SUMMARY AND CONCLUSIONS

Present work pertains to cytomorphological studies in the plants of Polypetalae from the cold deserts of Lahaul-Spiti and its adjoining areas. Lahaul-Spiti, a part of the cold deserts in western Himalaya is the largest district of Himachal Pradesh with an area of ca. 13, 835 km$^2$, situated between $31^\circ 44' 57''$ and $32^\circ 59' 57''$ N latitude and $76^\circ 29' 46''$ and $78^\circ 41' 34''$ E longitude. On the basis of geographical conditions, Lahaul-Spiti district is divided into two main regions, i.e., Lahaul Valley and Spiti Valley which differ in many aspects. Lahaul Valley is greener compared to the barren Spiti Valley. The area remained covered with snow for more than 6 months. Temperature during winter falls as low as $-30^\circ$ C. Air is very dry and strong winds blow almost throughout the year at higher elevations. The mountain ranges here consist of rugged mountains, snow clad peaks, bare rocks, and steep sandy slopes with rock gravels. As a consequence of harsh climatic conditions prevailing in the region, plants tend to become prostrate, thick, hairy, bushy, hardy, sturdy, mat and cushion forming, and spiny with long roots and small succulent or woolly leaves. Majority of the plants perennate through rootstocks, runners, bulbs, rhizomes and tubers. The area is under considerable pressure of human intervention and natural disasters which include agriculture, heavy grazing, snow avalanches, windstorms, landslides, increasing entry of tourists and transport vehicles, and overexploitation of medicinally important plants. So far no serious attempt has been made to explore the cytomorphological diversity in the flora of Lahaul-Spiti and its adjoining high altitudinal areas.

Present cytomorphological investigations included 140 species belonging to 72 genera of the Polypetalae. All the species have been studied meiotically for the first time from the area. Chromosome counts in 14 species are the first ever reports viz., Aquilegia pubiflora (n=7), Corydalis govaniana (n=8), C. thyrsiflora (n=8),
Hedysarum astragaloides (n=7), H. microcalyx (n=7), Oxytropis thomsonii (n=8), Rhodiola tibetica (n=10), R. wallichianum (n=16), Rosularia alpestris (n=14), Epilobium chitalense (n=18), E. leiospermum (n=18), Heracleum brunonis (2n=33), H. thomsonii (n=11) and Pleurospermum govanianum (n=9).

As many as 41 species have been counted chromosomally for the first time from India viz., Delphinium brunonianum (n=8), Corydalis vaginans (n=8), Arabis glabra (n=6), A. pterosperma (n=8), A. recta (n=8), Barbarea vulgaris (n=8), Descurainia sophia (n=14), Lepidium apetalum (n=16), L. virginicum (n=16), Sisymbrium brassiciforme (n=21), Hypericum perforatum (n=16), Malva neglecta (n=21), Cerastium glomeratum (n=36), Minuartia kashmirica (n=13), Biebersteinia odora (n=5), Geranium rotundifolium (n=13), Astragalus bicuspis (n=8), A. frigidus (n=8), Melilotus officinalis (n=8), Medicago falcata (n=8), Oxytropis lapponica (n=8), Trigonella emodi (n=8), Geum urbanum (n=21), Potentilla atrisanguinea (n=21, 28, 35, 42), P. gelida (n=14), P. multifida (n=21), Rosa webbiana (n=7), Bergenia stracheyi (n=17), Saxifraga hirculus (n=16) Parnassia laxmanii (n=9), Hylotelephium ewersii (n=11), Sedum oreades (n=11), Epilobium anagallidifolium (n=18), E. cylindricum (n=18), E. tetragonum (n=18), E. tibetanum (n=18), E. sikkimense (n=18), Datisca cannabina (n=11), Angelica glauca (n=11), Chaerophyllum aromaticum (n=11) and Ligusticopsis wallichianum (n=11).

