

National Mission on Himalayan Studies (NMHS)

PERFORMA FOR THE QUARTERLY PROGRESS REPORT

(Reporting Period *from April to June 2020.*)

1. Project Information

Project ID	NMHS/2017-18/MG43/27
Project Title	Ecology, monitoring, recovery and bio-prospection of some threatened plant species of Jammu, Kashmir and Ladakh in relation to regional socio-economic development
Project Proponent	Dr. Manzoor A. Shah Department of Botany University of Kashmir Srinagar, J&K-190 006
Co-Investigators	Prof. Namrata Sharma Department of Botany University of Jammu Dr Aijaz H Ganaie Assistant Professor Kargil Campus Department of Botany University of Kashmir

2. Objectives

- Assessment of traditional uses, population status, habitat characteristics, biotic interactions and causes of rarity of the target species;
- To predict suitable habitats through species distribution modeling vis-à-vis the impact of climate change;
- To recover the target species through active participation of local communities;
- Bioprospection of target species for value added products;
- Mass awareness of mountain communities through conservation education for upscaling the propagation and utilization.

3. General Conditions

- The project must be implemented in consultation with the State government.
- Biology and conservation methodologies of threatened species as per IUCN need to be incorporated.
- A report based on baseline data of all selected species in 3 zones should be submitted by the project proponent in first quarter of the project.
- The Periodic Progress Report of the NMHS Project needs to be submitted and updated on the Online Portal of the NMHS (<http://nmhsportal.org>) by the PI/ Project Proponent on Quarterly basis consistently. Monitoring indicators for the project should be able to quantify the difference made on ground.
- A Certificate should be provided that this work is not the repeat of earlier work (as a mandatory exercise).

- The roles and responsibilities of each implementing partners should be delineated properly with their budget. The budget allocations to partners should be done in accordance with the MoEF&CC guidelines (Max. 30% for salary, 30% for equipment and 5% contingency). The same should be communicated to NMHS-PMU, before start of the project.
- The PI must also submit the baseline data, past work done and all the supporting data generated under the NMHS Project along with the quarterly progress report to NMHS-PMU.
- On completion of the study, a Seminar/ Conference/ Workshop should essentially be organized by the PI/ Proponent to discuss and disseminate the findings among the experts and concerned beneficiaries/stakeholders.

4. **Progress (fill only 3rd and 4th Column)**

Quantifiable Deliverables	Monitoring indicators	Progress made against deliverables in terms of monitoring indicators	Supportive documents (PDF, Word, Excel, JPG, TIFT)
1	2	3	4
<ul style="list-style-type: none"> • Knowledge products on the actual population status of the target rare species in 3 regions i.e., Ladakh, Jammu & Kashmir; • Predictive model for species distribution in the context of projected climate change for 3 regions in state; • Improved conservation status of threatened target species through participatory community involvement in all 3 regions of J&K. 	<ul style="list-style-type: none"> • No. of New Database/ Datasets generated on the identified dynamics, i.e. threatened plant species linking with Livelihood Options (No. of species) 	<p>Threat status of three target plant species including (A) <i>Eremurus persicus</i> (B) <i>Gloriosa superba</i> and (C) <i>Acorus calamus</i>.</p> <p>Data on Phenological events, plant and floral morphometry of <i>Eremurus persicus</i> was recorded.</p> <p>cytological studies and meiotic behavior of the <i>Eremurus persicus</i> was carried out.</p> <p>Pollination behavior of <i>E. persicus</i> was noticed.</p>	<p>Appendix I</p>
	<ul style="list-style-type: none"> • Regular, periodic updates on region-specific best practices/ models developed (No.) 	<p>Threat status maps generated.</p>	<p>Figure 2</p>

	<ul style="list-style-type: none"> • No. of Stakeholders benefitted/ trained (No. of Rural Youth, No. of Women, and Total No. of Beneficiaries) 	<p>Already two workshops involving different stakeholders was done in the first year. The programs involving knowledge based training did not happen because of Covid19 pandemic and will be done in near future</p>	
	<ul style="list-style-type: none"> • Other Publications and knowledge products (Nos.) 	<p>In progress.</p>	

5. Financial Progress (fill only 3rd and 4th Column)

Head	Total Budget Allocated (Rs)	The Balance Amount (Rs)	Supportive documents (PDF, Word, Excel, JPG, TIFT)
1	2	3	4
Salary			
Travel			
Consumables			
Contingency			
Activities & other project cost			
Institutional Charges			
Equipments			
Total	6194200.00	3926356.00	

UC Submitted YES (2nd year)

*The financial statements will be sent with final UC

Appendix I

Work done from April to June, 2020

During the last 3 months (**April to June**, 2020) the summary of work is given as under:

- New sites/locations of target plants and actual threats to the plant species have been recorded.
- Threat status of three target plant species including (A) *Eremurus persicus* (B) *Gloriosa superba* and (C) *Acorus calamus*.
- Data on Phenological events, plant and floral morphometry of *Eremurus persicus* was recorded.
- cytological studies and meiotic behavior of the *Eremurus persicus* was carried out.
- Pollination behavior of *Eremurus persicus* was noticed.

