Closure Report

File No.	**CRG/2018/003919**
Title of the project	Assessment of cytological, biochemical and molecular variability in Viola species from Jammu and Kashmir
Principal Investigator	**Dr. Geeta,** Jammu University, Jammu, jk, Jammu, Jammu and kashmir-180006
Total Sanctioned Amount	31,97,181 **INR**

Approved Objectives of the project

• • Exploring Jammu and Kashmir Himalaya for collection, characterisation and documentation of different species of Viola.
• Assessing diversity of different accessions and species using morphological and cytological parameters.
• Qualitative and quantitative analysis of biochemicals in different accessions for identifying elite germplasm.
• Assessing diversity in different accessions using ISSR markers to determine genetic relatedness between different accessions and species.

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Date of start of the Project	20 May, 2019
Date of Completion	19 May, 2022
Total Released Amount	28,36,580 **INR**
Total Expenditure (as on date)	2469134
Duration of Project	36 months

Deviation made from original objectives

nil

Ph.D. Produced/Likely to be Produced (from this project only)	1
Technical Personnel Trained (from this project only)	4

Concise Research Accomplishment

Morpho-cytological, biochemical & molecular studies in 11 V. pilosa populations from Khellani, Pranoo, Jai-ghati, Sartingal, Kapra, Sanasar, Bhairon-ghati, Manyal-gali & Dera-gali, Bhimber-gali, Bafliaz of Jammu revealed marked variation in foliar traits, with Kapra plants bearing max. 7.8 leaves. Minimum 4.3 leaves are formed by Manyal-gali & Bafliaz plants. Sanasar plants (37.92cm2) bear largest leaves, followed by Jai-ghati (19.84cm2), Bhairon-ghati (16.7cm2), Manyal-gali (14.9cm2). Max. 4 flowers are produced by Kapra plants, 2.8-3.1 by Jai-ghati, Khellani, Pranoo, Dera-gali & Bhairon-ghati plants & largest flowers are differentiated by plants of Jai-ghati (5.48cm2) followed by Bhairon-ghati (4.98cm2) & Sanasar (4.59cm2). Since leaf & flower no. and their sizes are more in Bhairon-ghati, Sanasar & Jai-ghati plants, growing between 1998-2877m, these sites are suitable for cultivation.Cytologically, V. pilosa PMCs contain 20 chrs. except few cells of Dera-gali & Bhimber-gali being aneuploid. Presence of 10II at metaphase I and equal segregations indicates its diploid nature. Presence of few cells with multivalents indicates prevalence of translocations which reduce pollen viability to 39-50%.Biochemical studies in V. pilosa revealed that flavonoid, phenol and stigmasterol content is high in Bhairon-ghati plants (5.26 mg GAE/g, 9.91 mg QE/g, 0.019726µg/ml) followed by Manyal-gali, Sartingal which grow between 1759-1998m. These sites seem suited for V. pilosa cultivation. ISSR analysis reveals existence of high total species diversity (Ht=0.2678), greater proportion of diversity amongst groups of populations (Gst=0.7134) & less gene flow (Nm=0.2009, indicating presence of high inter-population variability. V. canescens plants from Jammu, Devi-pindiyan, Natha-top, BGSBU & Channi-prat reveal marked differences in leaves, with Channi-prat bearing max. 11 leaves and Jammu producing min 6. The largest leaves (19.6cm2) & flowers (3.76cm2) are produced by BGSBU and smallest ones (6.14 cm2) by Natha-top and smallest flowers (1.81cm2) by Jammu plants. Cytologically, 5 V. canescens populations contain max. PMCs with 20 chromosomes which depict regular behaviour, indicating its diploid status. Presence of multivalents in few PMCs point that the complements has undergone translocations. Pollen viability is more than 90%. Biochemical analysis of V. canescens reveals maximum TPC (13.87±0.021mg GAE/g), TFC (19.77±0.032 mg QE/g) in Devi-pindiyan plants, followed by BGSBU and Natha-top, the sites occurring between 1136-2265m. As plants of these sites have high antioxidant potential & stigmasterol (IC50 values 8.79, 9.89 and 13.54 µg/ml),these seem better for V. canescens cultivation. ISSR analysis in V. canescens reveals high total species diversity (Ht=0.2252), greater proportion of diversity among groups of populations (Gst=0.3697) lesser gene flow (Nm=0.8525) and greater diversity between populations.

Closure Details

Experimental/ Theoretical Investigation carried out

Experimental Setup:
Six Viola species viz. V. pilosa, V. canescens, V. betonicifolia, V. biflora, V. odorata & cultivated V. wittrockiana (V. tricolor) growing in Jammu J&K (India) have been studied. Currently, plants of ten V. pilosa populations from 6 districts (Doda, Kishtwar, Ramban, Reasi, Rajouri & Poonch) and five of V. pilosa populations inhabiting 4 districts (Jammu, Reasi, Ramban & Rajouri) were tagged. Overall, plants of 32 populations of 6 species of genus Viola have been analysed for morphological, cytological, biochemical and molecular attributes. Geo-coordinates of the sites inhabited by individual populations are given in table 1. Morphological studies were carried out on 10 plants per population.
Methods adopted:
Cytological Studies
Karyotypic details were made from dividing root-tip cells pre-treated in 0.5% aqueous colchicine solution for 3 hours, fixed in ethanol and acetic acid in 3:1 ratio) for 24 hours & preserved in 70% ethanol at 4-6ºC. For staining, root tips were washed and hydrolysed in 1N HCL at 60ºC for 12 minutes and stained in feulgen for 10-15 minutes. Stained tips were squashed in 1% acetocarmine and karyotypic details were worked out from photomicrographs.
For studying meiosis, young buds were collected, fixed in Carnoy's fluid for 24 hours, washed in water & stored in 70% ethanol at 4°C. Anthers of these were separated and squashed on a glass slide in 1% acetocarmine. Images were captured using Zeiss Primostar trinocular microscope fitted with Nikon FM3A (Japan) camera and analysed for chromosome number & behaviour.

Biochemical studies

For preparation of leaf extracts in ethanol, 10g leaves of plants of each populations were collected, shade dried for 15 days and ground to coarse powder. Powder was put to a beaker containing absolute ethanol, kept for 3 days with occasional stirring & filtered. The filtrate was dried using rota-evaporator into a semisolid paste which was dissolved in ethanol to get a concentration of 5mg/ml. Qualitative analysis was done for phenols (FeCl3 test), flavonoids (NaOH test), alkaloids (Dragendorff's test), glycosides (Keller Killiani test) & steroids (Salkowski test).

Quantitative analysis:

Samples were prepared in triplicates & for each analysis, mean value of absorbance was determined.

Determination of total phenolic contents in the plant extracts

Ethanolic extracts were prepared as 1 mg/ml. To 1.5ml of Folin-Ciocalteu (FC)'s reagent, 50µl of this extract was added and solution was kept in dark for 30 min. Then, 1 ml of 2% Na2CO3 solution was added and absorbance taken. The blank was prepared which contained 50 µl ethanol, 1.5 ml FC reagent and 1 ml of 2% of Na2CO3. The absorbance was measured at λmax (680 nm). The same procedure was repeated for serial dilutions of gallic acid and the calibration line was constructed. The phenolic concentration was determined (mg/ml) from calibration line which was expressed as mg of GA/g of extract.

