

**FINAL REPORT ON THE MAJOR RESEARCH  
PROJECT**

**EVALUATION OF CYTOMORPHOLOGICAL  
DIVERSITY IN THE SEDGES OF NORTH INDIA**

**DURATION: 01-07-2015 TO 30-06-2018**

**Submitted to:**

**UNIVERSITY GRANTS COMMISSION, NEW DELHI**

**by**

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**September 2018**

**Annexure -VIII**

**FINAL REPORT ON THE PROJECT**

**EVALUATION OF CYTOMORPHOLOGICAL DIVERSITY IN THE  
SEDGES OF NORTH INDIA**

1.	Project report No. 1 <sup>st</sup> /2 <sup>nd</sup> /3 <sup>rd</sup> / Final	Final
2.	UGC Reference No.	43-120/2014(SR)
3.	Period of report:	1July, 2015 to 30 June, 2018
4.	Title of research project	Evaluation of cytomorphological diversity in the sedges of North India
5.	(a) Name of the Principal Investigator  (b) Deptt.  (c) University/College where work has progressed  (d) Co-Investigator	Dr. Paramjeet Cheema  IAS & Allied Services Training Centre, Punjabi University, Patiala- 147002  Punjabi University, Patiala  Prof. M.I.S. Saggioo Department of Botany, Punjabi University, Patiala - 147002.
6.	Effective date of starting of the project	1 <sup>st</sup> July, 2015
7.	Grant approved and expenditure incurred during the period of the report: a. Total amount approved  b. Total expenditure  c. Report of the work done:  i. Brief objective of the project	Rs.10,55,000 ( Ten Lakh and fifty five thousand only) Rs.9,02,436 (Nine Lakh two thousand four hundred thirty six only)  1. To investigate cytological diversity in wild germplasm of Cyperaceae from North India.  2. To investigate genetic diversity in species with

		chromosomal races using DNA based markers.
	ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication	<b>Refer Appendix 1</b>
	iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons	Yes
	iv. Please indicate the difficulties, if any, experienced in implementing the project	None
	v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.	Not Applicable, as the project has been completed
	vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to University Grants Commission.	<b>Summary:</b> The project Evaluation of Cytomorphological Diversity in the Sedges of North India was started in July 2015. During the three year tenure of the project extensive and intensive field surveys were made to different localities in Punjab, Himachal Pradesh and Uttarakhand. The plants for cytomorphological studies on population basis were collected during the months of May to October. Preliminary field identification of the members of Cyperaceae was made using various floras. Then the identification of different species was confirmed by comparing with authentic specimens lodged in the Herbarium of Department of Botany, Punjabi University, Patiala. The relevant literature about the research topic was accessed from the library of Punjabi University, Patiala; Punjab University, Chandigarh; PAU, Ludhiana as well as Internet sources. Young flower buds were fixed in Carnoy's fixative (6 parts Ethanol:

3 parts Chloroform: 1 part Acetic acid) for meiotic studies. Chromosome number and meiotic abnormalities were confirmed from observations made on several pollen mother cells (PMCs) at different stages in each population. Presently the chromosome number through meiotic studies along with detailed meiotic course has been studied in 103 taxa belonging to 50 species of Cyperaceae. New chromosome numbers were reported in 16 species: *Carex filicina*, *C. foliosa*, *C. fedia*, *C. remota*, *Cyperus kyllingia*, *C. compressus*, *C. iria*, *C. pygmaeus*, *C. digitatus*, *C. tenuispica*, *C. laevigatus*, *Kyllingia brevifolia*, *Pycneus pumilus*, *Bulbostylis capillaries*, *Scirpus roylei* and *S. tuberosus*. Variable chromosome numbers were recorded for in eight species: *Cyperus alulatus*, *C. paniceus*, *C. difformis*, *C. digitatus*, *C. tenuispica*, *C. rotundus*, *Scirpus roylei* and *S. tuberosus*.

Meiotic abnormalities like chromatin bridges, cytotoxicity, laggards, stickiness and reduced pollen fertility were observed. Chromatin bridges and laggards were seen in four species: *Cyperus tenuispica*, *C. rotundus*, *Kyllingia brevifolia* and *Schoenoplectus mucronatus*. Cytotoxicity is recorded in *Cyperus paniceus*. Eight species: *Cyperus alternifolius*, *C. alulatus*, *C. cuspidatus*, *C. iria*, *C. tenuispica*, *Kyllingia brevifolia*, *Eriophorum comosum*, and *Fimbristylis miliacea*.

Karyomorphological studies on 4 species of genus *Fimbristylis* namely *F. bisumbellata* (n=5), *F. dichotoma* (n=10), *F. miliacea* (n=5) and *F. quinqueangularis* (n=5) genus have been made. The parameters like position of centromere, secondary constrictions, haploid chromatin length, absolute and average chromosome size and the type of symmetry based on GI and SI are used. Karyotypic polymorphism was reported for two species *Fimbristylis bisumbellata* and *F. dichotoma*.

The cytotypes of *Cyperus alulatus*, *C. paniceus*, *C. difformis*, *C. digitatus*, *C. tenuispica*, *C. rotundus*, *Scirpus roylei* and *S. tuberosus* had been studied morphometrically with different

		<p>parameters <i>i.e.</i> plant height, length of leaves, size of involucre bracts, numbers of spikelets, numbers of glumes, etc. and these reflect significant difference.</p> <p>In order to evaluate the genetic diversity in accessions of <i>Cyperus rotundus</i> and <i>S. tuberosus</i> DNA markers were used. Different molecular markers such as random amplified polymorphic DNA (RAPD) for assessment of genetic diversity at intraspecific level. Molecular evaluation of above intraspecific cytotypes was carried out. No significant results were obtained. It is a case of applying new markers especially ISSR, ITS and <i>rbcl</i>.</p>
	<p>vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any</p>	<p>(a) <b>MAN POWER TRAINED:</b> One research fellow Mr. Neeraj Kumar was enrolled for Ph.D programme of Punjabi University, Patiala on September 16, 2016. He has completed course work at Botany Department, Punjabi University, Patiala and is registered for Ph.D. on 9/2/2018 (Registration no. PU(P) 2013-629).</p> <p>(b) <b>PH. D. AWARDED:</b> Research fellow is registered for Ph.D.</p> <p>(c) <b>PUBLICATIONS:</b> Two research papers in peer reviewed journals were published</p>

**Annexure -IX**

**INFORMATION ON THE MAJOR RESEARCH PROJECT**

**EVALUATION OF CYTOMORPHOLOGICAL DIVERSITY IN THE  
SEDGES OF NORTH INDIA**

<b>1.</b>	<b>TITLE OF THE PROJECT</b>	Evaluation of Cytomorphological Diversity in the Sedges of North India
<b>2.</b>	<b>NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR</b>  <b>CO-INVESTIGATOR</b>	Dr. Paramjeet Cheema IAS & Allied Services Training Centre, Punjabi University, Patiala – 147002.  Prof. M.I.S. Saggo Department of Botany, Punjabi University, Patiala - 147002.
<b>3.</b>	<b>NAME AND ADDRESS OF THE INSTITUTION</b>	Department of Botany, Punjabi University, Patiala
<b>4.</b>	<b>UGC APPROVAL LETTER NO. AND DATE</b>	43-120/2014(SR), Dated 9 <sup>th</sup> Aug,2015
<b>5.</b>	<b>DATE OF IMPLEMENTATION</b>	1July, 2015
<b>6.</b>	<b>TENURE OF THE PROJECT</b>	Three year
<b>7.</b>	<b>TOTAL GRANT ALLOCATED</b>	Rs.10,55,000 ( Ten Lakh and fifty five thousand only)
<b>8.</b>	<b>TOTAL GRANT RECEIVED</b>	Rs.8,67,901 (Eight lakh and sixty seven thousand nine hundred one only)
<b>9.</b>	<b>FINAL EXPENDITURE</b>	Rs.9,02,436 ( Nine Lakh two thousand four hundred thirty six only)
<b>10.</b>	<b>TITLE OF THE PROJECT</b>	Evaluation of Cytomorphological Diversity in the Sedges of North India
<b>11.</b>	<b>OBJECTIVES OF THE PROJECT</b>	I. To investigate cytological diversity in wild germplasm of Cyperaceae from North India  II. To investigate genetic diversity in species with chromosomal races using DNA based markers.