Chromosome counts in Clematis orientalis var. acutifolia (n=16), Potentilla sericea var. polyschista (n=28), Saxifraga diversifolia var. diversifolia (n=8), S. diversifolia var. parnassifolia (n=8), S. flagellaris ssp. komarovii (n=16), S. hirculus var. hirculus (n=8), Epilobium latifolium ssp. latifolium (n=18) and E. latifolium ssp. speciosum (n=18) are the first ever records at sub-species/variety level.
New intraspecific diploid or polyploid cytotypes are recorded in 13 species, *Thalictrum foetidum* (n=21, 6x), *Thlaspi cochlearioides* (n=14, 4x), *Dianthus angulatus* (n=15, 2x), *Stellaria media* (n=14, 2x), *Geranium pratense* (n=28, 4x), *Astragalus ladakensis* (n=8, 2x), *Agrimonia eupatoria* (n=35, 5x), *Geum elatum* (n=28, 8x), *Potentilla bifurca* (n=21, 6x), *P. fruticosa* var. *rigida* (n=7, 2x), *Potentilla sericea* var. *polyschista* (n=28, 8x), *Sibbaldia micropetala* (n=14, 4x) and *Sinocrassula indica* (n=33, 6x). The chromosome counts for *Indigofera Gerardiana* (n=24, 6x), *Potentilla supine* (n=21, 6x) and *Epilobium angustifolium* (n=18, 4x) add new intraspecific cytotypes from India.

New intraspecific aneuploid cytotypes (indicated as in bold) are recorded in eight species viz., *Erysimum hieracifolium* (2n=16, 32, 40, 44, 46, 48), *Hypericum elodeoides* (2n=16, 18), *Malva verticillata* (2n=73, 84, 104,126), *Impatiens bicolor* (2n=14, 18), *I. thomsonii* (2n=12, 14, 16, 18, 20), *Filipendula vestita* (2n=14, 18), *Saxifraga diversifolia* (2n=16, 20) and *Bupleurum candollei* (2n=12, 14, 16). From India such variable/aneuploid chromosome counts are recorded in *Cerastium cerastioides* (2n= 36, 38), *Geranium wallichianum* (2n=26, 28) and *Impatiens sulcata* (2n=18, 20).

Presence of variable number of B-chromosomes are recorded for the first time in *Sedum oreades* (n=11+0-4B) and *Delphinium vestitum* (n=8+0-1B).

Morphogenetic variation involving morphological characters like plant height, number of branches/plant, length of internodes, leaf size and hairiness, flower colour, number of flowers/inflorescence and length of inflorescence are detected in *A. eupatoria* (4x,5x), *Potentilla atrisanguinea* var. *atrisanguinea* (8x,10, 12x), *P. atrisanguinea* var. *argyrophylla* (6x, 12x), *Ranunculus hirtellus* (2x, 4x), *Geranium wallichianum* (2x), *Impatiens thomsonii* (2x) and *Thalictrum foetidum* (6x). Such
variation noticed in *A. eupatoria*, *Potentilla atrisanguinea* var. *atrisanguinea*, *P. atrisanguinea* var. *argyrophylla* and *Ranunculus hirtellus* are attributed to ploidy level. On the other hand, the morphovariation involving flower colour (*Geranium wallichianum*, *Impatiens thomsonii*), plant size; colour and size of leaf/leaflet; leaflet lobe dentation; and degree of leaf pubescence (*Thalictrum foetidum*) seem to have a genetic basis. The intraspecific cytotypes detected presently in *R. hirtellus* (2x, 4x), *A. eupatoria* (4x, 5x), *P. atrisanguinea* var. *atrisanguinea* (8x, 10x, 12x) and *P. atrisanguinea* var. *argyrophylla* (6x, 12x) can be distinguished in the field on the basis of various morphological characters/flower colour. In *R. hirtellus*, the diploid cytotype showed restricted distribution in the Manimahesh hills compared to the tetraploid cytotype which showed much wider distribution (Manimahesh hills, Manali hills and Lahaul Valley). In *A. eupatoria*, 5x cytotype is restricted to only one locality in the Manimahesh hills compared to 4x individuals which are widely distributed in the Himalayas. The high polyploid cytotypes in *Potentilla atrisanguinea* (8x, 10x, 12x) showed much wider distribution in high altitude areas compared to 6x cytotype which showed restricted distribution in the Manimahesh hills.