Selected plant species

Eight species have been selected for the present study, three each from Kashmir and Jammu and two species from Ladakh (Table 1). Most (5 species) of the selected plant species are monocots and 3 species are dicots. All the selected species are native and two species namely: *Trillium govanianum* Wall. ex D. Don and *Rheum tibeticum* Maxim. ex Hook. f. are endemic to Himalayas.

Table 1: List of selected plant species

Region	Species	Family	Plant Group
Kashmir	<i>Euryale ferox</i> Salisb.	Nymphaeaceae	Monocot
	<i>Acorus calamus</i> L.	Acoraceae	Monocot
	<i>Trillium govanianum</i> Wall. ex D. Don	Trilliaceae	Monocot
Jammu	<i>Eremurus persicus</i> (Jaub. & Spach) Boiss.	Asphodelacea	Monocot
	<i>Gloriosa superba</i> L.	Liliacea	Monocot
	<i>Crotalaria mysorensis</i> Roth	Fabaceae	Dicot
Ladakh	<i>Rheum tibeticum</i> Maxim. ex Hook. f.	Polygonaceae	Dicot
	<i>Arnebia euchroma</i> (Royle) I.M.Johnst.	Boraginaceae	Dicot

Field survey

Methodology

Survey and collection

Extensive survey has been carried out in the Jammu region of the J&K state in order to collect the selected plant species and to record their distribution.

Record of Population structure

During field surveys the size, extent, habit, habitat and life form of selected plant species has been recorded following the standard methodology. Mature individuals have also been counted in each site, only those individuals which bear flowers or fruits were counted as mature (IUCN, 2010).

Areas surveyed and the selected plant species collected from various sites

In the present study new collection sites have been recorded in three regions of J&K state where the selected plant species are growing making a total of Collection sites so far. The geographical co-ordinates of the selected site were recorded using geographical positioning system (GPS) (Table 2).

Table 2: New collection sites with geo-coordinates

Plant species	Collection site	Latitude(N)	Longitude(E)	Altitude (masl)
<i>Eremurus persicus</i> (Jaub & Spach,) Boiss.	Sarmega II	33°07'46''	074°51'00''	961
	Ponsli I	33°07'54''	074°50'19''	1215
	Ponsli II	33°08'02''	074°51'58''	1175

Threat status

Species evaluated

Three medicinal plant species *Eremurus persicus*, *Gloriosa superba* and *Acorus calamus* (Fig. 1) were evaluated for the threat categories. These three species were assessed in accordance with IUCN red list categories and criteria (2017) version 13 and IUCN Red List criteria at regional levels (2010) version 4.0.

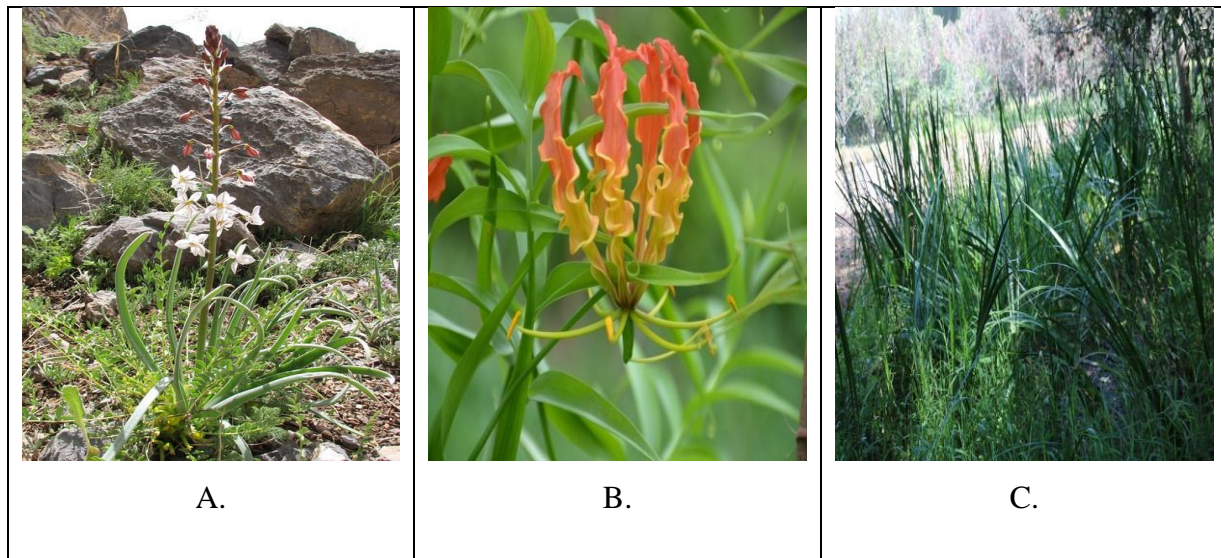


Fig. 1. Target plants (A) *Eremurus persicus* (B) *Gloriosa superba* (C) *Acorus calamus*.

Data collection

The field survey data was supplemented with occurrence records of target plant species obtained from various sources including the online source Global Biodiversity Information Facility (GBIF) database (<http://www.gbif.org>), ENVIS (Environmental Information System), Centre on Medicinal Plants (<http://envis.frlht.org/frlhtenvis.nic.in>) and herbarium records from Botanical survey of India, Kolkata (CNH), Dehradun (BSD) and; Forest Research Institute Dehradun (DD), University of Kashmir (KASH), published research articles. The occurrence data from all sources was then used to calculate the various parameters for assessment of threat status of target plant species.