Total flavonoid content (TFC):

In a test tube containing 5ml leaf extract, 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, 2.8 ml of distilled water was added and kept for 30 min at room temperature. Its absorbance reaction was taken at 415 nm was taken, Quercetin hydrate is used as standard and TFC is expressed as mg rutin equivalent (RE)g−1 dry weight.

Total tannin content (TTC):

To 1 ml of ethanolic extract,7.5 ml distilled water, 0.5 ml FC reagent and 1ml of 35% Na2CO3 was added and volume raised to 10 ml. The solution was shaken well, kept at room temperature for 30 min. and absorbance was taken at 700nm. Tannic acid was used as standard & its total tannin content (TTC) is expressed as mg Tannic acid equivalent (TE)/g dry weight.

Quantitative estimation of stigmasterol using HPLC:

Quantitative analysis of stigmasterol was initiated to check extent of its variability in different populations. About 10mg of crude ethanolic extract was dissolved in 1ml ethanol and the solution was syringe filtered using 0.45µm Millipore filter. Stigmasterol standard was prepared by dissolving 0.1g powder in 10ml ethanol. The HPLC analysis was performed using Shimadzu LC-10 system.

The presence of stigmasterol was detected at 236 nm (λ max) using variable wavelength UV detector and the content of stigmasterol was expressed as percentage peak area. The retention time was recorded at 17.311. HPLC profile of ethanolic extract of Viola pilosa have characteristics peaks at retention time 17.539, 17.544, 17.772, 17.530, 17.256, 17.450 and 17.8.

ISSR analysis

Genetic diversity parameters of Viola species were determined by using ISSR markers. For isolation, purification and quantification of DNA, different chemical constituents were used (Table 2).

DNA isolation

Leaf samples of plants of each population were collected in 15ml centrifuge tubes and preserved in liquid nitrogen. Genomic DNA was isolated using CTAB method. 3g leaves were powdered using liquid N2 and put in a 50 ml centrifuge tube containing 15 ml of pre-heated extraction buffer with 2% Cetyl Trimethyl Ammonium Bromide (CTAB, w/v), 100 mM Tris at pH 8, 1.4 M NaCl, 20 mM ethylenediamine tetra acetate (EDTA) at pH 8, 2% β-mercaptoethanol (v/v) and 2% PVP (w/v). The suspension was vortexed for 5 minutes and kept at 65˚C for 45 minutes with occasional shaking. The mixture was removed from the water bath, cooled and an equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by mixing for 10 minutes. The suspension was centrifuged (9000 rpm-15 minutes-room temperature) and supernatant was collected in a fresh sterile centrifuge tube, followed by precipitation with 2/3rdvolume of chilled isopropanol. Each tube was kept at -20˚C for 3-5 hours so that DNA could precipitate. The DNA was pelleted by centrifugation at 9000 rpm for 15 minutes at 25˚C. The supernatant was decanted off & pellet washed twice with 70% alcohol, dried at room temperature for 6-8 hours and re-suspended in 500µl of Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). After dissolution, DNA samples were stored at -20ºC ti.

D. Quantification and dilution of genomic DNA

The integrity of isolated DNA was determined during gel electrophoresis of these samples in 0.8% w/v agarose and concentration was determined using nanodrop (25ng/µl with appropriate amount of milliQ water).

E. ISSR analysis of the DNA samples

ISSR reaction was carried out with 50 ng of genomic DNA in 25µl reaction volume containing 1XTaq buffer, 3 mM MgCl2, 0.2 mM dNTPs, 1µM primers and 3 units of Taq polymerase. Amplification of each DNA sample was carried out in thermocycler with initial denaturation at 95°C for 5 minutes followed by 35 cycles, each with denaturation at 94°C for 1 minute and annealing at 38-47°C for 1min. and extension at 72°C for 2 mins and final extension at 72°C for 7 mins. After this, the samples were cooled to 4°C (Table 3 and 4).

The products so amplified were electrophoresed on 1.8% agarose gel with 0.5µg/ml ethidium bromide in 1% TAE buffer and visualized under UV light and photographed by using Gel documentation system (Vilber).

i) Analysis of morphological data

From morphological data, mean, standard deviation and standard error was determined. One-way analysis of variance (ANOVA) was applied to find the significance among the differences in mean values of various populations. Phenotypic (PCV) & Genotypic (GCV) coefficients of variance and Heritability (H2) in broad sense were also computed

ii) Analysis of ISSR based data

The data obtained from the ISSR marker analysis were scored for presence (1) or absence (0) of bands. Population wise Shannon's information index (I= -∑pilog2pi, where pi is the frequency of a given amplified fragment) and Nei's genetic diversity (H= 1-∑pi2, here also pi is the frequency of an amplified fragment) (Nei, 1973) were calculated using POPGENE version 1.32. Degree of polymorphism at the population and species level was calculated using (i) percentage polymorphic loci (P), (ii) Shannon's information index (I), (iii) Nei's genetic diversity index (H).

To analyse the genetic diversity in subdivided populations, total genetic diversity (Ht/Hpop), intrapopulation genetic diversity (Hs/Hsp)were determined using Nei's (1978) genetic diversity. Genetic differentiation between populations, also called as gene differentiation (Gst) was calculated as Gst = 1- Hsp/Hpop. The amount of gene flow between populations was determined using population differentiation [(Nm= 0.5(1-Gst)/Gst]. Here Nm<1 indicates a local differentiation of populations whereas Nm>1 is evidence of little differentiation among populations. Genetic distance (GD) estimates among population were calculated using Nei's unbiased genetic distance coefficient. Fst based pair-wise distances between group of populations were estimated using the GenAlex version 6.51 based on 999 permutations.

For two Viola species, optimum number of clusters, which depends on internal and stability validation measures were computed. Redundancy analysis was performed to study the similarity between populations in morphological and molecular datasets using Biodiversity R package (Kindt, 2020).

Data Collected:

The species of genus Viola namely growing in Jammu province have been scrutinized for morphological, cytological and molecular variability.

1. V pilosa

In total, eleven V. pilosa populations from five districts have been screened (table 1). At these locations, plants were found growing on moist slopes, along road-sides, stone walls, water channels, ditches and in the shade of Quercus, Cedrus and Pinus trees.

(i) Phenology

Plants of V. pilosa are semi-evergreen which produce leaves nearly throughout the year. This species bears two kinds of flowers viz. chasmogamous and cleistogamous in different non-overlapping seasons. Buds of chasmogamous flowers start appearing during January, reaching full bloom from February-March and fruits start maturing during first week of April. Cleistogamous flowers, on the other hand, start appearing during May and fruit is formed within a month. Initiation of chasmogamous and cleistogamous flowers is delayed by nearly two weeks in the plants growing on higher altitudes or with prolonged snow cover (Jai-ghati, Dera-gali and Sanasar).

