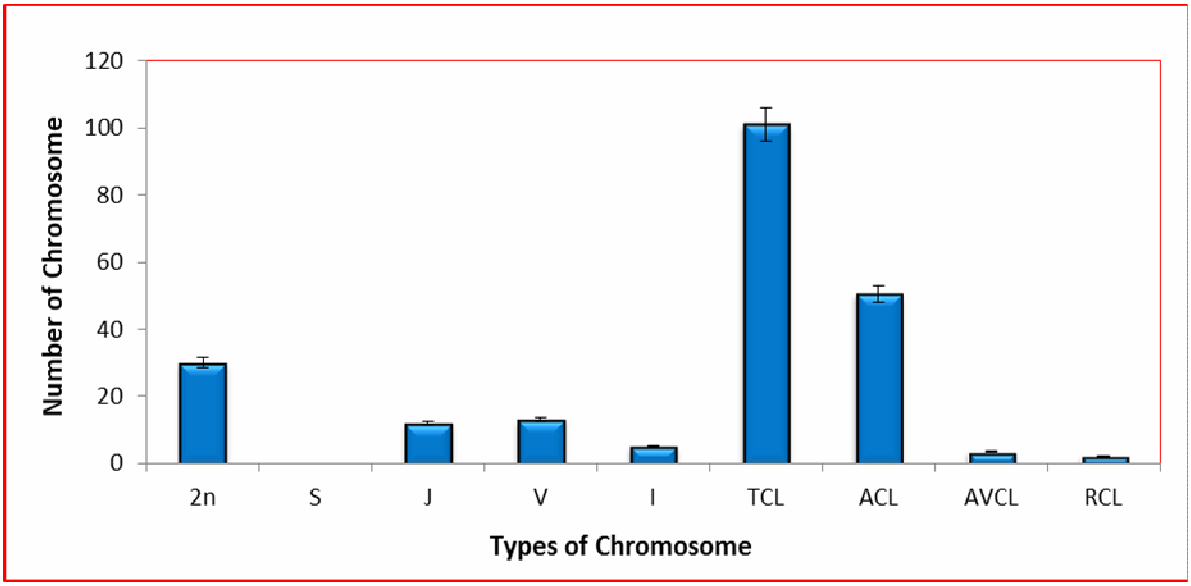

Fig. 3 : Number of chromosomes in *Brachycorthis obcordata* (Lindl), summarh on Kolli hills