Calculation of geographic range parameters

The R software package ‘Con R’ (<https://gdauby.github.io/ConR/>) with default parameters supplemented with data collected from various sources was used for calculation of various parameters for estimating threat category using criterion B of IUCN risk assessment. The grid cell size for estimating AOO is by default 2 x 2 km as recommended by IUCN guidelines (2016).

Location and sub-population

Each unique occurrence was buffered with a circle of 5 km radius and overlapping circles were combined to form a single subpopulation, the number of locations is the number of occupied cells of a grid for a given resolution. By default, the size of grid cells is 10 km. The output results of different calculated and estimated/approximated parameters are then visualised along with threat assignment and distribution map (Fig.2).

Criteria used for assigning a threat

For threat assessment, data obtained during the field surveys and other sources was prepared in view of the red list categories and criteria 2017 version 13 and following the regional guidelines of IUCN 2012 version 4.0

Data analysis

IUCN extinction risk assessment’s five criteria (A-E), In criteria B, a geographic range should be in the form of either B1 (EEO) or B2 (AOO) and taxon should also follow two of the three (a, b and c) conditions as shown in Table 2.

Con R supposes the condition “b” to be true i.e., continue future decline in habitat quality (Dauby et al 2017). So, in Con R the assessment of risk status to any taxon follows B1 or B2 and condition “a” and “b”. The condition (decline in the population of species) which Con R supposes to be true was found to be true during field surveys and literature survey.

Geographic range in the form of either B1 (extent of occurrence) and/ or B2 (area of occupancy)			
	Critically Endangered (CR)	Endangered (EN)	Vulnerable (VU)
B1 Extent of Occurrence	<100 km ²	<5,000 km ²	<20,000 km ²
B2 Area of Occupancy	<10 km ²	<500 km ²	<2,000 km ²
And at least two of the following three conditions			
(a) severely fragmented or number of locations	=1	≤5	≤10
(b) Continuing decline observed, estimated, inferred, or projected in any of (i) EOO; (ii) AOO area;(iii) extent and or quality of habitat; (iv) number of locations or subpopulations (v) number of mature individuals.			
(c) Extreme fluctuations in any of (i) EOO; (ii) AOO; (iii) number of locations or subpopulations; (iv) number of mature individuals.			

Table 2: Summary of criteria B used to evaluate if taxon belongs to any threat category (Critically Endangered, Endangered and Vulnerable).

Results

On the basis of output data of ConR, the number of sub-populations and locations for *Gloriosa superba* turned out to be 53 and 56 respectively, sub-populations and locations for *Acorus calamus* 18 and 26 respectively and in case of *Eremurus persicus* a total of 1 sub-population and number of locations were calculated to be 2. In case of *Gloriosa superba* the EOO and AOO as calculated by software ConR were 2499690 km² and 236 km² respectively (Fig 2), for *Eremurus persicus* the calculated EOO is 24 km² and AOO of 24 km² (Fig 2), similarly the EOO and AOO for *Acorus calamus* 301104 and 128 respectively (Fig 2).