<p><b>12. WHETHER OBJECTIVES WERE ACHIEVED</b></p>	<p>I. To investigate cytological diversity in wild germplasm of Cyperaceae from North India - Achieved</p> <p>II. To investigate genetic diversity in species with chromosomal races using DNA based markers – Partially achieved</p>	<p>Yes</p> <ol style="list-style-type: none"> <li>1. Cytological survey revealed chromosome number for 105 accessions belonging to 50 species of family Cyperaceae</li> <li>2. New chromosome numbers were reported in 16 species: <i>Carex filicina</i> (n=18), <i>C. foliosa</i> (n=28), <i>C. fedia</i> (n=52), <i>C. remota</i> (n=31), <i>Cyperus kyllingia</i> (n=28), <i>C. compressus</i> (n=24), <i>C. iria</i> (n=29), <i>C. pygmaeus</i> (n=27), <i>C. digitatus</i> (n=27), <i>C. tenuispica</i>(n=7), <i>C. laevigatus</i>(n=21), <i>Kyllingia brevifolia</i>(n=30), <i>Pycnus pumilus</i> (n=29), <i>Bulbostylis capillaries</i> (n=22), <i>Scirpus roylei</i> (n=36) and <i>S. tuberosus</i> (n=24, 27,26,)</li> <li>3. Variable chromosome numbers were recorded for in eight species: <i>Cyperus alulatus</i> (n=40, 57), <i>C. paniceus</i> (n=21, 42), <i>C. difformis</i> (n=17, 18), <i>C. digitatus</i> (n=27, 54), <i>C. tenuispica</i> (n=7, 8), <i>C. rotundus</i> (n=40, 48, 50, 52, 54, 55. 56), <i>Scirpus roylei</i> (n=11, 36) and <i>S. tuberosus</i> (n=24, 26, 27)</li> <li>4. Karyotypic polymorphicity was reported for two species: <i>Fimbristylis bisumbellata</i> (n=5) and <i>F. dichotoma</i> (n=10)</li> </ol> <ol style="list-style-type: none"> <li>1. Genetic diversity was detected on cytological basis in two species: <i>Cyperus rotundus</i> and <i>Scirpus tuberosus</i></li> <li>2. DNA was isolated and amplified and as many as 20 RAPD markers were tested for molecular evaluation of above intraspecific cytotypes. RAPD markers did not revealed significant results</li> <li>3. It is a case of applying new markers especially ISSR, ITS and <i>rbcl</i>.</li> </ol>
<p><b>13. ACHIEVEMENTS FROM THE PROJECT</b></p>		<ol style="list-style-type: none"> <li>1. Two species have been cytologically worked out for the first time from India: <i>Carex filicina</i>, and <i>C. foliosa</i></li> <li>2. New chromosome numbers were recorded in 16 species: <i>Carex filicina</i>, <i>C. foliosa</i>, <i>C. fedia</i>, <i>C. remota</i>, <i>Cyperus kyllingia</i>, <i>C. compressus</i>, <i>C. iria</i>, <i>C. pygmaeus</i>, <i>C. digitatus</i>, <i>C. tenuispica</i>, <i>C. laevigatus</i>, <i>Kyllingia brevifolia</i>, <i>Pycnus pumilus</i>, <i>Bulbostylis capillaries</i>, <i>Scirpus roylei</i> and <i>S. tuberosus</i></li> </ol>

3. Morphological diversity and/or cytological diversity was recorded in *Cyperus alulatus*, *C. paniceus*, *C. difformis*, *C. digitatus*, *C. tenuispica*, *C. rotundus*, *Scirpus roylei* and *S. tuberosus*
4. Two research papers were published:
  - Cheema, P; Saggoo, M.I.S. and Kumar, N. 2017. Cytomorphology of some medicinal sedges from North West India. International Journal of Pharmacy and Pharmaceutical Research. 10 (2): 231-243.
  - Cheema, P; Kumar, N and Saggoo M.I.S. Aneuploidy in *Cyperus tenuispica* from North India. Cytologia 83 (4): Accepted
5. Eight Papers were presented in conferences/symposium:
  - Chromosomal status of some medicinal sedges from North Western Himalayas in National conference on Basic and Applied Researches in Plants and Microbes at Punjabi University, Patiala from November 3-5, 2016.
  - An overview of chromosomal diversity in cytologically investigated species of *Fimbristylis* Vahl in National conference on Basic and Applied Researches in Plants and Microbes at Punjabi University, Patiala from November 3-5, 2016.
  - Karyomorphology of *Fimbristylis* Vahl from Punjab, North Western India in Swarna Jayanti National conference on Biodiversity and Sustainable Development at Kurukshetra University, Kurukshetra from 17-18 February 2017.
  - Cytomorphological diversity in some sedges (Cyperaceae) from North Western Himalayas in Swarna Jayanti National conference on Biodiversity and Sustainable Development at Kurukshetra University, Kurukshetra from 17-18 February 2017.
  - Review of *Cyperus rotundus* Linn.- Common Nut Sedge in 40<sup>th</sup> All India Botanical Conference of the Indian Botanical society & National symposium of Evaluation and Conservation of Plant Germplasm at Punjabi University, Patiala from 15-17 September, 2017.
  - Cytomorphology and ethanobotanical notes of some Sedges from North West India in 40<sup>th</sup> All India Botanical Conference of the Indian



		<p>Botanical society &amp; National symposium of Evaluation and Conservation of Plant Germplasm at Punjabi University, Patiala from 15-17 September, 2017.</p> <ul style="list-style-type: none"> <li>• Male meiosis in cytomorphotype of <i>Cyperus tenuispica</i> Steud. from North India in National conference research in plant sciences for sustainable development at Sri Guru Granth Sahib World University, Fatehgarh Sahib from 16-17 March, 2018.</li> <li>• Nutsedge : Weed with Tremendous Therapeutic Potential in DBT sponsored National Conference on Recent Trends in Plant Sciences at Sanmati Government College of Science Education and Research, Jagraon from 19-20 April, 2018.</li> </ul>
<p><b>14. SUMMARY OF THE FINDINGS</b></p>		<p>The project Evaluation of Cytomorphological Diversity in the Sedges of North India was started in July 2015. During the three year tenure of the project extensive and intensive field surveys were made to different localities in Punjab, Himachal Pradesh and Uttarakhand. The plants for cytomorphological studies on population basis were collected during the months of May to October. Preliminary field identification of the members of Cyperaceae was made using various floras. Then the identification of different species was confirmed by comparing with authentic specimens lodged in the Herbarium of Department of Botany, Punjabi University, Patiala. The relevant literature about the research topic was accessed from the library of Punjabi University, Patiala; Punjab University, Chandigarh; PAU, Ludhiana as well as Internet sources. Young flower buds were fixed in Carnoy's fixative (6 parts Ethanol: 3 parts Chloroform: 1 part Acetic acid) for meiotic studies. Chromosome number and meiotic abnormalities were confirmed from observations made on several pollen mother cells (PMCs) at different stages in each population. Presently the chromosome number through meiotic studies along with detailed meiotic course has been studied in 103 taxa belonging to 50 species of Cyperaceae. New chromosome numbers were reported in 16 species: <i>Carex filicina</i>, <i>C. foliosa</i>, <i>C. fedia</i>, <i>C. remota</i>, <i>Cyperus kyllingia</i>, <i>C. compressus</i>, <i>C. iria</i>, <i>C. pygmaeus</i>, <i>C. digitatus</i>, <i>C. tenuispica</i>, <i>C. laevigatus</i>, <i>Kyllingia</i></p>