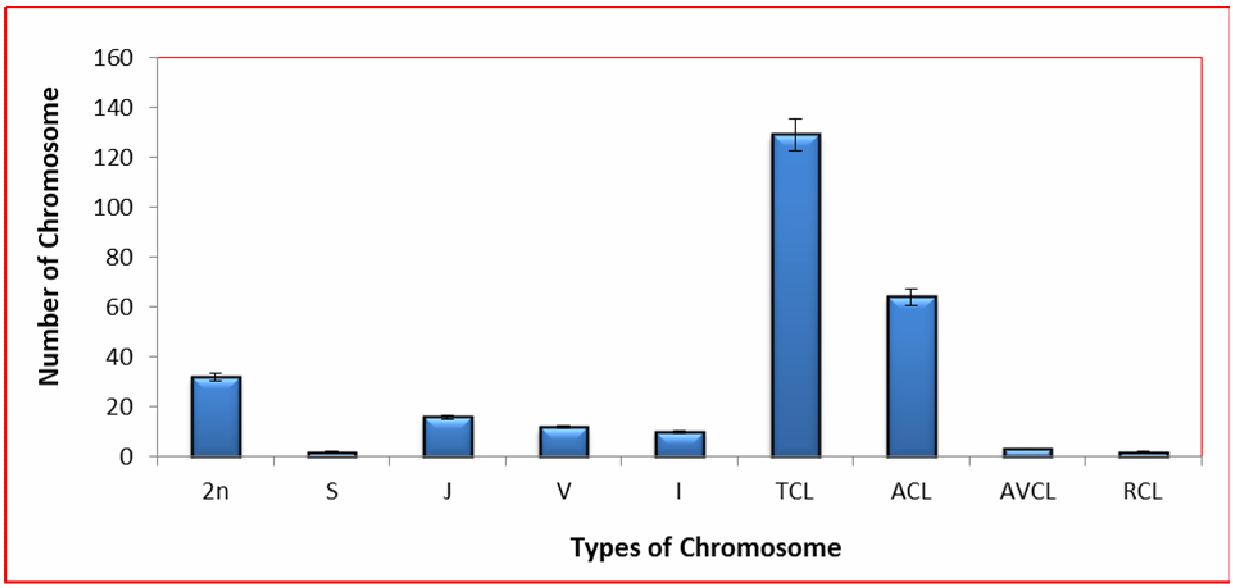

Fig. 4 : Number of chromosomes in *Calantheplan taginea Lindl* on Kolli hills

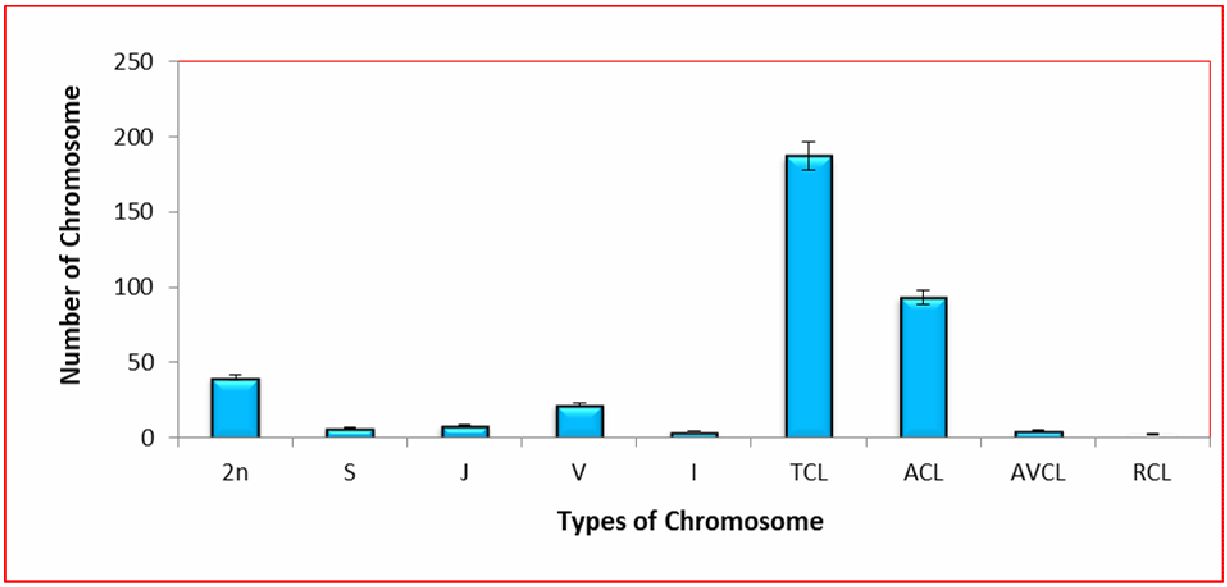

Fig. 5 : Number of chromosomes in *Calanthe purberula Lindl* on Kolli hills