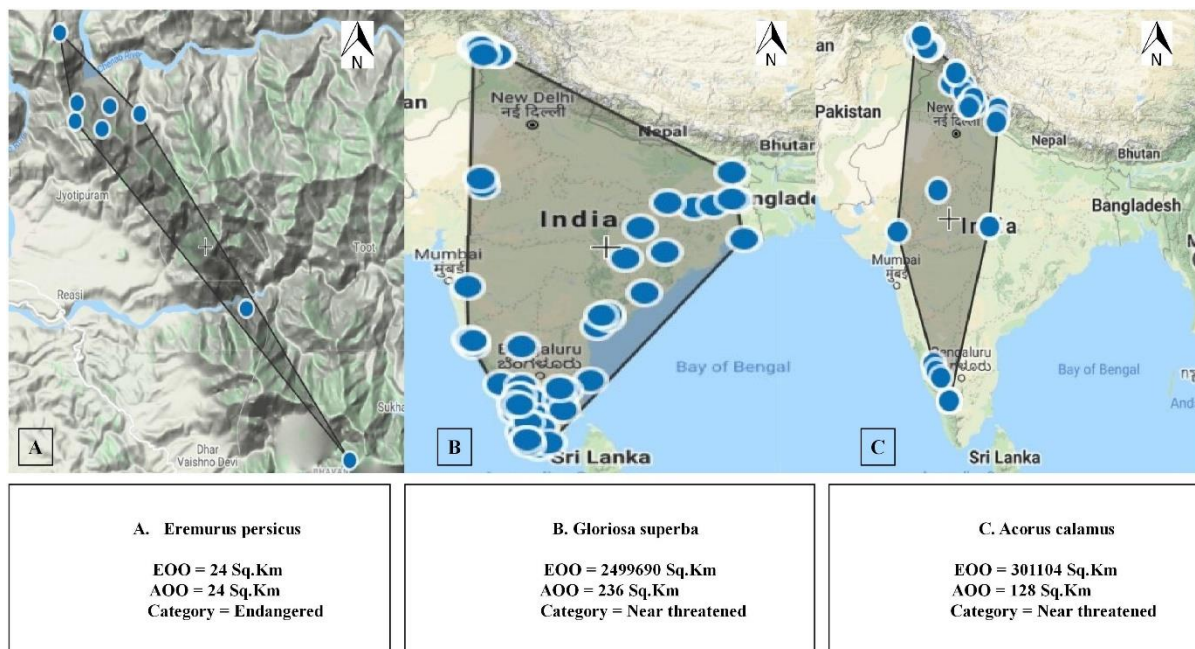


Fig 2: Occurrences of the species are shown as blue dots. The convex hull used for calculating the EOO is shown for the species as a black polygon.

(A) *Eremurus persicus* (B) *Gloriosa superba* (C) *Acorus calamus*

Of the three evaluated species, *Gloriosa superba* and *Acorus calamus* qualified for the IUCN category ‘Near Threatened’ as the taxon meets the area requirements under criterion B for threatened ($AOO < 2,000 \text{ km}^2$) (Fig 2, Table 3). While as *Eremurus persicus* qualified for the category as ‘Endangered’ with very low EOO and AOO.

Table 3: Occurrence sites, population size, EOO, AOO and threats of target plants.

Taxa	EOO	AOO	No of sub-Population	No of locations	Category	Category code
<i>Gloriosa superba</i>	2499690	236	53	56	LC or NT	LC or NT B1a+B2a
<i>Eremurus persicus</i>	24	24	1	2	EN	EN B1a+B2a
<i>Acorus calamus</i>	301104	128	18	26	LC or NT	LC or NT B1a+B2a

Morphology:

Plants were studied for vegetative and floral morphology in the field. Data was collected on various morphological features including plant height, number of branches/plant, number of leaves/branch, leaf size, number of inflorescence/branch, number of flowers/inflorescence (Fig 3). Flowers in the field were collected randomly and studied later with special emphasis on reproductive structures by using stereo-zoom microscope. All the measurements were made by using scale/ocular and stage micrometer (Fig 4, Table 4).

Table 4: Data on plant morphometry of *E. persicus*.

S.No.	Characters (n=20)	Average
1.	Plant height(cm)	69.84±4.65* (39-100)**
2.	Leaf size(cm)	38.82±2.00*×0.77±0.09*
3.	No. of leaves per plant	21.4±1.97* (9-35)**
4.	No. of scapes per plant	2.4±0.22* (1-4)**
5.	No. of flowers per scape	60.15±3.17* (34-80)**

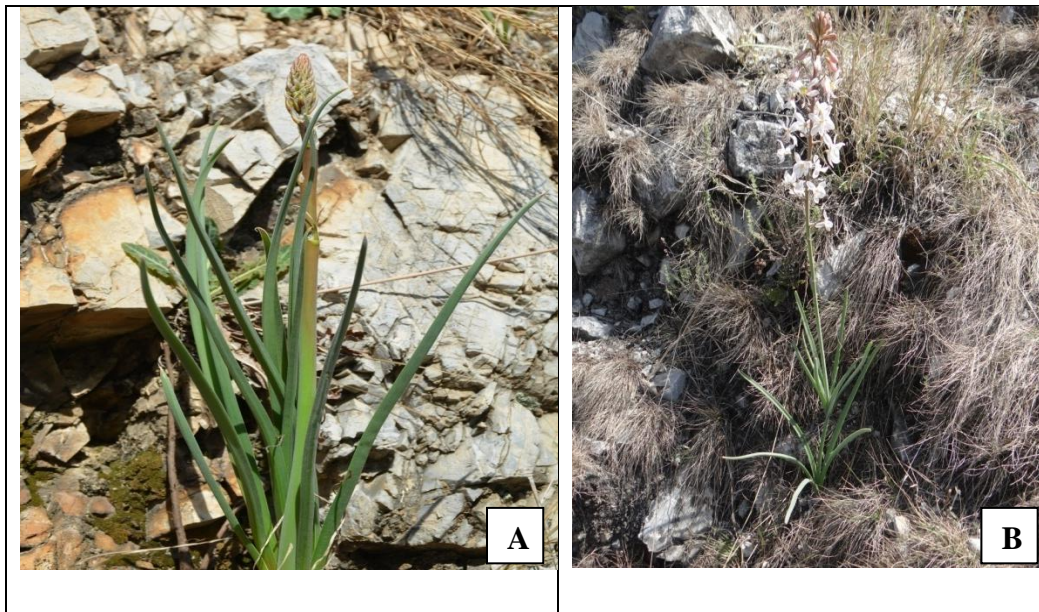
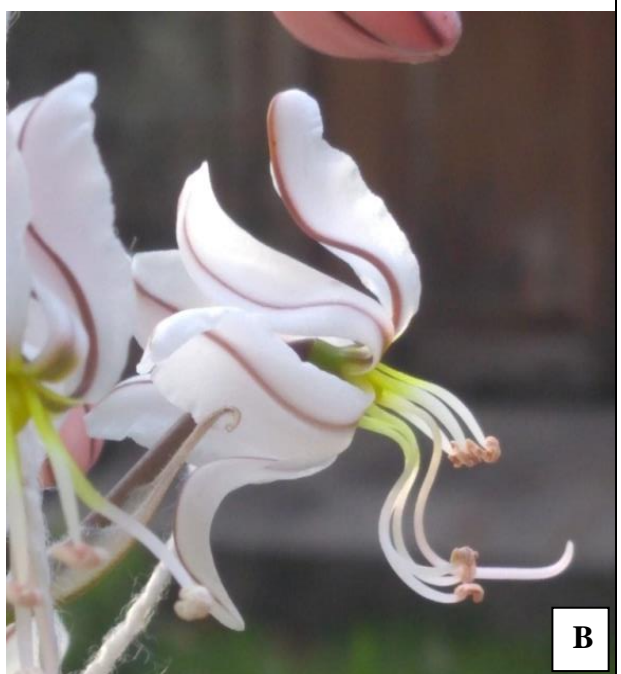