		<p><i>brevifolia</i>, <i>Pycreus pumilus</i>, <i>Bulbostylis capillaries</i>, <i>Scirpus roylei</i> and <i>S. tuberosus</i>. Variable chromosome numbers were recorded for in eight species: <i>Cyperus alulatus</i>, <i>C. paniceus</i>, <i>C. difformis</i>, <i>C. digitatus</i>, <i>C. tenuispica</i>, <i>C. rotundus</i>, <i>Scirpus roylei</i> and <i>S. tuberosus</i>.</p> <p>Meiotic abnormalities like chromatin bridges, cytomixis, laggards, stickiness and reduced pollen fertility were observed. Chromatin bridges and laggards were seen in four species: <i>Cyperus tenuispica</i>, <i>C. rotundus</i>, <i>Kyllingia brevifolia</i> and <i>Schoenoplectus mucronatus</i>. Cytomixis is recorded in <i>Cyperus paniceus</i>. Eight species: <i>Cyperus alternifolius</i>, <i>C. alulatus</i>, <i>C. cuspidatus</i>, <i>C. iria</i>, <i>C. tenuispica</i>, <i>Kyllingia brevifolia</i>, <i>Eriophorum comosum</i>, and <i>Fimbristylis miliacea</i>.</p> <p>Karyomorphological studies on 4 species of genus <i>Fimbristylis</i> namely <i>F. bisumbellata</i> (n=5), <i>F. dichotoma</i> (n=10), <i>F. miliacea</i> (n=5), and <i>F. quinqueangularis</i> (n=5) genus have been made. The parameters like position of centromere, secondary constrictions, haploid chromatin length, absolute and average chromosome size and the type of symmetry based on GI and SI are used. Karyotypic polymorphicity was reported for two species <i>Fimbristylis bisumbellata</i> and <i>F. dichotoma</i>.</p> <p>The cytotypes of <i>Cyperus alulatus</i>, <i>C. paniceus</i>, <i>C. difformis</i>, <i>C. digitatus</i>, <i>C. tenuispica</i>, <i>C. rotundus</i>, <i>Scirpus roylei</i> and <i>S. tuberosus</i> had been studied morphometrically with different parameters <i>i.e.</i> plant height, length of leaves, size of involucre bracts, numbers of spikelets, numbers of glumes, etc. and these reflect significant difference.</p> <p>In order to evaluate the genetic diversity in accessions of <i>Cyperus rotundus</i> and <i>S. tuberosus</i> DNA markers were used. Different molecular markers such as random amplified polymorphic DNA (RAPD) for assessment of genetic diversity at intraspecific level. Molecular evaluation of above intraspecific cytotypes was carried out. No significant results were obtained. It is a case of applying new markers especially ISSR, ITS and rbcL.</p>
15.	<b>CONTRIBUTION TO THE SOCIETY</b>	<ol style="list-style-type: none"> <li>1. The present work is of basic nature. It has enhanced the chromosomal database.</li> <li>2. <i>Cyperus alternifolius</i>, <i>C. compressus</i>, <i>C. digitatus</i></li> </ol>

		and <i>C. rotundus</i> are medicinally important plants. The cytological variants were recorded in them. These variants can be evaluated for future exploitation by the society.
<b>16.</b>	<b>WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT</b>	Mr. Neeraj Kumar was <b>enrolled for Ph.D</b> programme of Punjabi University, Patiala on September 16, 2016. He has completed course work at Botany Department, Punjabi University, Patiala and is registered for Ph.D. on 9/2/2018 ( <b>Registration no. PU(P) 2013-629</b> ).
<b>17.</b>	<b>NO. OF PUBLICATIONS OUT OF THE PROJECT</b> (Copy attached)	<ol style="list-style-type: none"> <li>1. Cheema, P; Saggoo, M.I.S. and Kumar, N. 2017. Cytomorphology of some medicinal sedges from North West India. International Journal of Pharmacy and Pharmaceutical Research. 10 (2): 231-243</li> <li>2. Cheema, P; Kumar, N and Saggoo M.I.S. Aneuploidy in <i>Cyperus tenuispica</i> from North India. Cytologia 83 (4): Accepted.</li> </ol>

**Appendix 1**  
**ITEM 7(c) (ii): WORK DONE SO FAR AND RESULTS ACHIEVED AND**  
**PUBLICATIONS RESULTING FROM THE WORK**

**Work Done**

**I. FIELD SURVEYS AND EXCURSION UNDERTAKEN**

The present cytomorphological analysis has been carried out in the Family Cyperaceae, growing wildly. Extensive and intensive field surveys were made to different localities in Punjab, Himachal Pradesh and Uttarakhand. The plants for cytomorphological studies on population basis were collected during the months of May 2016 to June 2018. The present investigations were based on wild materials collected from various localities in North India (Map of area surveyed)



**Fig. 1 Area Surveyed**

**Identification of Plants**

Preliminary field identification of the members of Cyperaceae was made using various floras and identification of different species was confirmed by comparing with authentic specimens lodged in the Herbarium of Department of Botany, Punjabi University, Patiala. Field photographs of some sedges are shown in Fig. 2 and 3.



**Fig. 2.** Field photographs of some sedges



**Fig. 3.** Field photographs of some sedges

## II. CYTOLOGICAL STUDIES

### 1. Meiotic studies

Young flower buds were fixed in Carnoy's fixative (6 parts Ethanol: 3 parts Chloroform: 1 part Acetic acid). Chromosome number and meiotic abnormalities were confirmed from observations made on several pollen mother cells (PMCs) at different stages in each population.

Pollen studies were carried out on voucher specimens of cytologically worked out taxa. For determining the pollen fertility, mature pollen grains were treated with 1:1 glycerol-acetocarmine. Well filled and uniformly stained pollen grains were scored as apparently fertile while shriveled and unstained pollen were counted as apparently sterile.

Photomicrographs were taken from the temporary mounts using a Magnus MLX Plus Microscope. Field photographs of plants are taken and voucher specimens would be deposited in Punjabi University Herbarium (PUN), Patiala.

### Results

Presently the chromosome number through meiotic studies along with detailed meiotic course has been studied in 103 taxa belonging to 50 species of Cyperaceae (Plate 1-5). The data regarding the species, locality with altitude, chromosome number and Pollen fertility is given in table 1.

Meiotic abnormalities like chromatin bridges, cytomixis, laggards, stickiness and reduced pollen fertility were observed. Chromatin bridges and laggards were seen in four species: *Cyperus tenuispica*, *C. rotundus*, *Kyllingia brevifolia* and *Schoenoplectus mucronatus*. Cytomixis is recorded in *Cyperus paniceus*. Eight species: *Cyperus alternifolius*, *C. alulatus*, *C. cuspidatus*, *C. iria*, *C. tenuispica*, *Kyllingia brevifolia*, *Eriophorum comosum*, and *Fimbristylis miliacea*.

**Table 1. Data regarding the species, Locality with altitude from where plant was collected, chromosome number, Pollen fertility and remarks.**

S. No.	Species	Locality with altitude (m)	Chromosome number (n)	Pollen fertility%	Remarks
<b>Tribe: Cyperaceae</b>					
1	<i>Cyperus alternifolius</i> Linn.				
	POP 1	Punjab: Patiala (254)	16	86.40	Stickiness
	POP 2	Punjab: Rajpura (254)	16	87.65	
	POP 3	Punjab: Gobind garh (268)	16	90.52	
2	<i>Cyperus alulatus</i> Kern				
	POP1	Punjab: Patiala (254)	40	70.00	Stickiness
	POP2	Uttrakhand: Uttarkashi (1158)	57	78.68	
3	<i>Cyperus compactus</i> (Gaert.) Endl.				
		Punjab: Patiala (251)	21	88.45	
4	<i>Cyperus compressus</i> Linn.				
	POP 1	Punjab: Patiala (350)	24	95.80	
	POP 2	Uttrakhand: Uttarkashi Agora (2250)	24	91.23	
5	<i>Cyperus cuspidatus</i> Kunth				
		Uttrakhand: Uttarakshi, Tiuni (1200)	28	76.85	Stickiness
6	<i>Cyperus cyperoides</i> (Linn.) Kuntze				
		Himachal Pradesh: Shimla (2276)	41	76.87	
7	<i>Cyperus difformis</i> Linn.				
	POP 1	Punjab: Patiala (251)	17	81.54	
	POP 2	Punjab: Ferozpur (190)	18	80.45	
	POP 3	Punjab: Ludhiana (262)	18	80.50	
8	<i>Cyperus digitatus</i> Roxb.				
	POP 1	Punjab: Jalandhar (260)	54	88.75	
	POP 2	Punjab: Ludhiana, Nillon (260)	27		
9	<i>Cyperus exaltatus</i>				
	POP 1	Punjab: Ludhiana (262)	24	89.50	
	POP 2	Punjab: Machiwara (262)	24	87.45	
10	<i>Cyperus fuscus</i> Linn.				
	POP 1	Himachal Pradesh: Palampur (1250)	24	85.40	
	POP 2	Uttrakhand: Uttarkashi, Agora (2250)	24	87.65	
11	<i>Cyperus globsus</i> All.				
		Punjab: Sangrur (230)	42	96.76	
12	<i>Cyperus iria</i> Linn.				



