Project Report

Innovative and conventional approaches for identifying *Viola* species from Jammu Province and selection of superior forms for hill-farming

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Project Title:

Innovative and conventional approaches for identifying *Viola* species from Jammu Province and selection of superior forms for hill-farming

Objectives:

The immediate objectives were:

a. Exploring Jammu Province for collection, characterisation and documentation of different species of *Viola*.

b. Analysing different accessions and species using morphological and cytological parameters.

c. Studying different accessions and species using DNA-based markers.

Project Details

Inrroduction

Viola, a member of Violaceae, is represented in Jammu Province by *V. odorata, V. canescens, V. pilosa* (*=V.serpens*), *V. biflora* and *V. tricolor*. Interaction with local people revealed that these species have been used catering to the health needs of majority of hilly populace since time immemorial. While *V. odorata* is commercially used for treating cough, cold, sore throat, bronchitis, fever and nervousness, *V. pilosa* finds use in purifying blood, treating cough and ulcers. *V. tricolor* has an ornamental value.

Species of genus *Viola* are known for their taxonomic complexity because of (i) existence of few morphological characters used to delimit taxa, with most of these being overlapping (ii) presence of strong phenotypic plasticity and (iii) frequent inter-specific hybridisation. For identifying different *Viola* species, cytological parameters viz. chromosome numbers, their forms and sizes, can be of great help. While for identifying some taxa and distinguishing between hybrid and non-hybrid forms, chromosome count

may be sufficient, for authenticating others, complete karyotypic and meiotic details may prove significant.

Material and Methods:

Field Survey:

a) Field trips were made to explore, identify and tag various populations of *V. odorata*, *V. pilosa* and *V. tricolor* from Jammu province. Geo-tagging was performed with the help of altimeter. Some of the plants of tagged populations were transplanted into the Botanical garden (University of Jammu).

b) Plant specimen were collected, dried and preserved in herbarium sheets.

Morphological analysis:

a) Morphometric data like leaf length, leaf width, petiole length, leaf shape, leaf number per plant, flower colour, number of flowers per plant, presence or absence of cleistogamous flowers were taken for the two *Viola* species. Four to five mature plants of each accession were studied for morphological descriptors. Standard Deviation (S.D.) and Standard Error (S.E.) for different plant parts were calculated by using the formulae:

$$S.D.=\sqrt{\frac{\sum(X-\bar{X})^2}{N-1}} \qquad S.E.=\frac{S.D}{\sqrt{N}}$$

Meiosis in male track:

a) For studying meiotic details immature buds were collected in the morning hours, fixed in Carnoy's fixative (3:1, Ethanol: Acetic acid) for 24 hours and preserved in 70% Ethanol. Anthers from these buds were squashed in 1% propionocarmine and pollen mother cells with clear associations were analysed for percentage frequency of various chromosomal configurations. Photomicrographs of these were taken using Zeiss Trinocular microscope.

Pollen stainabilty of different accessions was determined by squashing anthers in 1% acetocarmine. Well stained and filled pollen grains were categorized as viable and shriveled and unstained pollens were taken as non viable.

Results:

During March 2019, five field surveys were made along Bhaderwah, Rajouri, Patnitop and collections were made from different locations. Date of collection, area scanned and populations collected are shown in the table 1 below.

Table 1: Areas scanned and populations located

S.No.	Date of field trip	Area scanned	Population collected
1.	02/03/2019	Bhaderwah-Chinta Road	Chinta Kapra
2.	06/03/2019	Bhaderwah- Chamba road	Thanala Dodu
3.	10/03/2019	Bhaderwah	Gupt Ganga Teligarh
4.	15/03/2019	Rajouri	BGSBU
5.	17/03/2019	Patnitop	Natha Top

Different collections were geo-tagged and their habitats were photographed (Fig.1). Besides, observations were made regarding their habitat as shown in table 2.