Fig 3. (A-B) Plants showing aerial rosette of leaves along with inflorescence.

Table 5: Data on floral morphometry of *E. persicus*.

S.No.	Characters	Average
1.	Length of pedicel(cm)	1.62±0.05* (1.3-2.0)**
2.	Size of Tepal(cm)	1.89±0.04×0.45±0.03* (1.6-2.2)×(0.2-0.7)**
3.	Length of bract(cm)	2.5±0.11* (1.8-3.5)**
4.	Androecium	(a) Long stamen(cm)
		(i) Length of filament (ii) Length of anther lobe
		(b) Short stamen(cm)
		(i) Length of filament (ii) Length of anther lobe
6.	Gynoecium	(a) Length of stigma+style(cm)
		(b) Size of ovary(cm)

n =Sample size, *=Mean±SE, **=Range



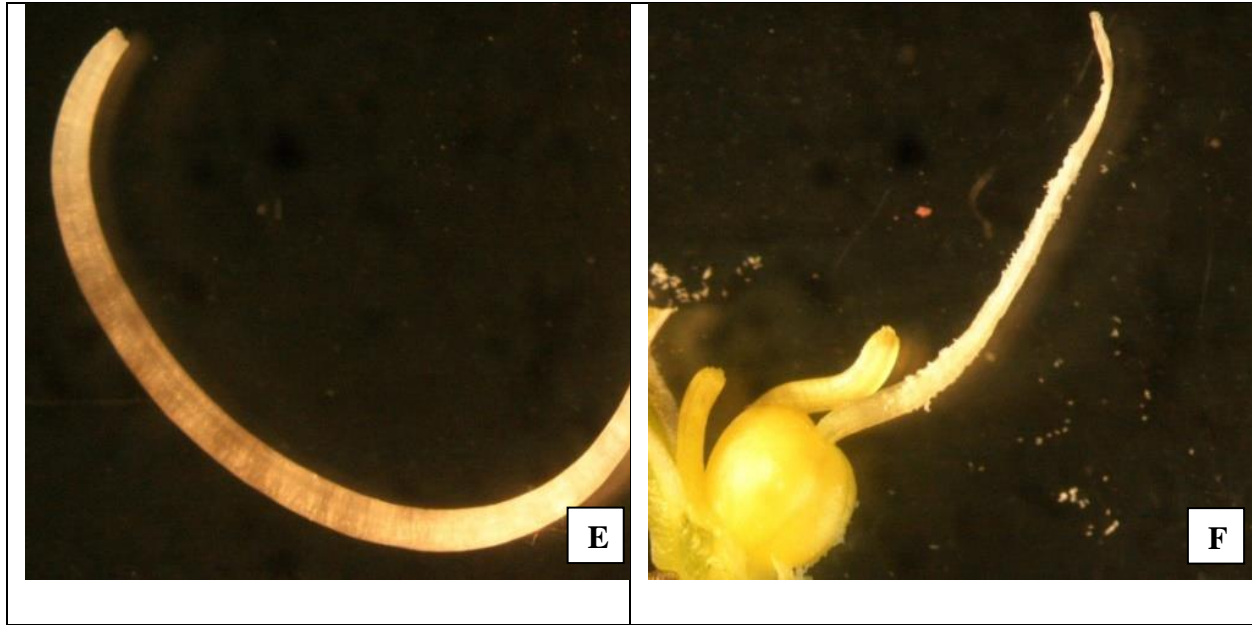


Fig 4 (A-F): (A) portion of flowering scape; (B) an open flower; (C, D) anther dehiscing through a longitudinal slit; (E) Sickle shaped style and (F) pistil showing stigma, style and ovary.

Pollination experiments:

To determine the breeding system, pollination experiments were conducted in the field. Different pollination experiments performed are as under:

- ❑ **Open pollination (OP):** Mature and unopened inflorescence were tagged before anthesis and left for pollination as it happens in nature. These were then monitored after few days to record the fruit set.
- ❑ **Unassisted selfing (US):** Mature and unopened flowers and inflorescences were covered with butter paper bags to exclude the visitors and left undisturbed to estimate fruit set after unassisted selfing.
- ❑ **Manual self-pollination (MSP):** Mature and unopened flowers were pollinated with self-pollen manually with the help of fine brush and then bagged and left undisturbed to estimated fruit set.