(ii) Morphology:

(a) Plant form, stem and leaf

V. pilosa plants are herbaceous, perennial and acaulescent. Each plant bears underground rhizome with reduced internodes and above ground stolons which serve as means of vegetative propagation. From the underground portion of rhizome arises roots and from above ground parts leaves emanate. Leaves borne in tufts, are cordate with acute tips and serrate-crenate margins. Dorsal surface of leaf is dark green and pubescent whereas ventral surface is light green (Fig. 1) and sparsely haired.

Eleven studied populations reveal variation in leaf number, size and petiole length (Table 5).

(b) Flower:

V. pilosa bears solitary flowers whose number and size vary at population level (Table 6). Maximum four flowers are differentiated by plants found in Kapra and minimum 1.9 by those from Sartingal and Bhimber-gali (Table 6). Floral traits like, flower size, anterior, lateral and posterior sepal and petal sizes, stamen and carpel sizes are given in the table 6.

(c) Fruit and seed:

The capsule opens via three longitudinal valves in non-explosive mode. Each fruit bears numerous brown tiny obpyriform seeds with aril on one side. Seed set per fruit differ from population to population. Seeds dispersed on the ground, frequently lodge deep in litter or soil crevices. As ants regularly move on the seed caruncles, Viola plants appear to disperse seeds with their help and through non-explosive fruit opening (table 7).

IV.B. Cytological studies in V. pilosa

a) Somatic complement: 3 root tip cells studied of V. pilosa reveal them having 2n=20 chromosomes (Fig. 2) which form 10 pairs. The matrices of single set are tabulated (Table 8).

b) Meiosis in male tract of V. pilosa: Presently, detailed chromosome behavior has been studied from PMCs of 10 of the 11 tagged populations except for Pranoo whose plants did not survive. Population-wise details of chromosome behavior are given in the table 9.

IV.C. Biochemical analysis in V. pilosa

Analyses of ethanolic leaf extracts of V. pilosa for phenols, flavonoids and sterols using specific color tests reveal their presence (Table 10).

(i) Total Phenolic Content (TPC):

TPC was calculated using the standard equation $y=2.1572x+0.0268$ ($r2=0.9373$). Population-wise values of TPC vary between 1.53 to 5.26 mg GAE/g (Table 11).

(ii) Total Flavonoid Content (TFC):

Perusal of Table 12. reveals that population-wise flavonoid content vary between 4.22 to 9.91 mg QE/g which was calculated using the equation $y=0.9164x+0.0949$ ($r2=0.9347$).

(iii) Free radical scavenging activity (%) using DPPH:

For different V. pilosa populations, Percentage Free Radical Scavenging Activity (%RSA) using DPPH has been determined, followed by estimation of IC50 values (Table 13)

(v) Stigmasterol concentration:

Stigmasterol is one of the important plant-based sterols having anti-inflammatory, anti-microbial and anti-cancerous activity. Therefore, its Quantitative estimation has been carried out presently from the samples of different populations through HPLC analysis. Chromatograms of the standard and leaf extracts of different populations obtained are shown in Fig.3a&b. From these chromatograms, retention time and mean area units (mAU) under curve have been noted and stigmasterol concentration calculated using standard equation $y = 751243x$ ($r2=0.99$) (Table 14).

IV.D Molecular variability in V. pilosa using ISSR markers

Eight selected primers, yield unambiguous and reproducible bands for 66 leaf samples in V. pilosa (Fig.4). A total of 1327 amplicons and 43 loci have been obtained. Out of 43 loci, 32 are polymorphic and 11 are monomorphic, with %age polymorphism being 74. For each population, data have been generated on number of polymorphic loci (PL), percentage of polymorphic loci (PPL), observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H) and Shannon information index (Table 15 and 16). Further, comparison of studied populations has been made for geographic and genetic distance (Table 17) which show overall significant positive correlation ($r=0.65$, $p<0.05$).

V. canescens

Of the 5 V. canescens populations studied presently, two belong to Rajouri (BGSBU, Channi-prat) and one each to Jammu (Botanical garden), Reasi (Devi-pindiyan) & Ramban (Natha-top) districts.

Phenology

The plants of V. canescens are semi-evergreen which bear leaves nearly throughout the year. They bear cleistogamous and chasmogamous flowers. Buds of chasmogamous flowers start initiating during December-January (shown in light green blocks) and plants depict flowering from February-April and fruits are formed during May-July. In temperate regions and at higher altitudinal sites like Natha-top, these events get delayed by 1-2 months. Cleistogamous flowers appear during August to September & mature into fruits from September to October.

Morphology

(i) Plant form, stem and leaf

V. canescens is a herbaceous, perennial and acaulescent plant with an underground rhizome giving rise to roots and stolons. Leaves petiolate, protected by lanceolate stipules, arise in tufts, are cordate and have serrate- crenate margins and obtuse tips (Fig.1) which vary from population to population with respect to size and number (table 18).

(ii) Flower:

Plants of V. canescens bear pedicellate, zygomorphic, beautiful solitary light-purple pentamerous, flowers, whose numbers vary from population to population (Fig. 1) with notable differences in flower number and size (table 19). Sepals and petals which have been further categorized as anterior, lateral and posterior. Amongst petals, the anterior petal is the smallest, two posterior ones are the largest two lateral ones have intermediate size. The anterior petal is further unusual in having dark blue streaks at the base and spur containing nectaries whereas lateral petals are bearded at the base. The three kinds of sepals and petals of V. canescens also differ in size at population level. Matrices of sepal and petal related traits are tabulated (table19).

Male reproductive organs of V. canescens are represented by five sessile stamens, having orange coloured appendages. Marginal differences exist in anther size of V. canescens from population to population (Table 19). The female reproductive part of flower called the gynoecium is represented by tricarpellary syncarpous ovary, style and beaked stigma. Carpel of this species reveals slight variations at population level (table 19).

(iii) Fruit and seed traits:

The fruit of this species is globose loculicidal capsule. Fruit number per plant varies slightly from population to population. Fruit size of this species varies marginally (Table 20). Seeds produced by Viola species are obpyriform & show variation in seed size and number (Table 20).

IV.F. Cytological studies in V. canescens

PMCs of 5 populations have 2n=20 chromosomes which show more or less regular behaviour

Biochemical analysis in V. canescens

Ethanolic leaf extracts of V. canescens when scruitinised for phenols, flavonoids and sterols indicates their presence (Table 25). These have been further analysed for total phenols, flavonoids, antioxidant potential and stigmasterol concentration (Table 22).

(A) Total Phenolic Content (TPC; Table 23)

At population level, value of TPC ranges from 1.32 mg GAE/g to 13.87 mg GAE/g (Table 23).

(B) Total Flavonoid Content (TFC; Table 23)

The value of TFC in studied leaf samples vary significantly ($p<0.05$) from population to population (3.82-19.77 mg QE/g extract) (Table 23).

(C) Free radical scavenging activity (%) using DPPH:

From percentage free radical scavenging activity (%RSA) of different populations, IC50 values were computed (Table 23).

(E) Stigmasterol concentration

Quantitative estimation of stigmasterol, a sterol of great pharmaceutical importance, has been done through HPLC analysis. Chromatograms of standard stigmasterol and leaf extracts of different populations are shown in Fig. 5. From chromatogram of each sample, retention time and mean area units (mAU) under curve has been noted and stigmasterol concentration calculated using the equation y=751243x (Table 24).