Table 2: Table showing study sites of *Viola pilosa* and *V. odorata* with their geographical coordinates.

Study site	Species name	Latitude	Longitude	Elevation (masl)	Habitat
Kapra	*V. pilosa*	33°0'38" N	75°42'38" E	1651	Sloping area under *Pinus* trees.
Gupt Ganga	*V. pilosa*	32°58'49" N	75°43'14" E	1667	Shady northern slope beside the road sides having moist soil.
Teligarh	*V. pilosa*	32°35'47" N	75°39'87" E	1771	Moist soil alongside the road under the shade of bushes.
Pranoo upper	*V. pilosa*	33°05'30" N	75°35'10" E	1140	Under the shade of *Quercus baloot* trees.
Pranoo Lower	*V. pilosa*	33°05'38" N	75°35'09" E	1068	Interspersed with other herbs under the shade of bushes.
BGSBU (Rajouri)	*V. pilosa*	33°23'44" N	74°20'51" E	1163	Luxuriant growth in most soil in *Pinus* forest.
Chinta	*V. pilosa*	33°01'13" N	75°43'11" E	2257	Growing sparsely in the shade of *Pinus* trees .
Thanala	*V. pilosa*	32°55'13" N	75°46'15" E	2202	Sparsely distributed under the shade of *Cedrus* trees.
Dodu (Sartingal)	*V. pilosa*	32°56'30" N	32°56'30" N	1943	Sparse growth under the shade of *Cedrus* trees as well as in the gritty slope along road-sides.
Natha Top	*V. odorata*	33°05'58" N	75°17'22" E	2265	Richly distributed in the sloping moist soil under the shade of *Cedrus* trees along with other grasses.