- ❑ **Manual cross pollination (MCP):** Mature and unopened flowers were cross pollinated manually with the help of fine brush and then bagged and left undisturbed to estimated fruit set

Results:

Percentage fruit set was recorded on different pollination experiments as shown in table 6.

Table 6: Data on reproductive output of *E. persicus*.

S.No.	Type of pollination	% Fruit set (initial)	%Fruit set (final)	% Fruit abortion
1.	OP (n=20)	26.46	19.47	80.52
2.	US (n=5)	0	0	0
3.	MSP (n=5)	25.32	7.69	92.30
4.	MCP (n=5)	60.66	21.97	78.02

n = Sample size

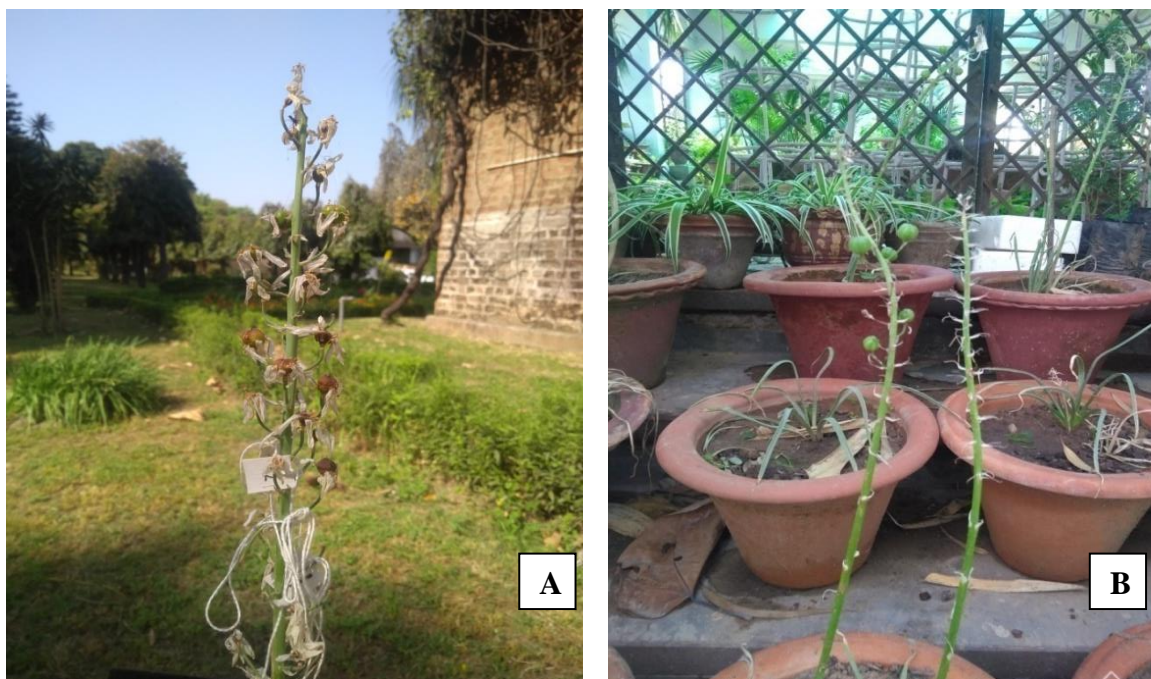


Fig 5 (A, B): Inflorescence showing healthy and aborted fruits.

Cytology:

Pollen mother cell meiosis was studied from young immature flower buds of *E. persicus* and were fixed in a mixture of three parts of ethyl alcohol and one part of acetic acid. After fixation of 24 hours, the buds were washed in water and preserved in 70% ethyl alcohol at 4-6° C. Finally, the anthers were squashed in 1% propiocarmine. All photomicrography of chromosomal preparations was done using unit-Nikon ECLIPSE E- 400 attached to a digital color camera SAMSUNG SDS-312.

Results:

Studies on pollen mother cell meiosis in *E. persicus* revealed that the species have chromosome number of $2n=14$ and the species is diploid $2n=2x=14$. Each pollen mother cell contains 2 small and 5 large bivalents. Pollen mother cells at diplotene, diakinesis, metaphase, early anaphase and anaphase were observed (Fig 6).

In Ponsli II population formation of univalents were observed at metaphase. However, at anaphase regular segregation of 7:7 was observed. Also, presence of bridges was observed at Telophase II (Fig 7). However more cytological studies need to be carried out to find out if there is any abnormality in the population.