Occurrence of some univalent chromosomes during first meiosis in the diploid individuals of *Saxifraga diversifolia* var. *parnassifolia* (n=8) are not associated with any multiple association and seems to be the result of asynapsis/desynapsis.

Multiple associations due to reciprocal translocations of chromosomes are recorded during male meiosis in the diploid individuals of *Astragalus chlorostachys* (n=8) and *Saxifraga diversifolia* var. *diversifolia* (n=8).

Presence of some multivalents in the polyploid cytotypes of *Potentilla atrisanguinea* var. *atrisanguinea* (12x), *P. atrisanguinea* var. *argyrophylla* (12x), *P. sericea* var. *polyschista* (8x), *Geranium pratense* (4x) and *Saxifraga flagellaris* ssp.
komarovii (4x) indicated to their segmental polyploid nature. On the other hand, the presence of large number of univalents and absence of multivalents in *Heracleum brunonis* (3x) and *Agrimonia eupatoria* (5x) indicated to their allopolyploid nature. Complete bivalent formation in rest of the 46 polyploids taxa indicated to their allopolyploid constitution.

Nonsynchronous disjunction behaviour (early or late) of some bivalents in *Aconitum heterophyllum*, n=8; *A. violaceum*, n=8; *Aquilegia fragrans*, n=7; *Astragalus chlorostachys*, n=8; *A. munroi*, n=8; *A. himalayanus*, n=8; *A. melanostachys*, n=6; *Biebersteinia odora*, n=5; *Caltha palustris*, n=16; *Clematis orientalis* var. *acutifolia*, n=16; *Corydalis thyrsiflora*, n=8; *Delphinium brunonianum*, n=8; *D. denudatum*, n=8; *D. vestitum*, n=8; *Heracleum thomsonii*, n=11; *Ligusticopsis wallichiana*, n=11; *Meconopsis aculeata*, n=28; *Ranunculus laetus*, n=16; *Saxifraga diversifolia* var. *diversifolia*, n=8; *S. flagellaris* ssp. *komarovii*, n=16 and *Sedum oreades*, n=11 could be attributed to the size of chromosomes and chromatin stickiness. Such irregular behaviour in disjunction of few bivalents resulted into some pollen sterility.

Chromosome clumping and interbivalent connections noticed in PMCs in *Astragalus bicuspis* (n=8), *Clematis orientalis* var. *acutifolia* (n=16), *Heracleum brunonis* (2n=33), *Meconopsis aculeata* (n=28), *Pleurospermum candollii* (n=11) and *Rosularia alpestris* (n=14) are associated with the phenomenon of cytomixis.

Secondary associations of chromosomes in the tetraploid cytotype of *Geranium pratense* (n=28) indicated to its secondary polyploid nature.