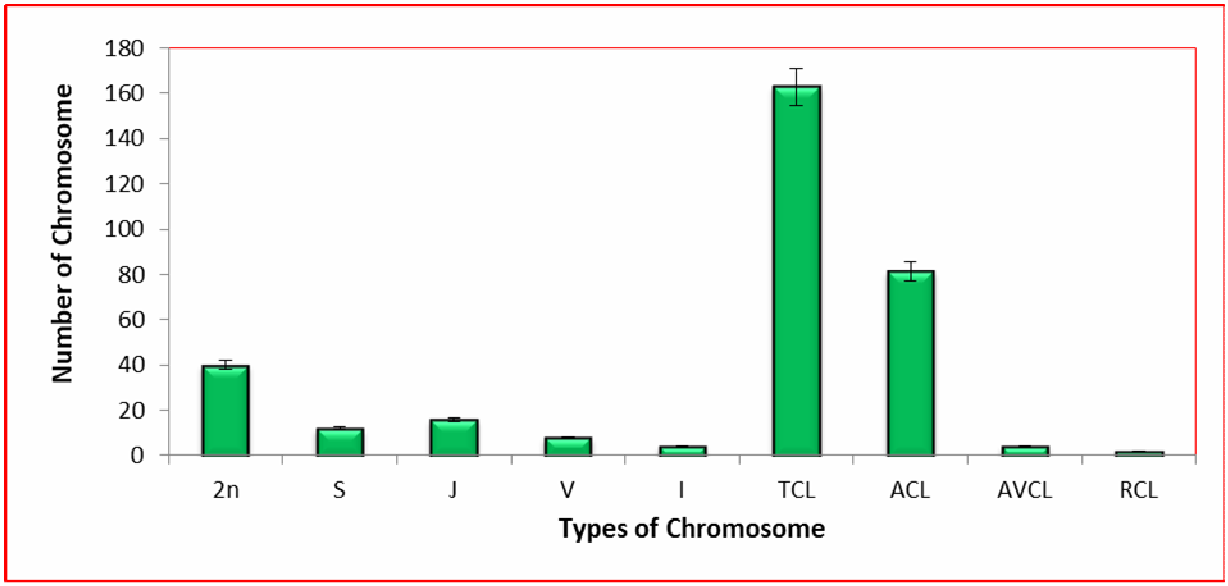

Fig. 6 : Number of chromosomes in *Calanthe sylvatica* Lindl on Kolli hills

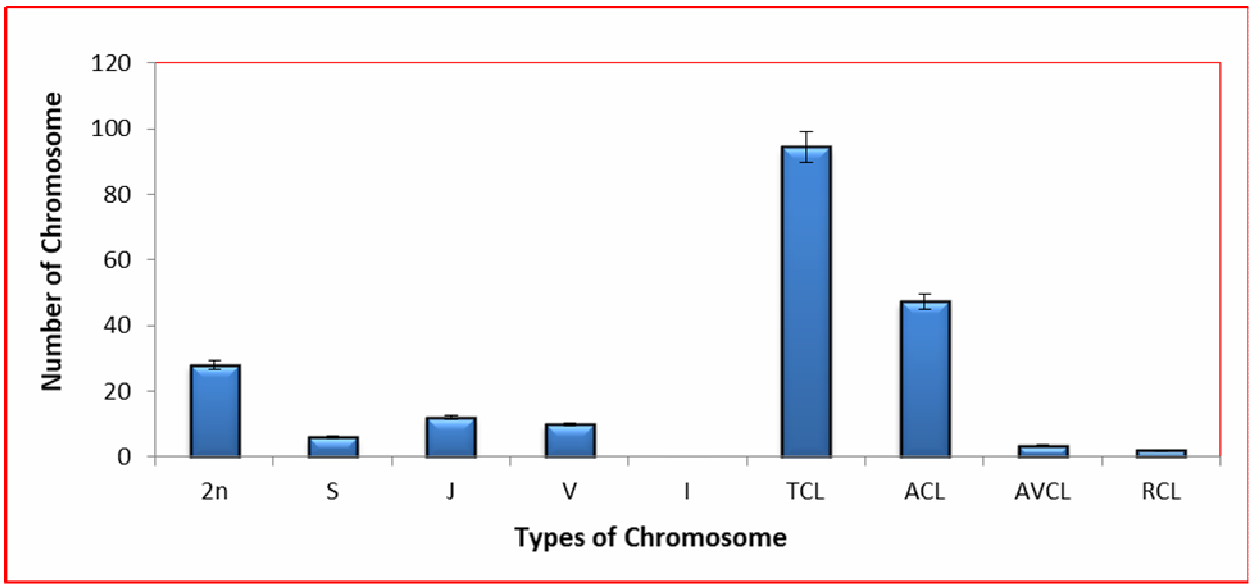

Fig. 7 : Number of chromosomes in *Calanthe tricarinata Lindl* on Kolli hills