IV.H. Molecular variability in V. canescens using ISSR markers

With five selected primers, yielding clear bands, 720 amplicons and 30 loci have been obtained (Fig.6). Out of 30 loci, 22 are polymorphic and 8 are monomorphic, with percentage polymorphism being 73.33%. Number and percentage of polymorphic loci, determined for each population, observed number of alleles (Na), effective number of alleles (Ne), Nei's genetic diversity (H) and Shannon's information indices (I) are tabulated (Table 25 and 26).

Viola x wittrockiana

Morphological and cytological analysis: Comparative morphometric studies on fourteen floral variants of V× wittrockiana have been carried out and results obtained are presented in table 27.

Cytological studies in V×wittrockiana: Five cultivars have been analysed for cytological aspects which reveal 2n=42 and 46 in various accessions and unequal (21:2:23) segregation at anaphase-I.

Viola betonicifolia

The species is acaulescent, herbaceous and perennial bearing five sepals, petals, stamens and 3 fused carpels. The leaves are arrow-head shaped with crenate margins and the sinus is broad. It differs from the above two species in having arrow-head shaped leaves, stipules attached to the base of petiole, stigma is three lobed and fruit is oblong which dehisces explosively. Morphometric data taken from 15 plants of a population from Thanala is given in table 28.

Cytology of V. betonicifolia

Meiotic analysis reveals that its diploid chromosome count is 2n=12 with equal segregation (6:6) at anaphase I.

Detailed Analysis of result

Morphological variability in V. pilosa

Variability analysis in V. pilosa regarding leaf number is important as they contain medicinal components. The maximum & second maximum leaves were noted in Kapra (7.81), Bhimber-gali (7.45) and Dera-gali (7.45) plants, followed by those of Jai-ghati(7), Khellani(6.81), Pranoo(6.45) and Bhairon-ghati (6.36). The largest leaves (37.92 cm2) were detected in Sanasar & second largest in Jai-ghati plants (19.84 cm2) followed by those of Bhairon-ghati (16.70 cm2) and Maniyal-gali (14.99 cm2).

As Viola flowers are used in preparation of therapeutic and perfumery items, determination of their number is equally important. Per plant, flower number vary from 1.9 (Sartingal & Bhimber-gali) to 4 (Kapra), with plants growing in Jai-ghati, Khellani, Pranoo, Dera-gali and Bhairon-ghati bearing 2.5-3.1 flowers per plant. As plants of these six populations also produce more leaves, these seem to be more vigorous than the rest. The flower size is maximum in Jai-ghati (5.48cm2) plants followed by Bhairon-ghati (4.98 cm2) and Sanasar (4.59cm2). As such, leaf and flower number and their sizes are greater in Jai-ghati plants. Similarly, number of leaves and flowers are more in Dera-gali and Kapra plants. Likewise, both leaf and flower-size is greater in Dera-gali, Sanasar and Bhairon-ghati plants. Therefore, on taking flower and leaf number and their sizes together, plants growing within altitudinal range of 1998-2877 masl, including Dera-gali, Bhairon-ghati, Jai-ghati and Sanasar along with another from Kapra (1651 masl) seems to be more vigorous. Greater vigorosity in the aforementioned plants might be due to prevailing environmental conditions or owing to their being genotypically different. As such, existence of plants bearing more and large sized plant parts between 1998-2877 masl indicates that plants growing there exhibit high adaptability and the sites are suitable for cultivation of V. pilosa.

Sexual apparatus of this species comprises of 5 stamens represented by sessile anthers and 3 fused carpels. While anther-size ranges from 2.36x1.4 (Bhairon-ghati) to 2.72 x 1.31 mm2 (Kapra), carpel length varies from 3.55 (Bhairon-ghati) to 4.11 mm (Dera-gali). Analysis of phenotypic traits using one-way ANOVA reveals existence of statistically significant differences in all traits, except anther and seed width, and seed number per fruit. For all traits, values of PCV are much higher than GCV which suggests a slightly greater impact of environmental factors on expression of traits. Heritability in the broad sense (H2) in current populations ranges from 0.008 to 0.71. It is quite high for posterior and anterior petal length (0.71 and 0.56) followed by leaf width (0.60), flower width (0.54), anterior sepal length (0.53) and anterior petal width (0.52). This depicts a considerable genetic control in expression of these phenotypic attributes. Redundancy analysis revealed that anterior petal length contributes to maximingly towards variability. Cluster dendrogram reveals grouping of 11 populations into 3 clusters. Though cluster-1 contains populations growing between 1181-2377 masl, its subcluster 1A contains populations inhabiting sites>1998 masl and subcluster1B contain populations growing between 1068-1651 masl. Apart from geographical location, altitudes at which plants of studied populations grow seem to have some role in grouping at subcluster level.

Cytological studies in V. pilosa : Somatic cells of V. pilosa contain 20 small (2.7-3.8 µm) chromosomes forming 10 homomorphic pairs. All/maximum meiocytes of ten populations contain 20 chromosmes which pair as 10 bivalents though few cells (5.8-9%) of 7 populations and 23% cells of Khellani contain multivalents. Occurrence of 10 bivalents and 10:10 segregations in maximum meiocytes at meta- and anaphase I is a pointer towards the diploid nature of V. pilosa. Six to seven V. pilosa populations contain few aneuploid (1.6-11.1) cells and those with multivalents which must have resulted from unequal segregation of chromatids during pre-meiotic mitotic division/s and strucrtural chromosomal changes respectively. In V. pilosa populations, data on meiocytes having variable chromosome numbers and multivalents (percent deviant cells) was compared with the pollen viability. Compared to the percent deviant cells which vary between 5.8-10% (except Khellani having 23% deviant cells), the pollen viability is quite low which ranges 39 to 50%.

Biochemical studies in V. pilosa

Leaf samples of V. pilosa largely contain phenols, flavonoids and sterols. For the studied samples, TFC was more than TPC. Besides TPC and TFC is maximum in leaves of Bhairon-ghati (5.26 mg GAE/g, 9.91 mg QE/g) and minimum in Bhimber-gali. Since plants of Manyal-gali, Sartingal and Bhairon-ghati plants growing between 1700-2000 masl contain more polyphenols, these sites seem more suitable for cultivation of V. pilosa and the Bhairon-ghati plants with maximum polyphenols can be considered for leaf collection for medicinal purpose.

Greater antioxidant potential of leaf/floral extracts of a medicinal species is important as it assists in quenching free radicals generated during cellular metabolism in an organism. Antioxidant potential of V. pilosa samples expressed in terms of IC50 values, is maximum in leaves of Bhairon-ghati, Sartingal, Manyal-gali plants, growing between 1759-1998 masl and one (Pranoo) found at 1068masl have lesser IC50 values viz. 11.46, 10.3, 9.61 µg/ml and 11.29 µg/ml respectively. In these four samples, the higher antioxidant capacity is probably on account of their having more polyphenols.