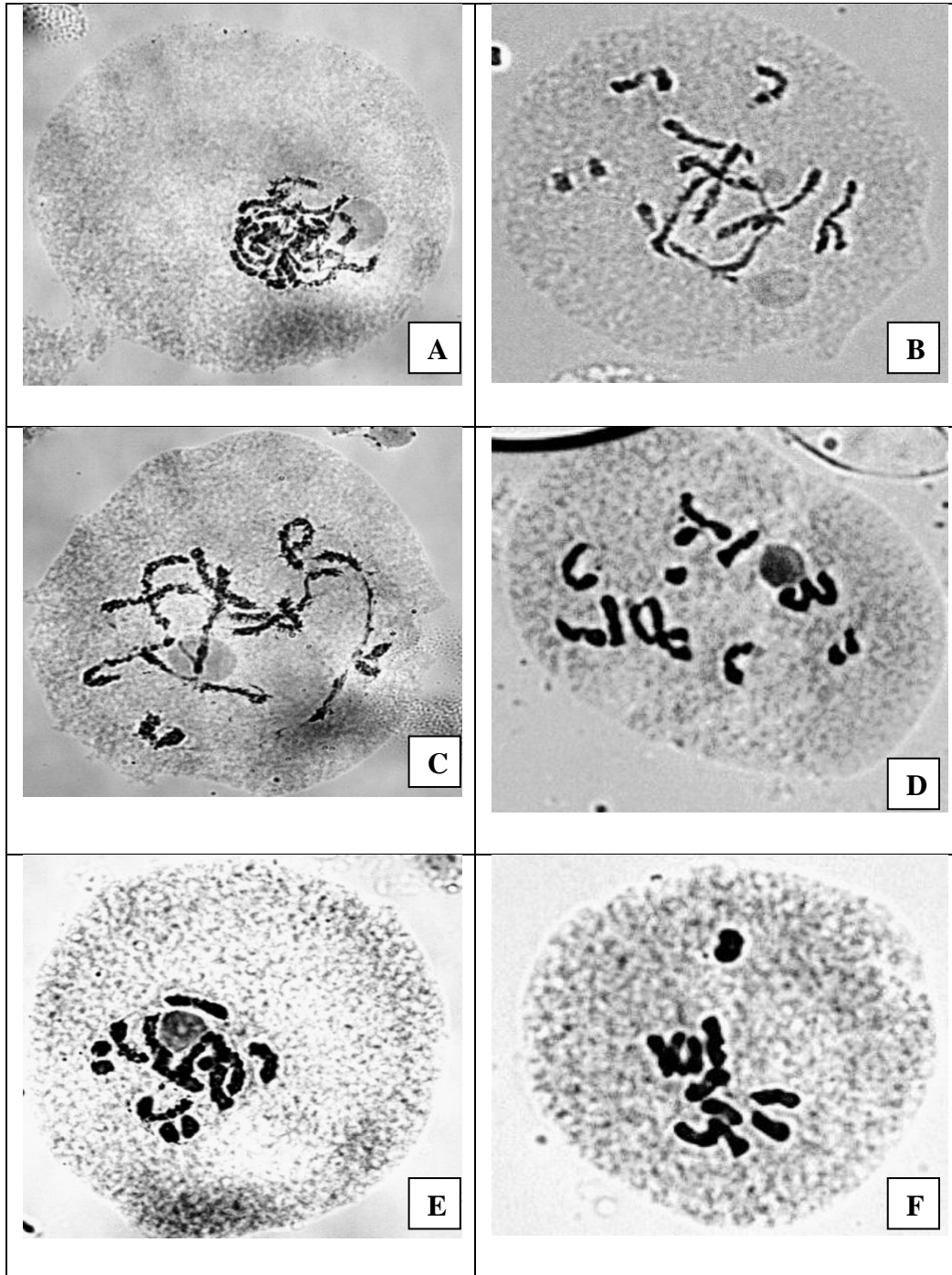
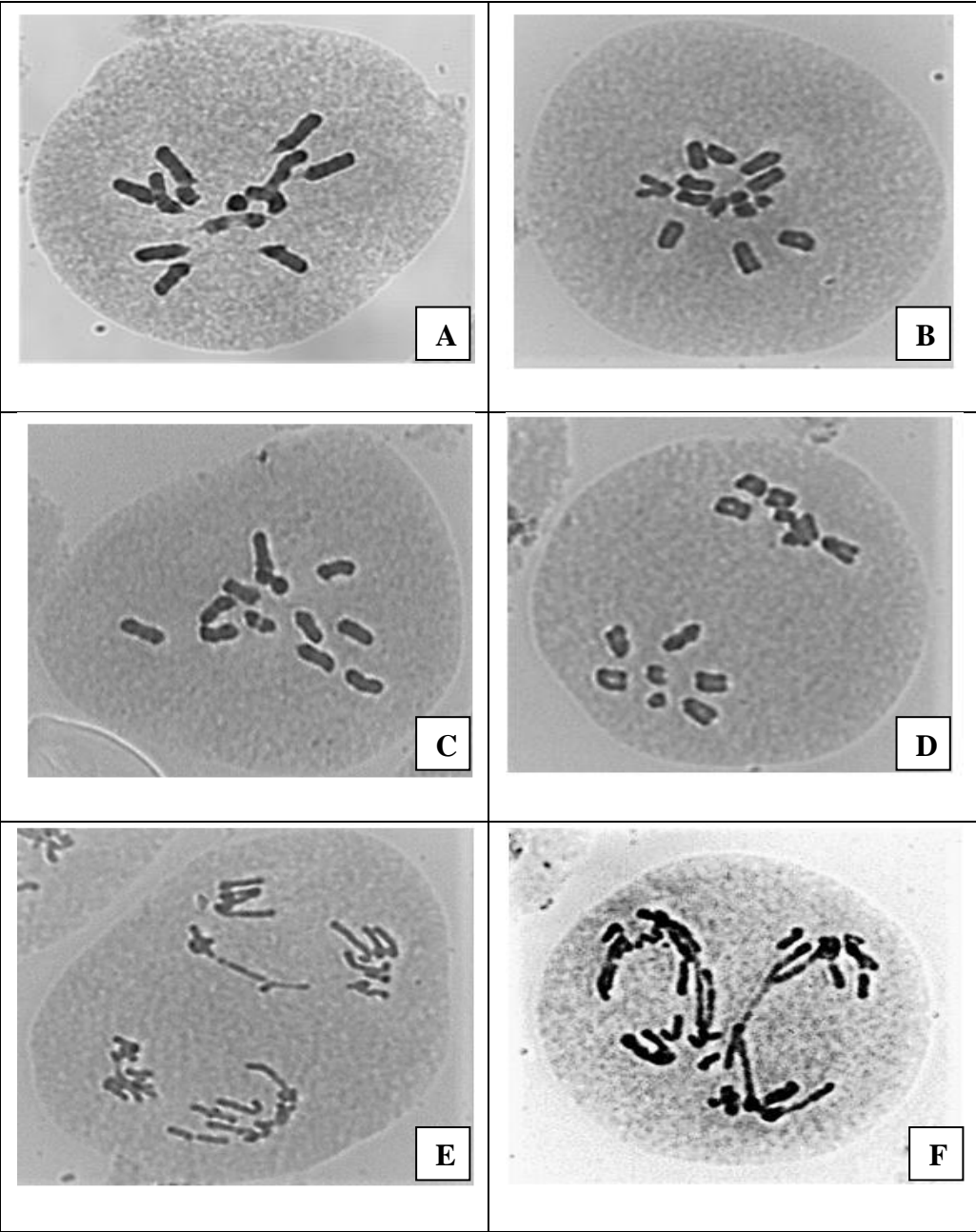