	POP1	Punjab: Machhiwara (262)	29	76.47	Stickiness
	POP 2	Punjab: Firozpur, MAllanwala (198)	29	80.50	
13	<i>Cyperus kyllingia</i> Endl.				
	POP 1	Punjab: Patiala (350)	28	83.69	
	POP 2	Himachal Pradesh: Palampur (1472)	28	85.54	
	POP 3	Uttarakhand: Uttarkashi, Agora (2250)	28	90.63	
14	<i>Cyperus laevigatus</i>				
		Punjab: Ropar (275)	21	81.34	
15	<i>Cyperus niveus</i> Retz.				
		Punjab: Ropar (275)	34	70.00	
	<i>Cyperus paniceus</i> (Rottb.) Boeck.				
	POP1	Punjab:: Patiala, Bahadurgarh (350)	42	93.56	
	POP2	Uttarakhand: Uttarkashi, Chirngi (1200)	21	90.55	
	POP 3	Himachal Pradesh: Palampur (1472)	21		Cytomixis
17	<i>Cyperus pygmaeus</i> Retz.				
		Uttarakhand: Uttarkashi, Agora (2250)	27	93.58	
18	<i>Cyperus rotundus</i> Linn.				
	POP1	Punjab:Ludhiana (250)	40	65.71	Micronuclei, Bridges present
	POP2	Punjab:Patiala (254)	56	72.46	
	POP3	Punjab:Patiala (254)	48	86.65	
	POP4	Himachal Pradesh: Nahan(1000)	55	81.74	
	POP5	Punjab:Sangrur(251)	50	74.87	
	POP6	Punjab:Jalandhar(260)	52	84.86	
	POP7	Punjab:Patiala (254)	54	88.75	
19	<i>Cyperus squarrosus</i> Linn.				
		Punjab: Patiala (254)	49	93.65	
20	<i>Cyperus tenuispica</i> Steud.				
	POP1	Uttarakhand: Parola (1200)	7	72.81	Bridges and Laggards
	POP2	Himachal Pradesh: Palampur (1472)	8	92.00	
	POP3	Himachal Pradesh: Dalhousie (1972)	8	89.67	
21	<i>Kyllinga brevifolia</i> (Rottb.) Endl.				
	POP 1	Punjab: Patiala (254)	30	87.57	Laggards, Bridges

	POP 2	Uttrakhand : Uttrakashi, Agora (2250)	30	79.65	Laggards
22	<i>Pycnus pumilus</i> (L.) Nees	Himachal Pradesh: Shimla (2276)	29	97.56	
23	<i>Carex filicina</i> Nees	Himachal Pradesh: Dharamsala (1457)	18	63.05	
24	<i>Carex foliosa</i> D. Don	Himachal Pradesh: Shimla (2276)	28	68.90	
25	<i>Carex nubigena</i> D. Don ex Tilloch & Taylor	Himachal Pradesh: Shimla (2276)	26	74.11	
26	<i>Carex fedia</i> Nees	Haryana: Karnal (350)	52	85.76	
27	<i>Carex remota</i> Linn.	Uttrakhand: Uttarkashi, Agora (2250)	31	84.56	
28	<i>Carex sp.</i>	Uttrakhand : Uttarkashi, Dharkot (2250)	17	89.65	
29	<i>Carex Sp.</i>	Uttrakhand: Uttarkashi, Chirngi (1291)	21	87.65	
30	<i>Carex Sp.</i>	Himachal Pradesh: Dharmshala, Dharnu (2082)	31	76.74	
31	<i>Carex Sp.</i>	Himachal Pradesh: Chamba, Bathri (489)	27	87.53	
32	<i>Carex sp.</i>	Uttrakhand : Uttarkashi ,Dodital (3310)	24	91.34	
33	<i>Carex sp.</i>	Uttrakhand: Uttarkashi road (1158)	13	90.46	
34	<i>Carex sp.</i>	Uttrakhand: Uttrakashi,Manjhi (2250)	21	89.64	
<b>Tribe: Scirpeae</b>					
35	<i>Bulbostylis barbata</i> Kunth				

	Uttrakhand: Uttarkashi Tiuni (1200)	5	73.57	
36	<i>Bulbostylis capillaries</i> (L.) Kunth ex C. B. Clarke			
	POP1 Uttrakhand: Uttarkashi Agora (2250)	22	85.75	
	POP2 Himachal Pradesh: Sirmaur (995)	22	89.56	
	<i>Eleocharis atropurpurea</i> (Retz.) Kunth			
	POP1 Himachal Pradesh: Palampur (1472)	10	81.31	
	POP2 Punjab: Patiala (250)	10	79	
	<i>Eleocharis congesta</i> D. Don			
	POP 1 Himachal Pradesh: Dharamshala Chambi (902)	10	87.45	Pollen mitosis
	POP 2 Uttrakhand: Uttarkashi, chirngi (2242)	10	85.53	
	POP 3 Himachal Pradesh: Dalhousie, khajjiar (1800)	10	87.50	
39	<i>Eleocharis palustris</i> (L.) R.Br. Punjab;Patiala (251)	8+1B	89.67	B-chromosome
40	<i>Eriophorum comosum</i> Wall. ex Nees			
	POP1 Himachal Pradesh: Narkanda (2710)	26	81.48	Stickiness
	POP2 Uttrakhand: Uttrkashi (1190)	26	80.54	
41	<i>Fimbristylis bisumbellata</i> (Forsk.) Bubani			
	POP1 Harayana: Dosarka (264)	5	77.58	Pollen mitosis
	POP2 Punjab: Anandpur Sahib(310)	5	88.23	
	POP3 Punjab: Patiala (254)	5	85.42	
	POP4 Punjab: Fridkot(205)	5	93.23	
	POP5 Punjab: Jalandhar (239)	5	93.45	
	POP6 Uttarkashi: Uttrakhand Agora (2250)	5	96.32	
41	<i>Fimbristylis dichotoma</i> (Linn.) Vahl			
	POP1 Punjab: Patiala (254)	10	84.11	Pollen mitosis
	POP2 Uttrakhand: Uttarkashi: Purola (1200)	10	86.12	
	POP3 Punjab: Zirkpur (250)	10	84.56	
	POP4 Punjab: Nangal(600)	10	96.86	
	POP5 Himachal Pradesh: Dharamshala (1480)	10	87.66	
	POP 6 Uttrakhand: Uttarkashi, Agora (2250)	10	87.67	
43	<i>Fimbristylis falcate</i> (Vahl) Kunth			

		Punjab: Jalandhar (260)	11	63	Pollen mitosis
44	<i>Fimbristylis miliacea</i> Vahl				
	POP1	Himachal Pradesh: Palampur (1472)	5	89.12	Stickiness Pollen mitosis
	POP2	Punjab: Patiala (254)	5	93	
	POP 3	Punjab Ludhiana (260)	5	96.56	
	POP 4	Himachal Pradesh Dalhousie Bathri (1557)	5	87.75	
	<i>Fimbristylis quinquangularis</i> (Vahl) Kunth				
	POP1	Himachal Pradesh: Palampur (1472)	5	92.52	Pollen mitosis
	POP2	Punjab: Patiala (251)	5	93	Pollen mitosis
	POP3	Punjab: Ropar (272)	5	91	
	POP4	Punjab: Firozpur, Mallan vala (198)	5	87.55	
	POP5	Punjab: Ludhiana (260)	5	89.60	
	POP 6	Haryana: Ambala (275)	5	90.34	Pollen mitosis
46	<i>Schoenoplectus mucronatus</i> (L.) J. Jung & H. K. Choi				
		Himachal Pradesh: Dalhousie, khajjiar (1800)	21	90.53	Laggard, Chromatin bridges, unoriented bivalents
47	<i>Scirpus roylei</i> (Nees) Parker				
	POP1	Punjab: Machhiwara (262)	11	72.60	Laggard
	POP2	Himachal Pradesh: Palampur (1472)	11	87.21	
	POP 3	Himachal Pradesh Dharamshala (1300)	36	80.34	Polyploid
	POP 4	Punjab: Ludhiana (260)	11	89.24	
48	<i>Scirpus supinus</i> Linn.				
		Punjab: Machhiwara (262)	14	74.00	
49	<i>Scirpus triqueter</i> Linn.				
		Jammu and Kashmir: Ganderbal (1620)	21	81.34	
50	<i>Scirpus tuberosus</i> Desf.				
	POP1	Punjab: Patiala, Rajpura (251)	26	91.74	
	POP2	Punjab: Patiala (251)	24	89.58	
	POP3	Punjab: Firozpur, MallanWala (180)	26	87.56	
	POP4	Haryana: Morni Hills (1074)	27	90.76	
	POP 5	Punjab: Ropar, Nurpur bedi (272)	27	87.42	

## 2. KARYOTYPIC STUDIES

For karyotype analysis, White (1945) and Stebbins (1971) was followed. Karyomorphological studies on 5 species of genus *Fimbristylis*, namely *F. bisumbellata* (n=5), *F. dichotoma* (n=10), *F. falcate* (n=11), *F. miliacea* (n=5), and *F. quinqueangularis* (n=5) genus have been made. The parameters like position of centromere, secondary constrictions, haploid chromatin length, absolute and average chromosome size and the type of symmetry based on GI and SI are used. Karyotypic differences have been noticed in *F. bisumbellata*, *F. dichotoma*, and *F. quinqueangularis* on population basis and in *F. bisumbellata* even on individual basis within the same population. This indicates the evolution at micro level.