Stigmasterol is an important sterol posessing blood purifying, anti-diabetic, anti-inflammatory properties. Presently, sterol concentration when determined from samples of V. pilosa plants growing at varying altitudes, revealed lesser quantity and variability (0.010414-0.019726 µg/ml). As such, concentration of stigmasterol is maximum in Bhairon-ghati plants (0.019726 µg/ml) followed by Jai-ghati (0.018171µg/ml) and Dera-gali (0.016231µg/ml) Sanasar (0.015699µg/ml), all growing between 1998-2377 masl. As concencentaration of phenolics and sterols is more in plants growing between 1998-2377 m, this range can be considered for cultivating V. pilosa for commercial purpose.

ISSR analysis in V. pilosa:

Screening of 11 V. pilosa populations and their 66 genotypes with 8 primers yielded 74% polymorphism. Total genetic diversity of studied V. pilosa populations (Ht) is 0.2678, with proportion of intra-population diversity being less (Hs=0.0768) than proportion of inter-population diversity (Gst) (0.7134). As V. pilosa plants producing chasmogamous flowers are likely to form seed on open pollination and cleistogamous ones by autogamy, high genetic differentiation of different populations seems to be the outcome of these having vegetative propagation and autogamy as major

modes of multiplication.

Gene flow between V.pilosa populations is quite low (0.2009). As in V. pilosa, vegetative and autogamy seem to be major means of propagation, in each population, some allele may got fixed or other may have lost, leading to lesser gene flow. Quite short flowering period, falling of seed near the parent plants and their short distance dispersal by ants are other factors accounts for low gene flow. The values of Nei's genetic diversity (H) and Shannon's (I) information indices are maximum (H=0.1610; I=0.2382) in Kapra population followed by Khellani (H=0.1297; I=0.1939) and minimum for Manyal-gali population (H=0.0296 and I=0.0432).

Molecular dendrogram revealed placement of studied populations in 3 clusters and additional subclusters. Five populations of 3 districts pooled in cluster-1 are further separated into 3 sub-clusters, with each sub-cluster having populations of separate district viz. Reasi (Bhairon-ghati), Poonch (Dera-gali), Doda (Sartingal, Pranoo, Jai-ghati). Cluster 2 contains populations of Poonch (except Manyal-gali) and cluster 3 contains populations of Doda except Sanasar. This separation seems to partly based on the geographical locations and partly on account of altitudes at which present populations grow.

For determining relationship between genetic and physical distance of studied V. pilosa populations, Mantel's test was performed which revealed existence of positive correlation between two parameters barring few exceptions. For example Dera-gali is less distant from Bafliaz (2.1Km), Bhimber-gali (12.1 Km) and Manyal-gali (3.2 Km) but has moderate genetic distance (0.2970, 0.2245, 0.2797).

Comparison of morphological and molecular dendrograms reveals similarities in some segregation patterns. It is indicated by (i) placement of Pranoo, Jai-ghati and Bhairon-ghati populations in cluster-1 (ii) presence of Bhimber-gali, Bafliaz and Manyal-gali populations in cluster 2 (iii) placement of Manyal-gali and Bafliaz in same subcluster (2B) of cluster-2. The placement of some populations in same cluster is, generally taken as an indicator of possible gene flow between them. In our population, there exists possibility of gene flow between some populations in same sub-cluster (Bafliaz and Manyal-gali), if other conditions (such as presence of enough pollinators) remain favourable.

Some differences, however, were noted regarding groupings based on morphological and molecular datasets. For example, Sanasar population stands apart in cluster 3 of morphological dendrogram but groups with Kapra and Khellani in cluster 3 of molecular dendrogram. This discord can be attributed to environment playing more role in expressing phenotypic traits. Because Sanasar plants experience annual winter snowfall and face more cold conditions, their separation at morphological level from others (Kapra) might be owing to prevailing specific climatic conditions and habitat (dense shade of Berberis). Secondly, morphological traits associated with few specific gene loci, might not have got detected while analysing large molecular data. Finally, genetic variation may result on account of capturing of different regions of genome by markers other than those responsible for morphological variability.

For detecting the contribution of specific markers towards significant level of variability (50%), Redundancy anlysis was performed. This detailed anaylysis indicates that F locus contributes maximingly towards genetic variability whereas towards morphological variability, petiole length and anterior petal length contribute to great extent. However, the behaviour of Locus F is not similar to anterior petal length or petiole length (e.g. maximum petiole length is noted in Sanasar plants which do not show maximum value of F locus). It indicates that the locus appears not to be part of genome which codes for petiole length.

Morphological variability in V. canescens

Studied five V. canescens populations also reveal marked differences in important vegetative and reproductive traits. Of the studied traits, leaf number and size depicted substantial variability. While the maximum leaves are differentiated by plants of Channi-prat (11.63), the least by Jammu (6.36) plants The largest flowers are borne by plants of BGSBU (3.76 cm2) followed by Channi-prat and the smallest (1.8cm2) ones by Jammu plants. Besides, the total petal size is maximum in flowers of Channi-prat followed by Devi-pindiyan and BGSBU plants. Since Devi-pindiyan plants bear considerable number of flowers having large sized plants, petal size, these appear important for cultivation purpose for personal use as well as for suppling to drug houses.

In this species, marginal differences have been noted reproductive traits. These include the anther size (1.71x1.07 mm2 in Natha-top to 2.12x1.21 mm2 in Devi-pindiyan) and the carpel length (1.62 mm in Jammu to 1.87 mm in Devi-pindiyan). Results of one-way ANOVA reveal significant differences of all the studied traits amongst populations except six (leaf length, leaf width, lateral sepal width, posterior petal length, anther width and seed width). More values of phenotypic co-efficient of variation (8.90-58.92) than genotypic ones (1.53-55.02) indicates slightly greater influence of environment on expression of traits. H2 (heritability in broad sense) values of some traits such as leaf length, flower width, pedicel length, number of seeds per plant and seed length is > 0.5 which is a pointer towards the considerable role of genetic factors in their expression. Further, redundancy analysis reveals that flower width contributes towards maximum variation. Using morphological data set of the studied V. pilosa populations, dendrogram prepared. This dendrogram reveals that sub-tropical Jammu population (304 masl0 falls in cluster 2, with remaining ones encompassimg in cluster 1. However, subclusters (1A and 1B) of cluster 1 do not separate populations based on altitudes, though populations of some districts get separated at sub-cluster level (Reasi in subcluster-I, Ramban in subcluster -II except Rajouri).

Cytological studies in V. canescens: Maximum cells of studied populations contain 20 chromosomes except Natha-top which contain few aneuploids cells (2.94%) and those with multivalents. Comparison on data onpercent deviant cells (aneuploid cells and with multivalent) and pollen viability reveals that plants contain no or few deviant cells (2-11.7%) and show good pollen viability (92 to 99%).

Biochemical studies in V. canescens

All V. canescens samples contain more flavonoids whose content is maximum in leaves of Devi-pindiyan plants (13.87±0.021mg GAE/g, 19.77±0.032 mg QE/g), followed by BGSBU and Natha-top. Existence of plants accumulating more phenolics in the regions falling between 1163-2265 masl is indicative of more adaptability in plants growing of these sites. Further, leaves of plants growing between 1163-2265 masl have lesser IC50 values (8.79, 9.89 and 13.54µg/ml) and more antioxidant potential.

