Material and Methods

MATERIALS AND METHODS

Different species of Cyanotis D.Don. were collected from different localities of Maharashtra, particularly from Kolhapur, Satara and Sangli districts of Maharashtra. The species collected and the localities from which they are collected are given in table No.2 and shown in Map. 1.

Amischophacepous cucullata, (Roth) [= Cyanotis cucullata (Roth) Kunth.] from cultivated fields with black soil. **Cyanotis cristata,** (L.) D.Don. was collected from campus area of Shivaji University, Kolhapur. **Cyanotis fasciculates,** Heyne, ex.Roth; Collected from Kolhapur, Panhala and Gaganbavda. **Cyanotis concanensis,** Hassk. [**Cyanotis sahyadrica** Blatt.], grows at higher altitudes in open areas on plateau and was collected from Gaganbavda (Kolhapur) and Kas Plateau (Satara). **Cyanotis tuberosa** (Roxb). Schult. is highly polymorphic species. The different morphological forms were collected carefully from above referred areas. The samllest from grows in grass land of plains, and was collected from Kolhapur. The other robust form of **Cyanotis tuberosa** (Roxb.) Schult F. was collected from hilly regions of Kartikiswami (Khatav taluka and Sagareshwar hills (Sangli district), And **Cyanotis tuberosa** (Roxb.) F. Var, adsendens Dalz. having prostrate habit was collected from Karnataka University Campus Bengalore.

The plants were collected in vegetative as well as in flowering and fruiting stages, during June to September. Observations on morphological characters, phenology, flowering and fruiting of different

species were carefully noted in the field, minimum fifty to hundred (50 to 100) plants were collected from each locality. After a careful study on morphological characters, Plants were planted in earthern pots and plots in Botanical Gardens of the Botany Department, Shivaji University, Kolhapur.

For morphological studies at least 25 randomly selected plants were analysed. Minimum 25 observations were made, for each characters, and the mean of all readings was computed with standered deviation by using calculator.

the leaf thickness of plant is measured by using leaf thickness meter. Morphological atributes of plants grown in Botanical garden, and from natural habitat were studied critically and changes in quantitative characters are shown Polygraphically in Figs. I and II.

The Karyotypic studies of **Cyanotis** D.Don, species have been performed from excised healthy root tips of soil cultured plants, during rainy season. The excised healthy roots treated with saturated aqueous solution of para - dichlorohenzen, (PDB) for 3 hours at 8°C. Then root tipes were hydrolysed in 1N. Hcl; (Hydrochloric acid), at 60°C for some times and then squashed it in 2% acetic orcein solution, which gave satisfactory results.

The slides were made parmanent after passing through usual grades of n Butanol, acetic acid, and were mounted in DPX, and deposited in Department of Botany of Shivaji University, Kolhapur.

Minimum 20 to 25 plants from each locality were used for karyotypic studies. Minimum 20 plates were analysed for each species.

For karyotypic analysis the nomenclature recommended by Levan et. al. (1964) for centromeric position has been adopted. Symmetry of Karyotypie has been analysed by using stebbin's (1958) system of classification. F% and TF% were calculated as given by Huziwara (1962). while TCL%, S% and relative length of chromosome were determined by using following formulae.

$$\text{TCL \%} = \frac{\text{Length of the chromosome}}{\text{Absolute length of the Complement}} \times 100$$

$$\text{S \%} = \frac{\text{Length of shortest chromosome,}}{\text{Length of longest chromosome.}} \times 100$$

$$\text{Relative length of chromosome} = \frac{\text{Length of chromosome}}{\text{Length of longest chromosome in the complement.}} \times 100$$

$$\text{F\%} = \frac{\text{Short arm length of Chromosome}}{\text{Total length of chromosome}} \times 100$$

$$\text{TF \%} = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

For the meiotic studies young floral buds were fixed in Acetic alcohol (1:3), after fixation a freshly fixed buds were used. The anthers were squashed in 2% aceto-orcine after hydrolysis in 1 N. Hcl.

Photomicrographs were taken from temparory and parmanent preprations using MFAKS system of JENAVAL, Carl-zeiss microscope. Cytological preprations were made parmanent by using usual grades of acetic acid and n Butanol, and were mounted in DPX.

TABLE NO. 2

Showing Time and Place of Collection of Different Species of Amischophaceleus, Rolla Raw, Kammathy, and Cyanotis D.Don. in MAHARASHTRA.

Sr. No.	Name of Species	Locality	Chromosome Number	Time of Collection
I)	**Amischophascelus** Rolla Raw			
1)	**Amischophacelus axillaris** (L.) D.Don. (**C.axillaris** D.Don.)	Malvan (Shindhudurg)	n=10	July 1991
2)	**Amischophacelus cucullata** (Roth.) Rolla Rao (C.cucullata Roth. Kunth.)	Kolhapur Kadepur Kadegaon, (Sangli,Dist.)	2n=20	August 1989 August 1990
II)	**Cyanotis** D.Don.			
1)	**Cyanotis concanensis,** itassk. (**C.Sahyadrica,** Blatt.)	Kas Plateau (Satara) Gaganbavada (Kolhapur Dist.)	2n=72	October 1989 August 1990 September 1990
2)	**Cyanotis cristata** (Linn.)	Kolhapur Shivaji Unive-rsity,Kolhapur. campas.	2n=24	September 1989 September 1990 October 1989 October 1990
3)	**Cyanotis fasciculata**	Kolhapur Panhala Gaganbavada	2n=24	September 1989 September 1990 October 1989 October 1990
4)	**Cyanotis tuberosa** Roxb. Schult.F.	Kolhapur Kadepur (Sangli Dist.)	2n=24	July 1990
5)	**Cyanotis tuberosa** Roxb.Schult.F.	Sagreshwar itills, itills of Kartikiswami Satara Dist.	2n=48	August 1990
6)	**Cyanotis tuberosa,** Rodb.vari adsendens (Dalz.)	Karnata Univ-ersity of Benglore.	2n=24	August 1989

MAP:-I Distribution of Cyanotis species in MAHARASHTRA.