## III. MORPHOMETRIC ANALYSIS

Morphometric analysis in *Cyperus alulatus*, *C. paniceus*, *C. difformis*, *C. digitatus*, *C. tenuispica*, *C. rotundus*, *Scirpus roylei* and *S. tuberosus* had been done based on plant height, leaf characters, inflorescence characters, etc. was done in species showing intraspecific number chromosomal variations. Detailed comparisons of different morphological traits of various variants of *Cyperus tenuispica* (n=7 and n=8) are provided in Fig 4 and Table. 2.

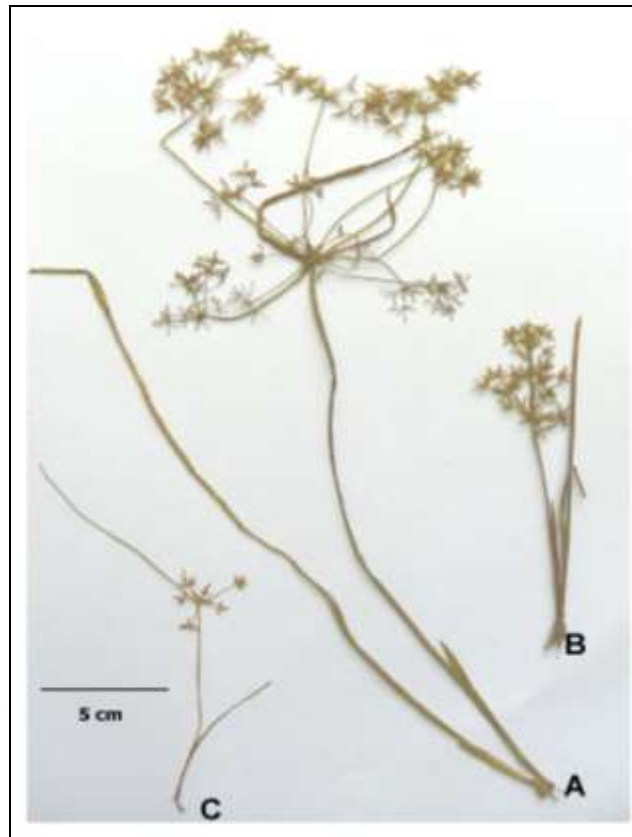


Fig. 4. Plants of *C. tenuispica* from Pop. I (A), Pop. II (B) and Pop. III (C), respectively.

**Table 2.** Cytomorphological comparison of three populations of *Cyperus tenuispica*.

Population	Large Sized		Small Sized
	Pop. I	Pop. II	Pop. III
Locality	HP: Palampur, Gopalpur	HP: Dalhousie Bathri	UK:Uttarakshi, Prola
Altitude	1472 m	1900 m	1220 m
PUN accession number	62160	62161	62162
<b>Chromosome Number</b>	<i>n</i> =8	<i>n</i> =8	<i>n</i> =7
<b>Plant Height (cm)</b>	30.23±3.92	11.7±1.66	8.93±0.94
<b>Culm</b>			
Length (cm)	19.75±3.52	7.50±0.96	7.28±0.90
Diameter (mm)	2	1	1
<b>Leaf</b>			
Length of leaf (cm)	6.30±1.40	3.10±0.73	4.58±1.72
Width of leaf (mm)	3	2	1
<b>Inflorescence</b>			
Length of involucre bract (cm)	7.30±1.49	4.75±1.22	5.75±1.55

Width of involucre bract (mm)	2	2	1
Number of rays	14±3	9±2	7±1
Length of ray (cm)	8.88±1.93	3.38±0.50	1.45±0.13
Number of spikelets per ray	11±4	10±2	7±3
Length of spikelet (cm)	0.45±0.13	0.45±0.13	0.33±0.06
Number of glumes per spikelet	24±3	24±3	15±3
Length of glume (mm)	0.85±0.01	0.85±0.04	0.95±0.01
<b>Pollen</b>			
Size (µm)	18.74×15.56	18.56 ×16.78	14.80 ×12.65
Fertility %	90.25	89.33	72.65

#### IV. GENETIC DIVERSITY

In order to evaluate the genetic diversity in accessions of *Cyperus rotundus* and *Scirpus tuberosus* DNA markers were used. Different molecular markers such as random amplified polymorphic DNA (RAPD) for assessment of genetic diversity at intraspecific level. The detailed protocol is as follows:

##### DNA Extraction

Genomic DNA was isolated from young dry leaves of four accessions of *C. rotundus* and two accessions of *S. tuberosus* collected from different localities in Punjab. 100 mg of dry leaves were taken and grind with pestle and mortar with liquid nitrogen to make fine powder. For further DNA extraction HipurA™ Plant Genomic DNA Miniprep Purification Spin Kit (HIMEDIA) was used and followed the procedure for dry material. Extracted DNA was stored at -20°C for further use.

##### DNA Quantification

The extracted DNA was checked by running samples on 0.8% agarose gel prepared in TAE buffer. The solution of gel was boiled and then cooled to 50°C and to this Ethidium bromide (0.5µg/mL of gel) was added and shaken well. Transfer the solution of gel in to casting tray, fixed the comb and allowed it to solidify. After solidification removed the comb and then shifted the tray to electrophoresis chamber filled with TAE buffer.

Load 5µl of DNA mixed with 2µl of Bromophenol blue in the wells of gel and run the electrophoresis after fixing the chamber with electric supply and after one hour presence of DNA was quantified by spectrophotometer. Single intact bands near the wells confirmed the presence of DNA.

**Table 3. PCR reaction mixture for DNA amplification in *C. rotundus***

S. No.	Ingredient	Amount (µL)
1	sterilized water	39
2	<i>Taq</i> buffer	3
3	dNTP	2
4	Forward primer	1
5	Reverse primer	1
6	DNA	2
7	<i>Taq</i> polymerase (5U/ µL)	2

### PCR Thermal cycling conditions

PCR machine thermal cycler (MyCycler™, Bio-rad) was set at following different parameters-

**Table 4. PCR amplification reaction using different primers for *C. rotundus***

Initial denaturation	Annealing	Extension	Final hold
94°C- 5min	94°C- 1min } 30cycles 60°C- 1min } 72°C- 1min45 sec	72°C- 10min	4°C- infinity

### Agarose gel electrophoresis

Electrophoresis was performed for 2-3 hours using 1.5% agarose gel loaded with 12µL PCR product mixed with 2 µL of Bromophenol. 100bp ladder (GENEI MERCK) was used as reference. Ethidium bromide was used at the concentration of 0.5 µg/ml of gel to visualize the bands and photographs of the gel were taken using UV light in BioRad Gel DOC System.



**RAPD markers tested:** DNA amplification of purified DNA for RAPD analysis was conducted using 20 random primers obtained from Bangalore GeNei™. Primarily, all the 20 primers were tested in one population of plant for RAPD amplification. The primers giving polymorphic bands were selected for scoring.

### **Results:**

- RAPD markers did not revealed significant results
- It is a case of applying new markers especially ISSR, ITS and rbcl.

## **V. PUBLICATIONS**

1. Cheema,P; Saggoo, M.I.S. and Kumar, N. 2017. Cytomorphology of some medicinal sedges from North West India. International Journal of Pharmacy and Pharmaceutical Research. 10 (2): 231-243.
2. Cheema, P; Kumar, N and Saggoo M.I.S. Aneuploidy in *Cyperus tenuispica* from North India. Cytologia 83 (4): Accepted

## **VI. PAPER PRESENTATION IN CONFERENCES/ SEMINAR**

1. Chromosomal status of some medicinal sedges from North Western Himalayas in National conference on Basic and Applied Researches in Plants and Microbes at Punjabi University, Patiala from November 3-5, 2016.
2. An overview of chromosomal diversity in cytologically investigated species of *Fimbristylis* Vahl in National conference on Basic and Applied Researches in Plants and Microbes at Punjabi University, Patiala from November 3-5, 2016.