The phenomenon of cytomixis involving inter PMC transfer of chromatin material has been found to be quite common in the species of these cold deserts. Presently cytomixis had been recorded in 31 species (*Anemone rivularis*, n=8; *Aquilegia pubiflora*, n=8; *Astragalus bicuspis*, n=8; *A. frigidus*, n=8; *A. himalayanus*,
n=8; A. rhizanthus, n=8; Caltha palustris, n=16; Clematis grata, n=8; C. orientalis var. acutifolia, n=16; Geranium pratense, n=28; Hedysarum astragaloides, n=7; Meconopsis aculeata, n=28; Medicago falcata, n=8; M. sativa, n=16; Melilotus officinalis; n=8; Parnassia laxmanii, n=9; Pleurosporum candollii, n=11; P. govanianum, n=9; Potentilla atrisanguinea var. atrisanguinea, n=42; P. atrisanguinea var. argyrophylla, n=42; P. cuneifolia, n=14; P. fruticosa var. rigida, n=7; Ranunculus hirtellus, n=16; R. laetus, n=14; Rosularia alpestris, n=14; Silene vulgaris, n=12; Thalictrum cultratum n=7; T. foetidum, n=21; Trifolium pratense, n=7; T. repens, n=8 and Trigonella emodi, n=8. In most of the cases chromatin transfer among proximate PMCs resulted into hypo-, hyperploid and enucleated PMCs. Cytomixis also induced various meiotic abnormalities in the meiocytes which include pycnotic chromatin, interbivalent/chromosomal connections, extra chromatin masses, laggards, chromatin bridges, chromatin stickiness, supernumerary nucleoli and spindle irregularities resulting in abnormal sporads (triads, polyads and tertrads with micronuclei). The products of such sporads resulted into varying amount of pollen sterility and heterogeneity in the size of pollen grains. Cytomixis in all these cases seem to be a natural phenomenon under the genetic control influenced by environmental factors especially freezing temperature stress prevailing in the area.

Stickiness of chromosomes involving varying number of bivalents is noticed at different stages of meiosis in Anemone rivularis, n=8; Astragalus frigidus, n=8; Caltha palustris, n=16; Clematis grata, n=8; C. orientalis var. acutifolia, n=16; Dianthus angulatus, n=15; Hedysarum microcalyx, n=7; Meconopsis aculeata, n=28; Medicago sativa, n=16; Ranunculus hirtellus, n=16 and R. laetus, n=14. In Meconopsis aculeata and R. laetus the chromatin stickiness is so severe that it interferes with the segregation of chromosomes during anaphases resulting into restitution nuclei, diads and ultimately
unreduced pollen grains. The stickiness in chromosomes in majority of the presently observed species seems to be associated with the phenomenon of cytomixis and other meiotic irregularities.

The meiotic course in *Ranunculus laetus* (n=14) and *Rosularia alpestris* (n=14) is highly abnormal and is characterized by the presence of univalents during first meiosis. The univalent chromosomes showed scattered distribution at diakinesis/M-I and failed to organize at the metaphase plate. PMCs in these species also showed irregular distribution of chromosomes at A-I/T-I and A-II/T-II and consequently abnormal sporads and high amount of pollen sterility and pollen grains of variable sizes. Such an irregular synaptic behaviour during early stages of meiosis-I in these species could be attributed to the malfunctioning of genes due to extreme cold conditions or to the interspecific hybrid nature of the taxon where genomes of the two putative parents remained unpaired.

Cytokinesis in *Potentilla atrisanguinea* var. *argyrophylla* (n=42) and *Ranunculus hirtellus* (2n=32) is abnormal resulting into the formation of heterogeneous sized pollen grains.

Syncyte meiocytes noticed presently in *Astragalus rhizanthus* (n=8), *Clematis orientalis* var. *acutifolia* (n=16), *Meconopsis aculeata* (=28) and *Medicago sativa* (n=16) are resulted due to direct fusion of PMCs during the earlier stages of meiosis. The products of such syncyte PMCs yielded large sized ('2n') pollen grains.

In *Caltha palustris* (n=16), *Meconopsis aculeata* (n=28), *Potentilla atrisanguinea* var. *argyrophylla* (n=21), and *P. sericea* var. *polyschista* (n=28) fusion among pollen grains resulted into large/giant sized pollen grains. Such large sized pollen grains could play an important role in the origin of polyploids.
The presence of supernumerary nucleoli in the PMCs of *Astragalus himalayanus* (n=8), *Caltha palustris* (n=16), *Medicago falcata* (n=8), *M. sativa* (n=16), *Potentilla gelida* (n=14), *Potentilla atrisanguinea* var. *argyrophylla* (n=21), *Sibbaldia micropetala* (n=14) and *Silene vulgaris* (2n=12) is associated with the phenomenon of cytomixis among meiocytes.