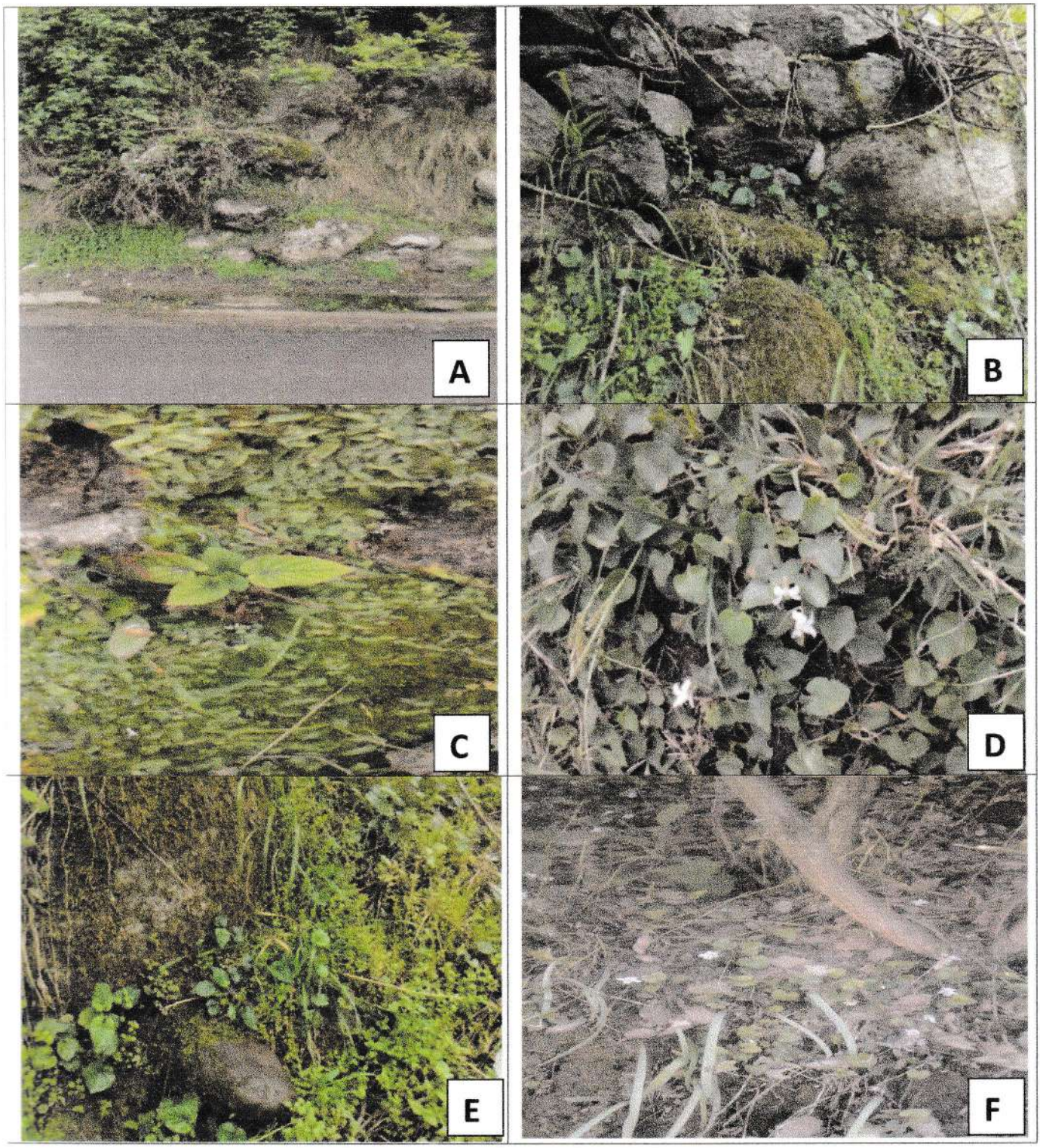


Fig.1 **Habitat of *Viola spp.* (A,B) *V. pilosa* growing along the roadside at Gupt Ganga (Bhaderwah), (C) *V. pilosa* at Ramnagar (Udhampur) alongwith bryophytes (D) *V. odorata patch* at Patnitop and (E) *V. pilosa* at Kapra (F) *V. pilosa* at Kishtwar.**

Morphological details:

The morphological data for some of the populations collected was taken as shown below in the table.

Table 3. Morphometric details of *Viola pilosa* and *V. odorata* from different regions

Study site	Species name	Leaf length (cm)±SE	Leaf width (cm)±SE	Petiole length (cm)±SE	Leaf number± SE	No. of veins/lamina±SE	No.o f flowers/ plant	Flower colour	Cleistogamous flowers
Kapra	*V. pilosa*	4.87±1.01	4±0.83	8.65±2.38	6.7±1.15	5.2±1.03	3	Whitish blue with purple streaks	+
Gupt Ganga	*V. pilosa*	4.02±0.64	3.52±0.71	6.59±1.35	6.0±0.57	5.38±0.86	2	Whitish blue with purple streaks	+
Teligarh	*V. pilosa*	4.82±1.40	3.91±1.21	5.94±2.34	6.1±1.19	5.5±0.52	3	Whitish blue with purple streaks	+
Pranoo Upper	*V. pilosa*	4.9±0.83	3.88±0.72	8.32±1.3	6.42±1.61	7.14±0.69	3	Whitish blue with purple streaks	+
Pranoo Lower	*V. pilosa*	5.45±0.58	4.16±0.60	6.55±1.22	8.5±1.64	5.33±0.51	4	Whitish blue with purple streaks	+
BGSBU (Rajouri)	*V. pilosa*	3.62±0.68	2.86±0.29	3.06±1.41	8.0±2.44	6±1	3	Whitish blue with purple streaks	–
Natha Top	*V. odorata*	4.3±0.65	3.42±0.35	4.76±1.23	6.4±2.07	6.4±0.54	4	White with purple streaks	+