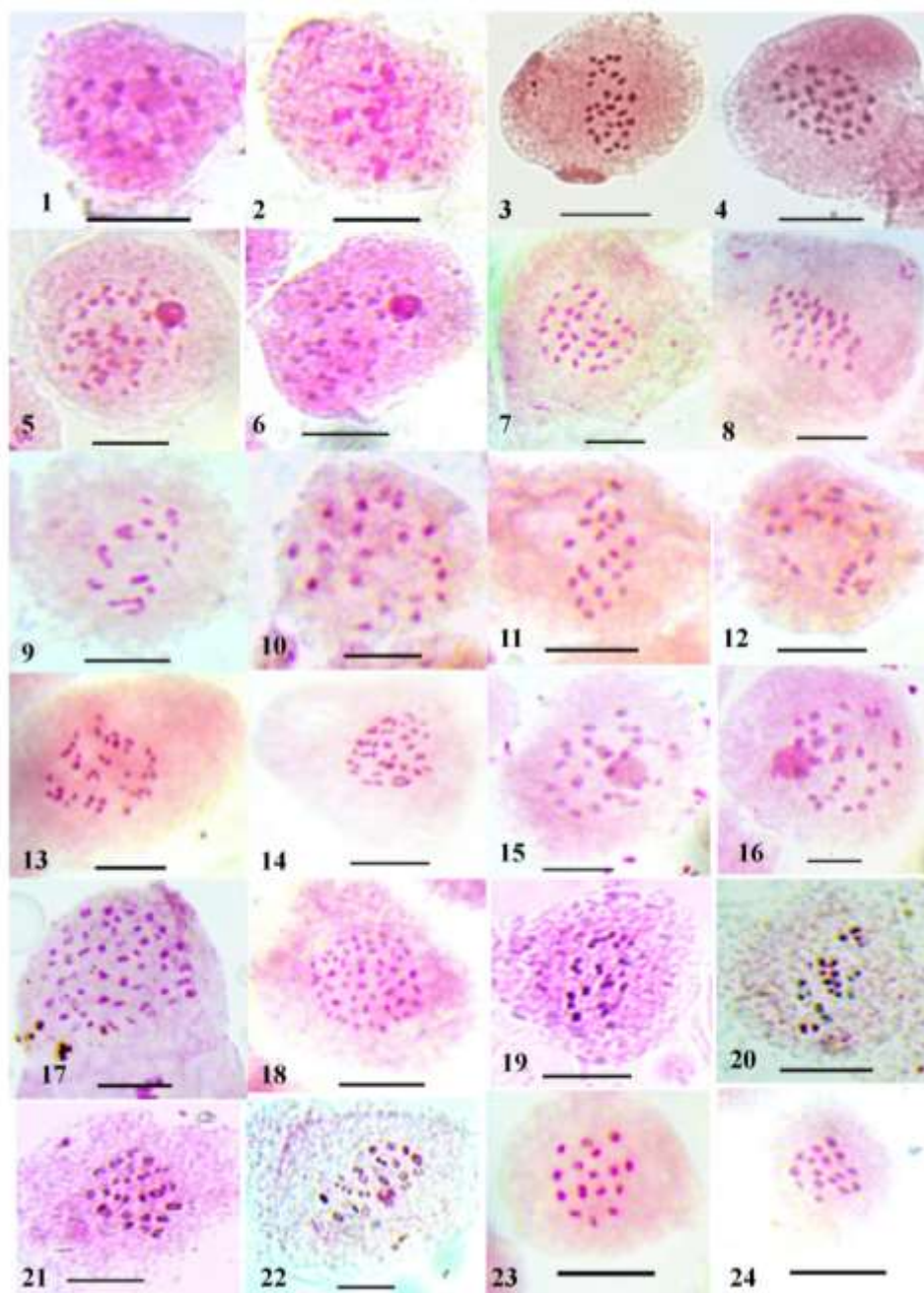
3. Karyomorphology of *Fimbristylis* Vahl from Punjab, North Western India in Swarna Jayanti National conference on Biodiversity and Sustainable Development at Kurukshetra University, Kurukshetra from 17-18 February 2017.
4. Cytomorphological diversity in some sedges (Cyperaceae) from North Western Himalayas in Swarna Jayanti National conference on Biodiversity and Sustainable Development at Kurukshetra University, Kurukshetra from 17-18 February 2017.
5. Review of *Cyperus rotundus* Linn.- Common Nut Sedge in 40<sup>th</sup> All India Botanical Conference of The Indian Botanical society & National symposium of Evaluation and Conservation of Plant Germplasm at Punjabi University, Patiala from 15-17 September, 2017.
6. Cytomorphology and ethanobotanical notes of some Sedges from North West India in 40<sup>th</sup> All India Botanical Conference of The Indian Botanical society & National symposium of Evaluation and Conservation of Plant Germplasm at Punjabi University, Patiala from 15-17 September, 2017.
7. Male meiosis in cytomorphotype of *Cyperus tenuispica* Steud. from North India in National conference research in plant sciences for sustainable development at Sri Guru Granth Sahib World University, Fatehgarh Sahib from 16-17 March, 2018.
8. Nutsedge : Weed with Tremendous Therapeutic Potential in DBT sponsored National Conference on Recent Trends in Plant Sciences at Sanmati Government College of Science Education and Research, Jagraon from 19-20 April, 2018.

## **VII. WORKSHOP ATTENDED**

Workshop on Systematics of Plants and Microbes organized by Department of Botany, PU Patiala from December 20- 26, 2015.

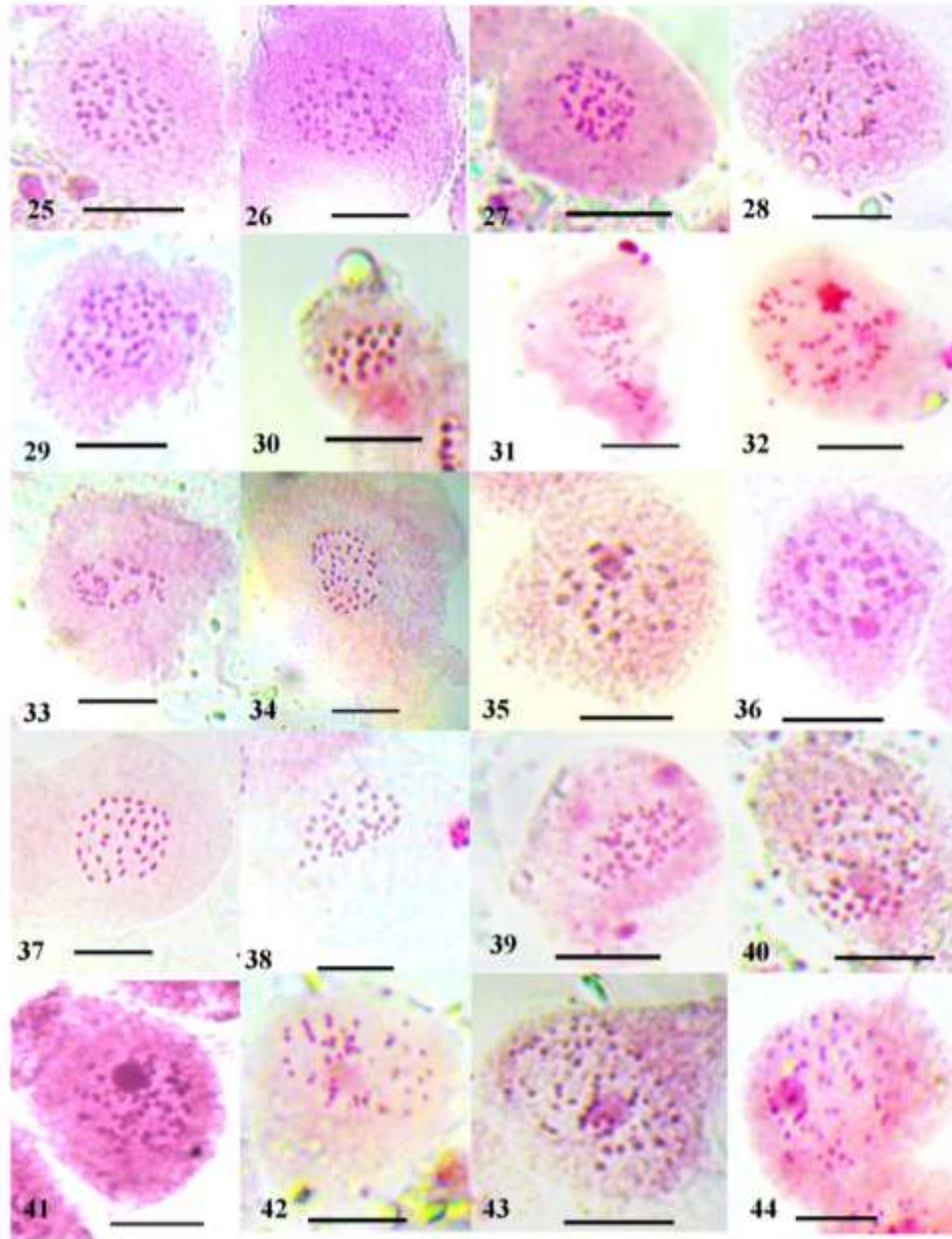
## **VIII. MAN POWER TRAINED**

One research fellow Mr. Neeraj Kumar was enrolled for Ph.D programme of Punjabi University, Patiala on September 16, 2016. He has completed course work at Botany Department, Punjabi University, Patiala and is registered for Ph.D. on 9/2/2018 (Registration no. PU(P) 2013-629).