Variation in the relative size of bivalents/chromosomes noticed in various species of the family Ranunculaceae, viz., *Aconitum heterophyllum*, *A. violaceum*, *Anemone rivularis*, *Caltha palustris*, *Clematis grata*, *C. orientalis* var. *acutifolia*, *Delphinium brunonianum*, *D. denudatum*, *D. vestitum*, *Ranunculus hirtellus* and *R. laetus* and species like, *Astragalus melanostachys*, *A. munroi*, *Saxifraga diversifolia* var. *diversifolia*, *S. diversifolia* var. *parnassifolia*, *S. flagellaris* ssp. *komarovii*, *S. hirculus* and *Sedum oreades* might be the consequence of simple translocations or unequal reciprocal translocations.

Role of polyploidy in the evolution of species of these cold deserts is quite significant as 51 species (36.43 %) existed at various polyploid levels. Some of the species depicted high level of ploidy viz., *Potentilla atrisanguinea* var. *atrisanguinea* (12x), *P. atrisanguinea* var. *argyrophylla* (12x), *P. sericea* var. *polyschista* (8x), *Geum elatum* (8x), *Potentilla bifurca* (8x) and *P. gerardiana* (8x). Polyploidy seems to be a common phenomenon in certain genera and families. Role of polyploidy in the evolution of these species is also apparent as 57 species exhibited intraspecific polyploidy at different levels. In *Potentilla, Caltha, Ranunculus, Malva* and *Trifolium* series of intraspecific cytotypes are existent.

Aneuploidy causing chromosomal variations is equally important as 37 species depicted the existence of intraspecific aneuploid cytotypes at diploid and/or polyploid level. *Caltha palustris* (2n= 28, 32, 34, 44, 47, 48, 52, 54, 55, 56, 57, 58, 60, 61, 64,
Trifolium pratense (2n= 14, 26, 27, 28, 29, 32, 48), T. repens (2n= 16, 22, 28, 30, 32, 48, 64), *Potentilla atrisanguinea* (2n=42, 56, 63, 74, 77, 84, 91, 98), *Hypericum perforatum* (2n= 14, 16, 18, 26, 32, 34, 36, 48) and *Bupleurum candollei* (2n=14, 16, 19, 20, 21, 25, 26, 28, 32, 36, 37, 40) are the species which depicted an array of chromosome numbers involving both aneuploidy and polyploidy, constituting species complexes. Majority of these species are observed to be perennial in nature and perennate by means of rootstocks, rhizomes and stolons. It thus indicates that the chromosomal variation is associated with the vegetative mode of reproduction. Another important finding recorded presently in the plants of cold deserts included synaptic irregularities, spindle abnormalities, syncytes, cytomixis, aberrant cytokinesis, pollen fusion and formation of restitution nucleus which resulted into the formation of unreduced ('2n') gametes in the form of large/giant pollen grains. These '2n' gametes may play a role in the production of polyploids. Harsh climatic conditions particularly freezing temperature prevailing in the area seem to have caused various meiotic abnormalities in the plants which affected the genetic constitution and viability of male gametes and lead to reduced reproductive success through seeds. In turn the plants of the area have adopted the alternate means of propagation through agamospermy (vegetative means like rootsuckers, rhizomes, stolons, bulbs and tubers).
Figure 802: Diagrammatic representation of conclusion

Plants of Cold Deserts

Abnormal meiosis (Meiotic irregularities)

Affecting genetic constitution and viability of male gametes

Reduced reproductive success through seeds

ALTERNATE MEANS OF REPRODUCTION

Reproduce/Regenerate through vegetative means like rootstocks, root suckers, runners, bulbs, rhizomes and tubers