+ = Present

– = Not observed

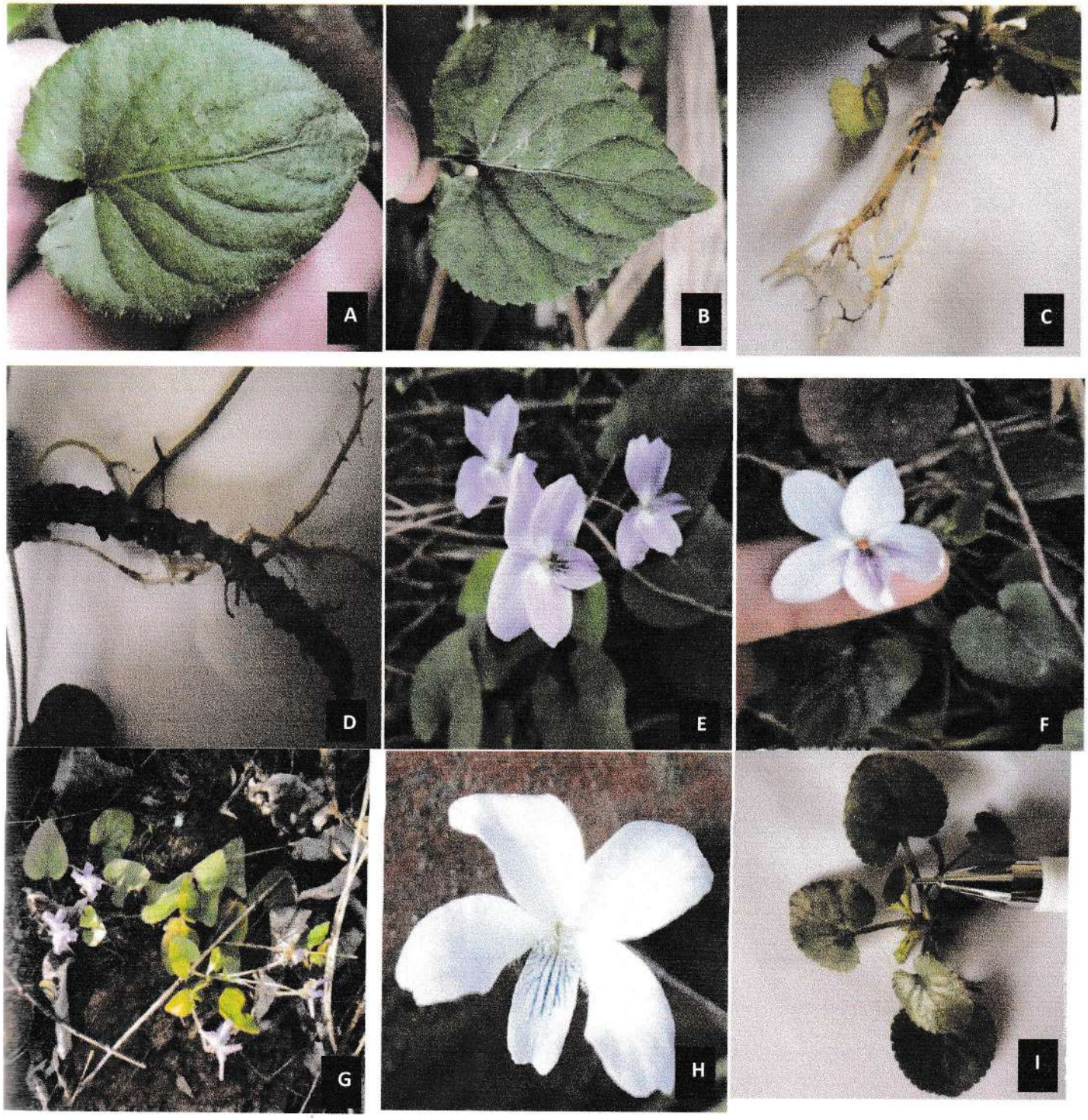


Fig.2 Morphology of *Viola* (A) leaf with serrate margins and obtuse tip in *V. odorata* (B) leaf with serrate margins and acuminate tip in *V. pilosa* (C)rhizome of *V. odorata* bearing roots (D) rhizome of *V. pilosa* (E-F) flowers of *V. pilosa* (G) habitat (H) flower having bearded lateral petals in *V. odorata* (I) whole plant of *V. odorata.*