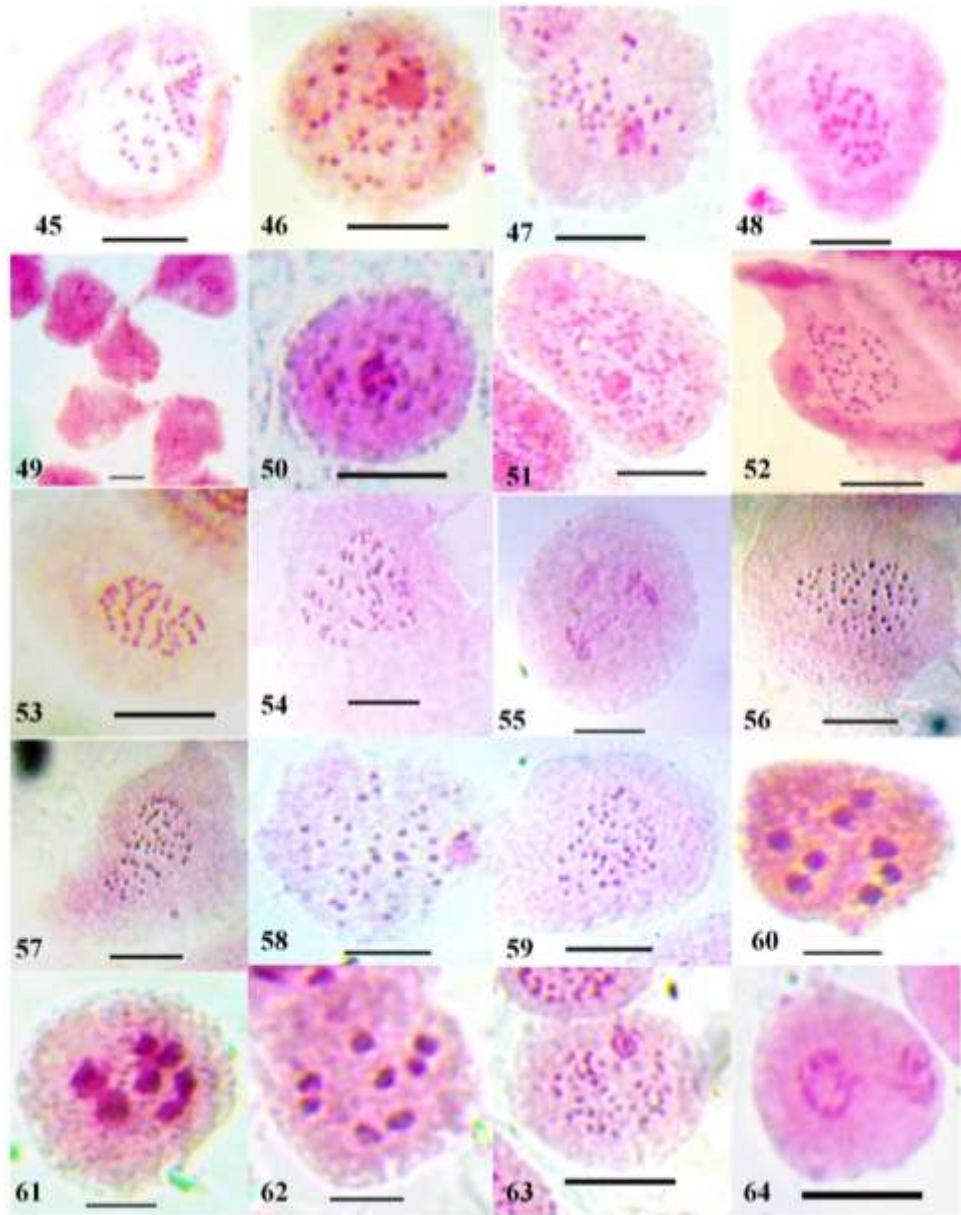


**Plate 1** *Carex filicina* (Fig. 1 and 2) showing n=18 at Diakinesis stage; *Carex foliosa* (Fig. 3 and 4) showing n=28 at Metaphase; *Carex nubigena* (Fig. 5 and 6) showing n=26 at diakinesis stage; *Carex remota* (Fig. 7 and 8) showing

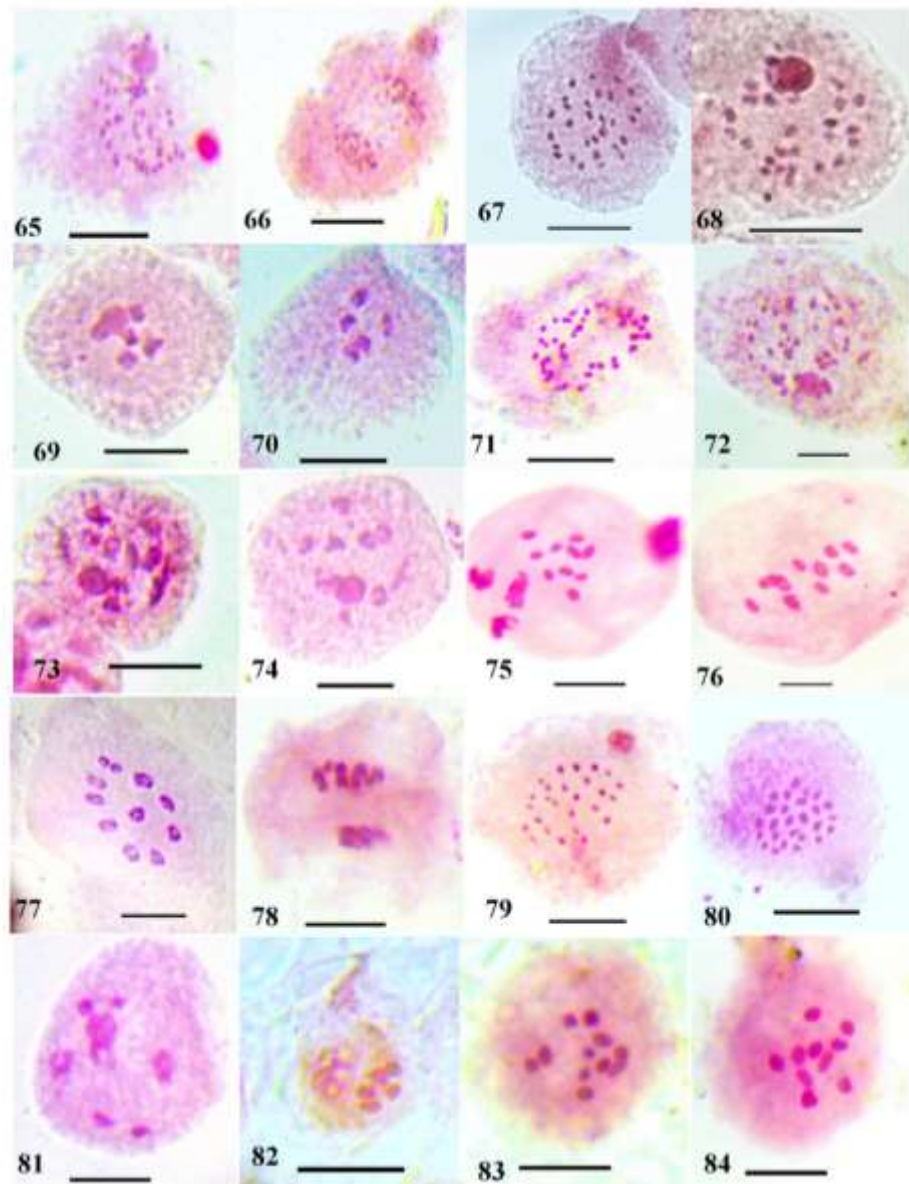
n=31 at Metaphsae; *Carex sp.* (Fig. 9 and 10) showing n=17 at Metaphase; *Carex sp.* (Fig. 11 and 12) showing n=21 at Metaphase; *Carex sp.* (Fig. 13 and 14) showing n=31 at metaphase; *Carex sp.* (Fig. 15 and 16) showing n=27 at Diakinesis stage; *Carex sp.* (Fig. 17 and 18) showing n=24 at Metaphase; *Carex sp.* (Fig. 19 and 20) showing n=13 Metaphase; *Carex sp.* (Fig. 21 and 22) showing n=21 Metaphase stage; *Cyperus alternifolius* showing n=16 at Metaphase I (Fig. 23; Gobindgarh and Fig. 24 ; Rajpura)