ISSR analysis in V. pilosa:

Present screening of 11 V. pilosa populations and their 66 genotypes with 8 primers yield 74% polymorphism. Total genetic diversity of studied V. pilosa populations (Ht) is 0.2678, with proportion of intra-population diversity being less (Hs=0.0768) than proportion of inter-population diversity (Gst) (0.7134).

Gene flow between different V.pilosa populationsis quite low (0.2009). As in V. pilosa, vegetative multiplication and autogamy are major means of propagation, in each population, some allele may got fixed or other may have lost, leading to lesser gene flow. Short flowering period, falling of seed near parent plants and their short distance dispersal by ants are other factors accounts for low gene flow. Nei's genetic diversity (H) and Shannon's (I) information indices, indicates maximum intra-population diversity (H=0.161; I=0.238) in Kapra population and minimum in Manyal-gali population (H=0.0296and I=0.0432).

Molecular dendrogram based on 43 loci and 11 polymorphic loci revealed placement of studied populations in 3 clusters. Five populations of 3 districts pooled in cluster-1 which got separated into three sub-clusters, with each sub-cluster having populations of separate district viz. Reasi (Bhairon-ghati), Poonch (Dera-gali), Doda (Sartingal, Pranoo, Jai-ghati). Cluster 2 contains populations of Poonch (except Manyal-gali) and cluster 3 contains populations of Doda except Sanasar. This separation is partly based on the geographical locations and partly on account of altitudinal sites.

Comparison of morphological and molecular dendrograms reveals some similarities such as (i) placement of Pranoo, Jai-ghati and Bhairon-ghati populations in cluster-1; Bhimber-gali, Bafliaz and Manyal-gali populations in cluster 2 and Manyal-gali and Bafliaz in same subcluster (2B) of cluster-2. The placement of some populations in same cluster/sub-cluster indicates possible gene flow between them (Bafliaz and Manyal-gali).

Some differences, however, existed regarding groupings based on morphological and molecular datasets. For example, Sanasar population stands apart in cluster 3 of morphological dendrogram but groups with Kapra and Khellani in cluster 3 of molecular dendrogram. This discord can be attributed to environment playing more role in expressing phenotypic traits. Because Sanasar plants experience annual winter snowfall and face more cold conditions, their separation at morphological level from others (Kapra) might be owing to prevailing specific climatic conditions and habitat (dense shade of Berberis). Secondly, morphological traits associated with few specific gene loci, might not have got detected while analysing large molecular data. Finally, genetic variation may result on account of capturing of different regions of genome by markers other than those responsible for morphological variability. As per RDA, F locus contributes maximingly towards genetic variability.

ISSR analysis in V. canescens:

For V. canescens populations, 5 ISSR primers were used and 30 loci were detected which exhibit 73.33% polymorphism. While total genetic

diversity (Ht) is 0.2252, intra-population genetic diversity is quite low (Hs=0.1420). Between different populations, there exists high genetic differentiation (Gst=0.3697) and low gene flow (Nm=0.2180) probably becuse of cleistogamy and clonal propagation as the multiplying means alongwith patchy distribution of plants, their geographic isolation and seed dispersal through myrmecochory.

Nei's genetic diversity (H) and Shannon's (I) information indices reveals least genetic diversity (H=0.0637, I=0.0946) in Jammu and maximum (H=0.2280; I=0.3411) in plants of Devi-pindiyan. In molecular dengrogram, studied populations were placed in different clusters, with plants of sub-tropical zone (Jammu in cluster 2 and Devi-pindiyan in subcluster 1A) getting separated from those inhabiting temperate zones. This separation seems to be largely influenced by geographical and climatic factors. RDA analysis reveals that locus I contributing maximingly towards total genetic variability. Comparison of dendrograms based on morphological and molecular data reveals segregation of Jammu from the rest. However, in molecular dendrogram, Natha-top and Channi-prat form one group, in morphological dendrogram, Devi-pindiyan pairs with BGSBU.

V. betonicifolia (Morphological studies)

Plants of V. betonicifolia from 4 districts (Rajouri, Ramban, Doda, Poonch) of Jammu bear short rhizome and tufted 9.5±0.41 bright-green leaves which are 4.2cm long and 3.1cm broad and have 5.1cm long petioles. Upper petiolar portion near attachment to lamina is winged which is nearly 0.25cm broad. To each petiole, two linear foliaceous and persistant 1.2cm long stipules that are are adnate and are 1-1.6 cm lonf. This species bloom from May-August and bear pentamerous and zygomorphic flowers.

Cytological studies in V. betonicifolia;

PMCs of this species have 2n=12 chromosomes which showed regular behavior.

iv)V ×wittrockiana (Morphological analysis)

Mmorphometric studies were carried out on 14 floral variants: White-purple, Light-Purple, White (purple blotch on 3 petals), White (purple blotch on 5 petals), Yellow with black streaks, Yellow with maroon blotch, Yellow Magenta (blue blotch), Royal blue with yellow blotch, Light blue Maroon with purple blotch, Red yellow with maroon blotch and Maroon.

Cytological studies in V×wittrockiana: The three cultivars with yellow, yellow with maroon blotch and maroon with purple blotch flowers contain 42 chromosomes and two with red-yellow with maroon blotch and light purple flowers contain 46 chromosomes which associated as multi-, bi- and univalents and exhibited irregular segregations.

iv) V. biflora: Morphological and cytological studies:

Few V. betonicifolia plants growing in hilly slopes of Gool (33°16' N 75°10' E; 1850 masl) of Ramban were studied cytologically which revealed them having 20 chromosomes which revealed normal chromosome behavior.

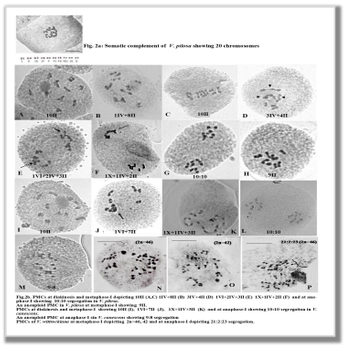

Fig.2.cyto-viola species

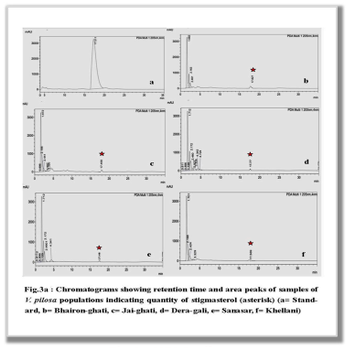

Fig.3a-HPLC-pilosa

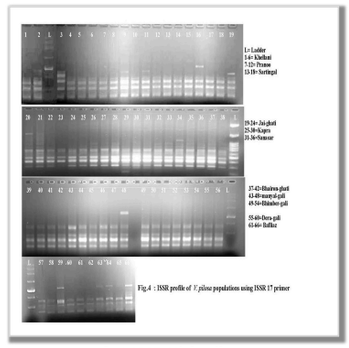

fig4-ISSR-Viola pilosa

Fig.1 Morphology of Viola

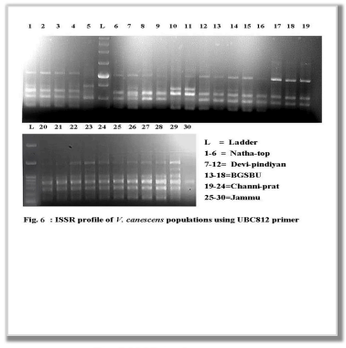

Fig.5 ISSR in Canescens

Conclusions

Presently different populations of V. pilosa, V. canescens, V. betonicifolia, V. biflora and Vx wittrockiana have been analysed for morphological, cytological, biochemical and molecular variability. Morphological studies in 11 V. pilosa and 5 V. canescens populations revealed marked variability. Maximum and larger leaves and flowers in V. pilosa are produced by plants of Bhairon-ghati, Sanasar & Jai-ghati, growing between 1998-2877m. As such, these plants can be selected as superior genotypes. Similarly, altitudinal sites between 1998-2877m can be selected for cultivation of these medicinal species. In case of V. canescens, more vigorous plants were found in Natha-top and the plants of smaller size were located in Jammu.