V. odorata:

As is evident from the above observations *V. odorata* (Fig. 2I) is found at higher altitudes (2265 masl) of Natha Top and inhabited shady forest floor under *Cedrus* trees. This rhizomatous (Fig. 2c) plant bears white flowers with purple streaks on the lower petal (2H). Lateral two petals are bearded and upper petals are wedge shaped. Leaves are serrate and obtuse as shown in Fig.2 (A).

V.pilosa:

The species shows wide range of distribution, in various habitats from 1068 to 2257 masl. It has whitish blue flowers with purple streaks on lower petals. The leaves are serrate and acuminate unlike *V. odorata* as shown n Fig.2 (B). Plants of BGSBU (Rajouri) population were observed smaller than the rest and cleistogamous flowers were not observed.

Cytological details:

Meiosis was carried out from cleistogamous flowers. Pollen Mother Cells (PMCs) of *V. pilosa* observed at Diplotene and Metaphase-I revealed that it contains 2n= 18 chromosomes with majority (98%) of pollen mother cells having 9 bivalents while 2% PMCs have 6 bivalents+ 2 trivalents. Segregation was normal as observed in Anaphase-I. Nucleolus was large sized with a trivalent attached with it. Pollen stainability was 95%.

In *V. odorata* 10 bivalents were observed at Metaphase-I. Apart from dividing meiotic cells , some large sized cells (1.5 times larger) were also noted. These cells had small sized chromosomes with 2-5 nucleoli. The chromosomes number ranged from 2n=40 to 2n= 60 and the nucleoli were persistent at metaphase.

DNA analysis:

Chemicals used for isolation of genomic DNA have been purchased and work on DNA isolation has been initiated.

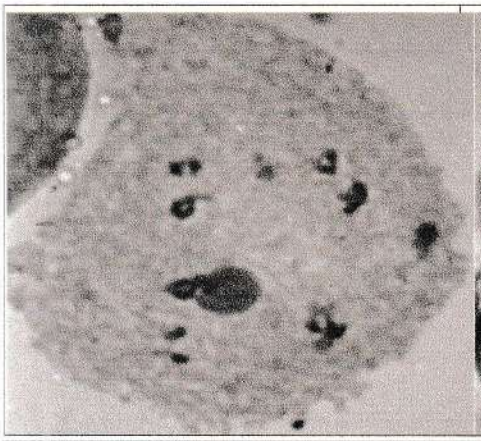


Fig.3(A). PMCs of *V. pilosa* at diplotene showing 2-III and6-II.

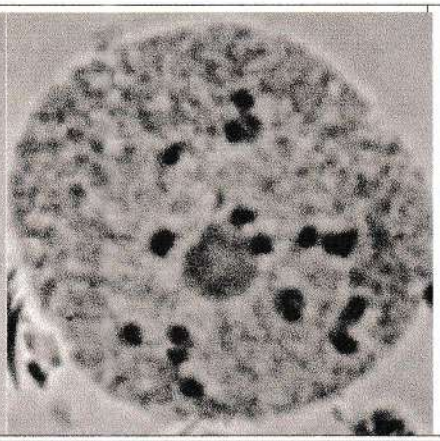


Fig.3(B). PMC of *V. pilosa* at Metaphase-I showing 9 II.