Fig 6 (A-F) pollen mother cells at diplotene (B, C) diakinesis (D, E) metaphase (F)



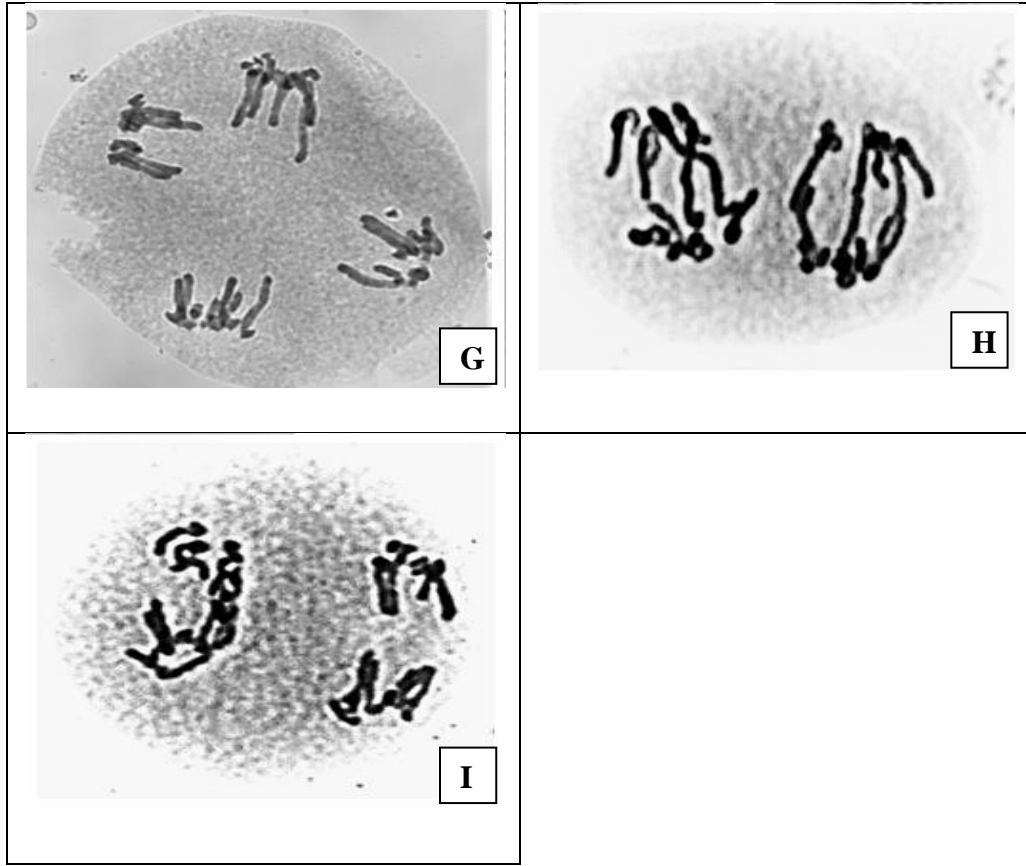


Fig 7 (A-I): pollen mother cells at metaphase (A-C), anaphase(D) and telophase II(E-I) in *Eremurus persicus* (Ponsli II population, $2n=14$)