Cytological studies revealed diploid chromosome number of 2n=20 in V. pilosa and V. canescens, though a few cells exhibit aneuploidy and contain multivalents. While V. pilosa plants exhibit 39-50% pollen viability, those of V. canescens depicts >90% viability. As such, chromosome number of these species has been established for the first time and also the few meiotic anomalies witnessed are hitherto not on the record.

Biochemical analysis revealed that V. pilosa plants of Manyal-gali, Sartingal and Bhairon-ghati (1759-1998 masl) and V. canescens plants of Devi-pindiyan contain more sterols, polyphenols and have greater antioxidant activity. These plants can be selected for cultivation and sites can identifed for growing elite genotypes. ISSR analysis in different populations of V. pilosa and V. canescens revealed that these exhibit high total species diversity than average diversity within groups of populations, depict lesser gene flow and greater inter-population variability. This appears due to tendency of these plants to multiply by vegetative means and falling of seeds near the proximity of parent plants. Besides, molecular dendrograms depict that the populations falling in the same group were either growing in geographically similar conditions or nearly similar altitudinal sites.

V. betonicifolia: Five populations of this species, revealing scarce distribution. These plants uniformly contain 2n=12 chromosomes which showed regular behavior at diakinesis, metaphase and anaphase-I.

Viola×wittrockiana hybrids: Fourteen Viola×wittrockiana cultivars have been analyzed for morphological aspects which revealed variations in

different traits. Of these, five were analysed cytological aspects. While five hybrid forms contained 42 chromosomes, two had 46 chromosomes. Besides, meiosis in these hybrids is unusual in having high frequency of pollen mother cells with complex associations.
V. biflora: A single population noted growing in Gool (33°16' N 75°10' E) of Ramban District (1850 masl) contain 2n=12 chromosomes which showed regular behavior.

Scope of future work

1. During present investigation, plants of Bhairon-ghati, Sanasar & Jai-ghati, growing between 1998-2877m are found to be more vigorous and the sites suitable cultivations. However, detailed studies are needed to be done for confirming this claim. Besides, attempts can be made to cultivate these for determining whether vigrosity is genotypically controlled or the climatic conditions where the studied plants grow.
2. For biochemical analysis, more signature bioactive compounds need to be investigated which during present investigation can be done due to paucity of time and funds.
3. Studies on molecular variability can be extended to include the codominant markers like Simple Sequence Repeats.
4. More explorations are required to identify more populations of Viola betonicifolia and Viola biflora from different altitudinal sites for determining whether or not altitudinal variations result in alteration of ploidy level or not.
5. As different species of genus Viola has few characters for identification and these also overlap at species level, precise identification of these is needed. For this purpose, DNA barcoding can be of great help.

Any Other Information :

List of Publications (only from SCI indexed journals)

Title of the Paper	List of Authors	Name of Journal	Type of Journal	Status
First detection of endopolyploidy in tapetal cells and chromosomal anomalies in meiocytes of Viola pilosa cytotypes (2n=20) from Pir-Panjal (Himalayas)	Iqbal T, Sharma G	JOURNAL OF GENETICS	National	Published

List of Papers Published in Conference Proceedings, Popular Journals

Title of the Paper	List of Authors	Name of Journal	Type of Journal	Status
Studies o morphological and biochemical variability of Viola pilosa Blume from Kishtwar and Rajouri districts (J&K)	Romica Bhandari and Sharma G	Dissertation of M. Sc	National	Published
Cultivation and Utilization of Viola tricolor var. hortensis: An Ornamental and Medicinally Important Herb	Ritika Jamwal and Geeta Sharma	Sustainable Agriculture: Recent Advances.-Edited book	National	Published
DISTRIBUTION, MORPHOLOGY AND UTILIZA TION OF MEDICINALLY IMPORTANT VIOLA BETONICIFOLIA SM. IN JAMMU PROVINCE	RITIKA JAMW AL, T ASIR IQBAL AND GEETA SHARMA	J. Indian bot. Soc.	National	Published

List of Patents filed/ to be filed

No Such Record Found

Equipment Details

Equipment Name	Cost (INR)	Procured	Make & Model	Utilization %	Amount Spent (INR)	Date of Procurement
Electronic balance	99,992	Yes	Mettler toledo-ME-204	80	91,35,000	01 November, 2019
Field Camera	96,305	Yes	Cannon-EOS 77D	80	95,998	15 May, 2020
Gel Documentation system	7,99,995	Yes	Eppendorf,s Quantum	80	7,56,000	13 May, 2020
Water bath cum Shaker	99,997	Yes	Scientech-SE136	85	27,000	13 May, 2020

Plans for utilizing the equipment facilities in future

Further studies at cytological, biochemical and molecular level in Viola species are in progress. We are working on morphological, cytological ad molecular aspects of Allium stracheyi, Carissa spinarum and Pyrus pashia. The equipment purchased will be used for carrying out these investigations. Besides, the research scholars of other laboratories are also working on molecular aspects of various angiospermic taxa and are also using the the purchased equipment. At the same time, a research scholar from central University, Jammu is also using the equipment received under the project

Table1:Geo-coordinates & habitat characteristics of sites inhabited by *Viola* speciesin J & K

No	Species	Distt.	Pops. Pop. Code)	Lat (N)	Long(E)	Alt. masl	Dist*	Locality
1.	*V. pilosa*	Doda	Khellani (Khe-P)	33°7.9'	75°31.25'	1181	Dense	Fields in shade of *Quercus* trees
2.			Sartingal (Sar-P)	32°56.3'	75°44.9'	1902	Scarce	Steep slope on roadside along *Pinus* trees
3.			Jai (Jai-P)	33°1.1'	75°46.1'	2377	Dense	Steep slope along *Pinus* trees
4.			Kapra (Kap-P)	33°0.5'	75°42.5'	1651	Dense	Sleep slopes alongwith*Pinus* trees
5.		Ramban	Sanasar (San-P)	33°7.4'	75°15.4'	2101	Dense	Steep slopes alongwith*Cedrus* trees
6.		Reasi	Bhairongh ati (Bha-P)	33°1.5'	74°56.7'	1998	Dense	Steep slopes alongwith*Quercus* trees
7.		Rajouri	ManyalGali (Man-P)	33°34.2'	74°22.5'	1759	Scarce	Steep slopes on road side
8.		Poonch	Bhimber Gali (Bhi-p)	33°34.3'	74°15.6'	1647	Scarce	Moderate slopes alongwith*Pinus* trees
9.			Dera Gali (DKG-P)	33°35.7'	74°21.2'	2085	Dense	Steep slopes alongwith*Pinus* trees
10.	*V.canescens*	Reasi	Devi Pindiyan (Dep-C)	33°0.6’	74°59.8’	1321	Scarce	Steep slopes alongwith*Juglans* trees
11.		Ramban	Natha Top (Ntu-C)	33°5.9’	75°17.3’	2265	Dense	Steep slopes alongwith*Cedrus* trees and grasses