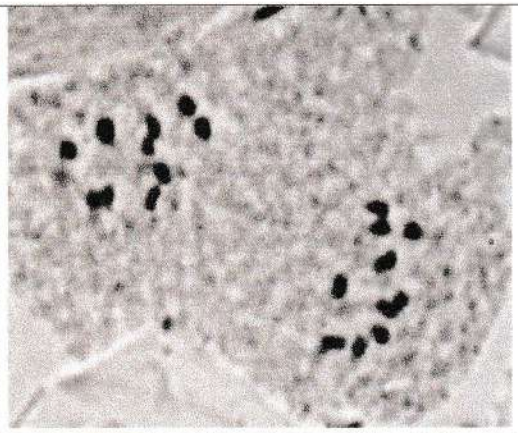
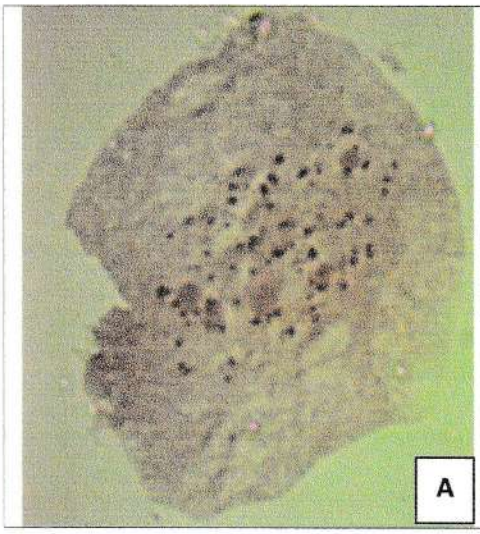
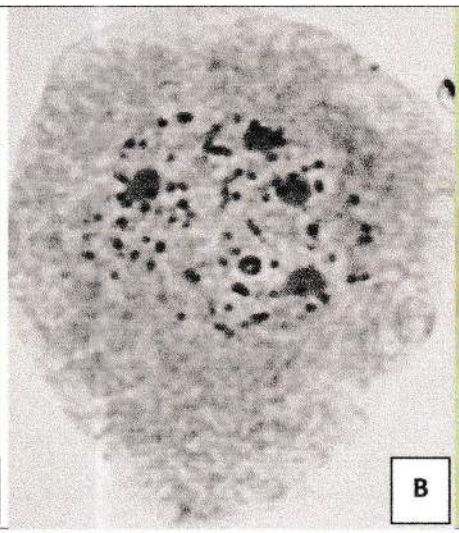


Fig.3(C). PMC of *V. odorata* showing 10 chromosomes at each pole in Anaphase-I.





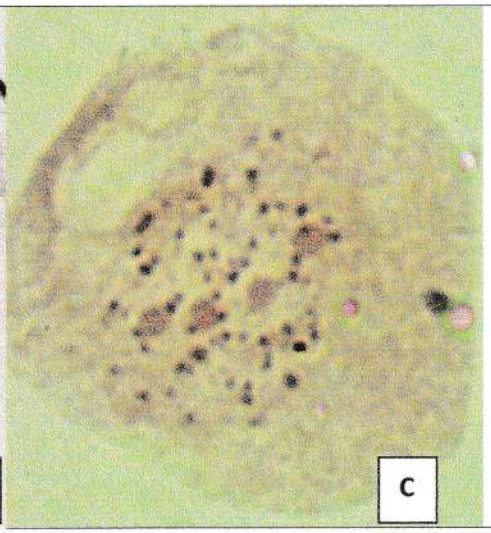


Fig.4. Tapetal cells of *V. odorata* at Metaphase having 2n=50 (A) and 2n=60 (B,C) chromosomes.

Utilization certificate

Certified that the grant of Rs. 55,000/- (Fifty-five thousand rupees) sanctioned during the year 2019-20 for *Innovative Project under RUSA* vide order no: RA/19/5907-18, dated 28-2-2019 has been utilized for the purpose for which it was sanctioned.

Signature 

Dr. Geeta (PI, RUSA project)