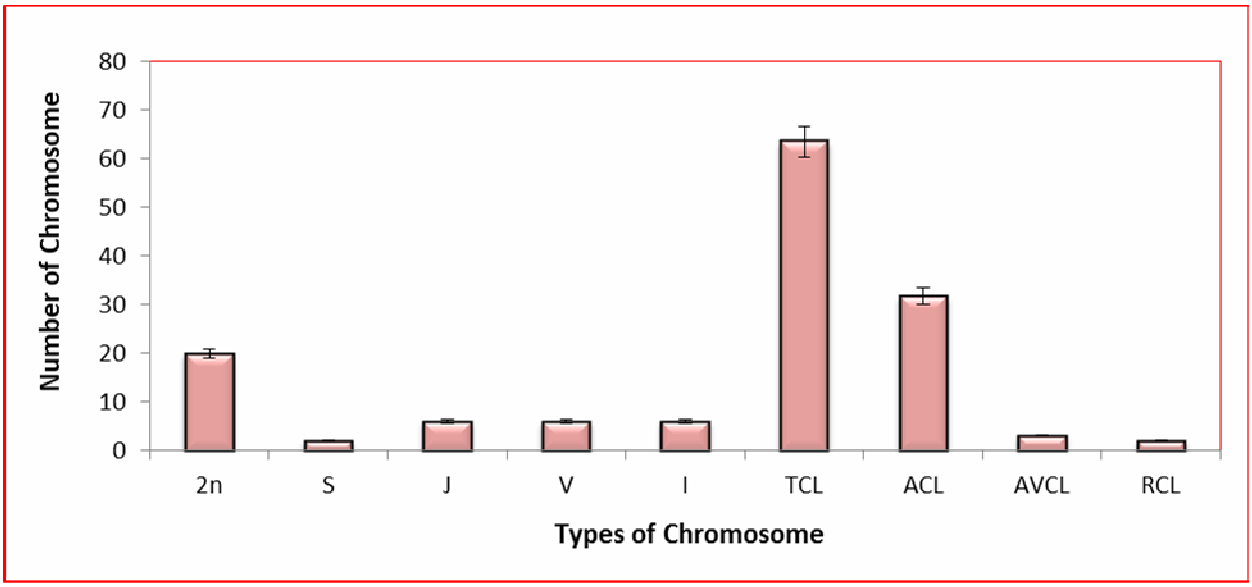

Fig. 8 : Number of chromosomes in *Cypripedium cordigerum* Lindl on Kolli hills

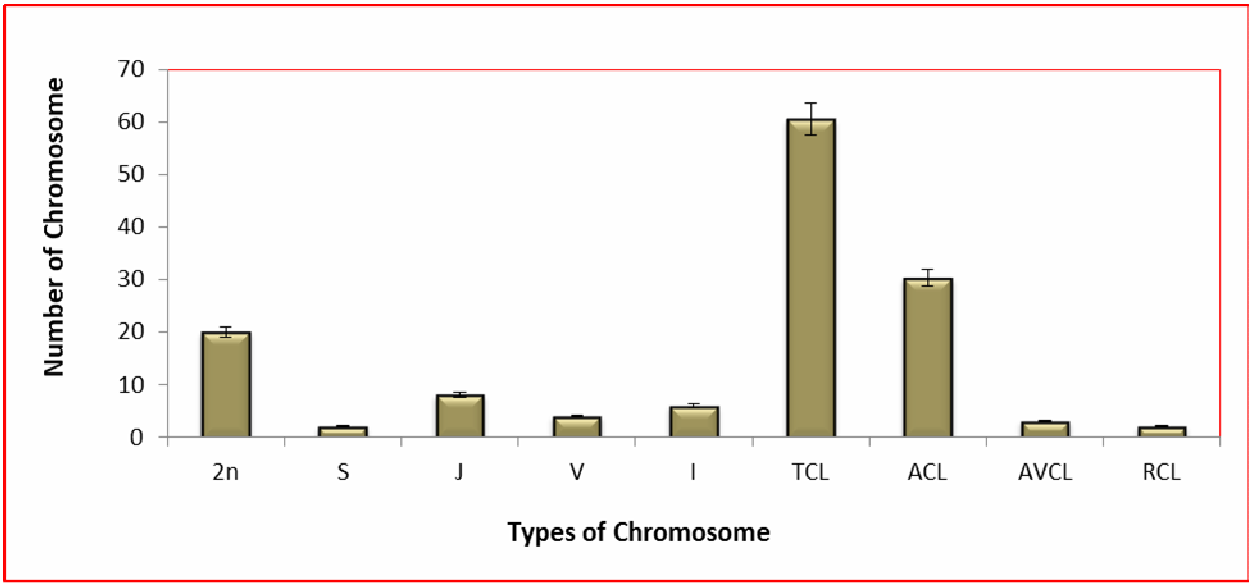

Fig. 9 : Number of chromosomes in *Cypripedium elegans Reichehb.f. Nep* on Kolli hills