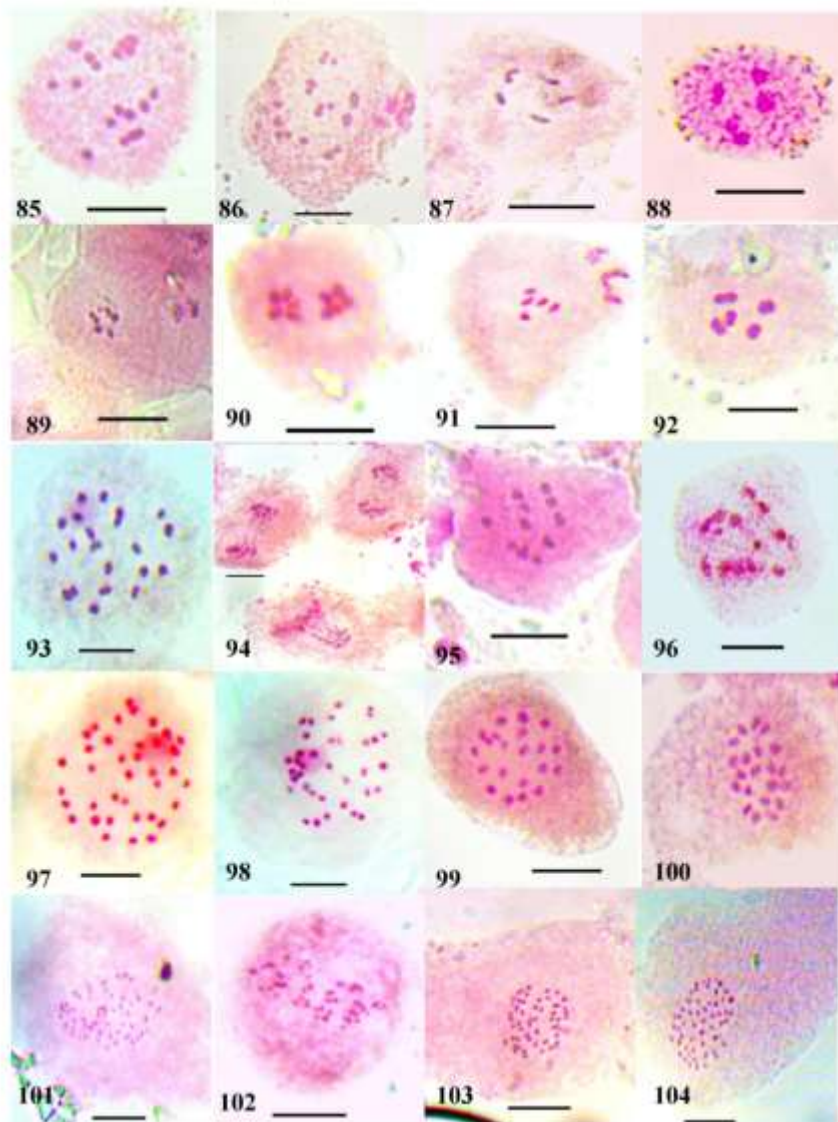
**Plate 2** *Cyperus alulatus* showing (Fig. 25; Patiala n=40 at M-I and Fig. 26; Uttarkashi n=57 at M-I). *Cyperus compressus* showing n=24 at Metaphase I (Fig. 27; Patiala and 28; Uttarkashi). *Cyperus cuspidatus* showing n=28 M-I stage (Fig. 29). *Cyperus difformis* showing n=18 M-I stage (Fig. 30). *Cyperus digitatus* (Fig. 31 and 32) showing *Cyperus exaltatus* showing n=24 at M-I (Fig. 33; Machiwara and Fig. 34 Ludhiana). *Cyperus fuscus* showing n=24 at Diakinesis (Fig. 35; Palampur and Fig. 36; Uttarkashi). *Cyperus globsus* showing n=42 at M-I (Fig. 37 and 38). *Cyperus iria* showing n=29 (Fig. 39; Machiwara at M-I and Fig. 40; Firozpur at diakinesis). *Cyperus kyllingia* showing n=28 at Diakinesis (Fig. 41; Patiala, Figs. 42, 43; Palampur and Fig. 44; Uttarkashi)



**Plate 3** *Cyperus laevigatus* showing n=21 (Fig. 45 at M-I and Fig. 46 at Diakinesis) *Cyperus niveus* showing n=34 at Diakinesis (Fig. 47). *Cyperus paniceus* showing (Fig. 48; Palampur n=21 at M-I, Fig. 49; Cytomixis, Fig. 50; Uttarkashi n=21 at Diakinesis and Fig. 51; Patiala n=42 at diakinesis) *Cyperus pygmaeus* showing n=27 at M-I (Fig. 52 and 53). *Cyperus rotundus* showing (Fig. 54; Ludhiana n=40, Fig. 55 Chromatin bridge at A-I, Fig. 56; Sangrur n=48 at M-I, Fig. 57; Ropar n=55 at M-I, Fig. 58; Nahar n=52 at Diakinesis and Fig. 59; Jalandhar n=48 at M-I). *Cyperus tenuispica* showing (Fig. 60; Palampur n=8 at M-I, Fig. 61; Dalhousie n=8 at M-I and Fig. 62; Uttarkashi n=7 At M-I). *Kyllinga brevifolia* showing (Fig. 63; Patiala n=30 at Diakinesis and Fig. 64; Chromatin Bridge)



**Plate 4** *Kyllinga brevifolia* showing (Fig. 65; Uttarkashi n=30 at Diakinesis and Fig. 66; laggards). *Pycreus pumilus* showing n=29 (Fig. 67 at M-I and Fig. 68 at Diakinesis). *Bulbostylis barbata* showing n=5 (Fig. 69 at Diakinesis and Fig.70 at M-I). *Bulbostylis juncooides* showing n=22 (Fig. 71; Uttarkashi at M-I and Fig.72; Sirmaur at diakinesis). *Eleocharis atropurpurea* showing n=10 at Diakinesis (Fig. 73 and 74). *Eleocharis congesta* showing n=10 (Fig. 75; Dharamshala at Pollen mitotic stage, Fig. 76; at M-I, Fig. 77; Uttarkashi at M-I and Fig. 78; Dalhousie at A-I). *Eriophorum comosum* showing n=26 at M-I (Fig. 79; Narkanda and Fig. 80; Uttarkashi). *Fimbristylis bisumbellata* showing n=5 (Fig. 81; Anandpur Sahib at Diakinesis and Fig. 82; Uttarkashi at A-I). *Fimbristylis dichotoma* showing n=10 at M-I (Fig. 83; Uttarkashi, Fig. 84; Dharmshala).



**Plate 5** *Fimbristylis dichotoma* showing  $n=10$  at A-1 (Fig. 85; Patiala and Fig. 86; Nangal). *Fimbristylis miliacea* showing  $n=5$  (Fig. 87; Ludhiana at Pollen mitotic stage and Fig. 88; Palampur at M-I). *Fimbristylis quinquangularis* showing  $n=5$  (Fig. 89; Patiala at A-I, Fig. 90; Ludhiana at A-, Fig. 91; Firozpur at Pollen Mitotic phase and Fig. 92; Ropar at M-1) *Schoenoplectus mucronatus* showing  $n= 21$ (Fig. 93 at Diakinesis and Fig. 94 Chromatin bridges). *Scirpus roylei* showing ( Fig. 95; Machiwara  $n=11$  at M-1 and Fig. 96; Ambala  $n=11$  at M-I, Figs. 97 and 98; Dharamshala  $n= 36$  at Diakinesis). *Scirpus triqueter* showing  $n= 21$  at M-I (Fig. 99 and 100). *Scirpus tuberosus* showing (Fig. 99; Rajpura  $n=26$  at M-I, Fig. 100; Patiala  $n=24$  at M-I, Fig.101; Morni Hills  $n=27$  at M-I and Fig.102; Ropar  $n=27$  at M-I).