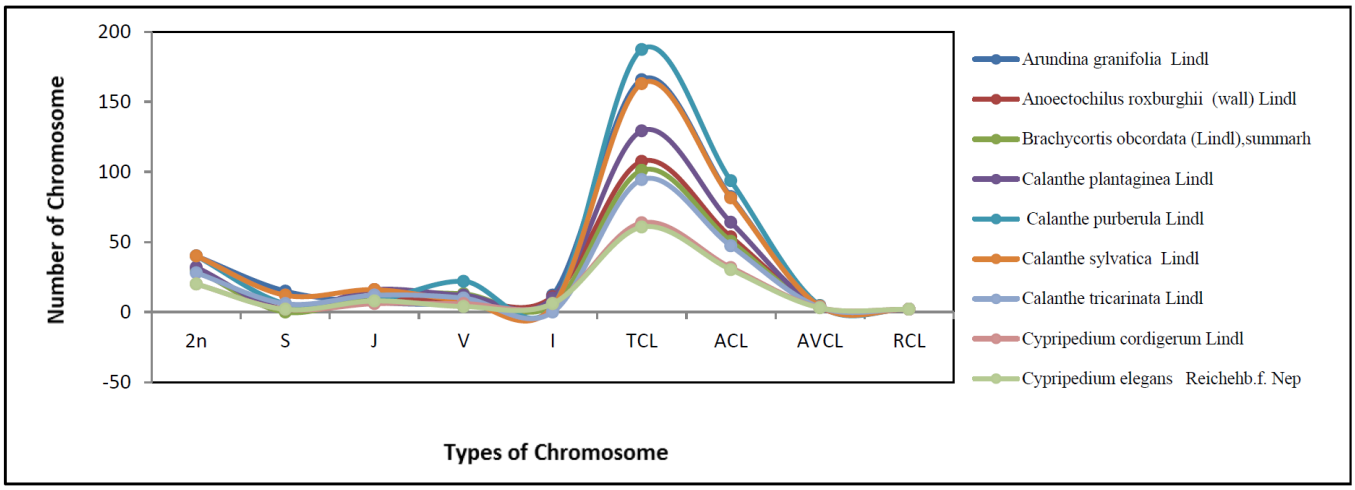

Fig. 10 : Number of taxa and number of chromosomes in Kolli hills.

References

Abraham, A. and Vatsala (1981). Introduction to orchids with illustrations and descriptions of 150 South Indian Orchids. *Tropical Botanical Garden* and *Research Institute*, Trivandrum.

Blumenschein, A. (1960). Numerodecranossames de algumasespecies de orquideas. *Publ. Cien. Univ. Sao.Paulo. Inst. Genet*., 1: 47-48.

Davis, P.H. and Heywood, V.H. (1963). Principles of Angiosperm Taxonomy, Oliver and Boyed Ltd., London.

Divakar, K.M. (1987). Cyto-embryological investigations on certain orchids of *Tribe epidendreae and Vandeae*. Ph.D. Thesis of the Karnataka University, Dharwad.

Foja Singh IOPB chromosome number reports. *Taxon*., 30: 704-705

Harmen, L. (1943). Studies on the cytology of arctic plants. Meddel Granland, 131(1): 1-15.

Jones, K. (1963). The chromosomes of Dendrobium. A.O.S. Bull., 32: 634-643.

Jorapur, S.M. and Kulkarani, A.L. (1979). Cytotaxonomical studies in five species of the genus Eria Lindl. *Cytologia*, 44(2): 479-485.

Jorapur, S.M. and Kulkaranil, A.L. (1980). OPB chromosome number report, Orchidaceae. *Taxon*., 29: 546-547.

Jorapur, S.M. and Garg (1980). In IOPB chromosome number reports. *Taxon*., 29: 533-547.

Jorapur, S.M. and Hegde, S.M. (1974). Karyomorphological studies in *Bulbophyllum neilgherrense* Wt. *Curr. Sci*., 43(14): 460-461.

Kamemoto, H. (1950). Polyploidy in cattleyas. *Bull. Amer. Orchid. Soc*., 19(7): 366-373.

Kamemoto, H. and Randolpl, L.F. (1949). Chromosome of the Cattlya tribe. *Bull. Amer. Orchid. Soc. Hawaii,* 18(6): 366-369.

Marimuthu, K.M. and Subramanian, M.K. (1960). A haematoxylin squash method for the root tips of *Dolichos lablab* L. *Curr. Sci*., 29: 482-493.

Mehra, P.M. and Viji, S.P. (1972). Cytological studies in the east Himalayan Orchidaceae-2. *Orchideae Caryologia*, 25: 335-351.

Roja Sigh I. (1983). OPB chromosome number reports. *Taxon.,* 32(1).

Santham, P. (1991). Systematics and karyomorphology of some South Indian Orchidaceae. *Ph.D.Thesis,* Presidency College, Madras University.

Stenar, H. (1937). On *Achroanthes manophyllos* (L). Greene, dessgeografis kautberdingoch embryologi *Heimbygdastidskr. Fornvardaven, Uppsala*, 6:177-231.

Sublabhapathak and Jorapur, S.M. (1983). Three chromosomal biotypes of *Habenaria marginata* coleb. (Orchidaceae).70th session of Ind. Sci. Cong. Assoc. Abstracts, P.87.

Sublabhapathak (1982). Polysomaty in *Habenaria ovalifolia* weight. Fifth All India Botanical Conference Dec: 27-29, Abstracts, 61 Supplement, p.64

Vatsala, P. (1964). Studies on the cytology and evolution of Orchidaceae with special reference to orchid of South India. *Ph.D. Thesis*, Kerala University.

Vijayakumar, M. (1986). Cytotaxonomical studies of South Indian Orchidaceae. *Ph.D. Thesis,* Annamalai University, Annamalainagar.