12.		Rajouri	BGSBU (Bgs-C)	33°23.75’	74°20.87’	1163	Dense	Steep slopes in the campus
13.		Rajouri	ChanniPrat (Chp-C)	33°04’	74°27’	535	Scarce	Moderate slopes along *Pinus* trees
14.		Jammu	Jammu (Jmu-C)	32°43.8’	74°51’	304	Scarce	Experimental fields
15.	*V. betoniciloia*	Rajouri	Dharal	33°27.57'	74°25.03'	1661	Scarce	Moist-gritty soil of slopes under Pinus trees
		Ramban	Gool	33°16.34'	75°10.44'	1850	Scarce	Moist-gritty soil of sloppy areas
		Doda	Thanala	33°55.13'	75°46.15'	2202	Scarce	Gritty soil on road-side
		Poonch	Molsar	33°52.39'	74°19.21'	2491	Scarce	Gritty soil of hilly slopes
			Mandi	33°46.28'	74°16.33'	2039	Scarce	Hilly slopes
16.	*V. odorata*	Kashmir	???					
17.	*V.biflora*							
18.	***V. wittrockiana***	Cultivars						
19.		White-purple	Bot. Gar.				Cultivated	Bot. Garden
20.		Light-Purple	Bot. Gar				,,	Bot. Garden
21.		White, purpleblotch on3 petals	Bot. Gar				,,	Bot. Garden
22.		White; purple blotch onpetal	Bot. Gar				,,	Bot. Garden
23.		Yellow, with blackstreaks	Bot. Gar.				,,	Bot. Garden
24.		Yellow, withmaroonblotch	Bot. Gar				,,	Bot. Garden
25.		Yellow	Bot. Gar				,,	Bot. Garden
26.		Magenta,withblueblotch	Bot. Gar				,,	Bot. Garden
27.		Royalblue	Bot. Gar					Bot. Garden
28.		Royal blue withyellowblotch	Bot. Gar.					Bot. Garden

29.		Lightblue	Bot. Gar					Bot. Garden
30.		Maroon with purpleblotch	Bot. Gar					Bot. Garden
31.		Redyellowwithmaroonblotch	Bot. Gar					Bot. Garden
32.		Maroon	Bot. Gar					Bot. Garden

Table 2. List of constituents in extraction buffer

S.No.	Name of the constituent (Manufacturing company)	Concentration	
		Stock solution	Working solution
1.	Tris (Himedia)	2 M	100 mM
2.	EDTA (Himedia)	0.5 M	20 mM
3.	NaCl (Himedia)	5 M	1.4 M
4.	CTAB (Himedia)	10%	2%
5.	β-mercaptoethanol (Himedia)	-	2%
6.	PVP (Himedia)	-	2%
7.	TAE (Himedia)	50x	1x

Table 3: Components of PCR reaction mixture

Reagents	Initial Concentration	Final Concentration	Total volume/25µl
Taq buffer	5X	1X	5 µl
dNTPs	10 mM	0.2 mM	0.5 µl
$MgCl_2$	25 mM	3 mM	3 µl
Primer	100 µM	1 µM	0.25 µl
Taq polymerase	5 U	3 U	0.6 µl
Template		50 ng	2.0 µl
Autoclaved ddH_2O			13.65 µl

Table 4: Thermal Cycler program

Steps	Process	Temperature	Duration (minutes)
1	Initial denaturation	95˚C	5
2	Denaturation	94˚C	1
3	Annealing	48˚C	1
4	Elongation	72˚C	2
5	Final extension	72˚C	7
6	Final hold	4˚C	

Table 5: Vegetative morphometric details of *V. pilosa* from Jammu province

Trait	Khellani	Sartingal	Bhairon-ghati	Kapra	Sanasar	Dera-gali	Manyal-gali	Bafliaz	Bhimber-gali	Jai-ghati	Pranoo
Leaf number /plant	6.81±0.44	5.54±0.45	6.36±0.62	7.81±0.44	5.27±0.54	7.45±0.62	4.36±0.38	4.36±0.41	7.45±0.62	7±0.57	6.45±0.45
Leaf length (cm)	3.92±0.11	5.64±0.41	4.43±0.5	2.93±0.13	7.01±0.21	3.49±0.11	5.70±2.33	3.76±0.36	3.5±0.12	5.35±0.36	3.6±0.33
Leaf width (cm)	2.94±0.11	2.26±0.13	3.77±0.28	2.38±0.11	5.41±0.23	2.8±0.13	2.63±0.29	3.02±0.31	2.60±0.13	3.71±0.21	3.06±0.32
Leaf size (cm^2)	11.52	12.74	16.70	6.97	37.92	9.77	14.99	11.36	9.1	19.84	11.01
Petiole length (cm)	4.5 ±0.39	1.84±0.15	8.5±1.03	1.96±0.16	11.64±0.61	5.48±1.03	5.35±1.3	6.06±0.74	6.09±0.81	7.4±0.69	5.47±0.73
Stipule length (cm)	1.34±0.8	0.76±0.47	1.33±0.81	0.95±0.45	0.84±0.57	1.03±0.41	1.21±0.42	1.08±0.69	0.88±0.42	1.18±0.69	1.12±0.66
Stipule width (cm)	0.25±0.25	0.18±0.15	0.36±0.2	0.30±0.22	0.33±0.20	0.30±0.25	0.34±0.24	0.28±0.22	0.24±0.21	0.3±0.35	0.34±0.34

Table 6: Matrices of floral traits of 11 *V. pilosa* populations

Traits	Khellani	Sartingal	Bhairon-ghati	Kapra	Sanasar	Dera-gali	Manyal-gali	Bafliaz	Bhimber-gali	Jai-ghati	Pranoo
Flower no./ plant	2.90±0.25	1.90±0.21	2.45±0.24	4±0.40	2.36±0.20	3.09±0.21	2.72±0.23	2.72±0.27	1.90±0.21	2.81±0.22	2.90±0.28
Flower L (mm)	20.45±0.62	18±0.53	20.18±0.40	18.81±0.64	20.81±0.81	18.72±0.51	18.90±0.45	16.81±0.37	18.18±048	20.72±0.60	20.45±0.57
Flower W (mm)	21.36±0.97	20.27±0.59	24.72±0.30	23.72±0.51	22.09±0.66	21.45±0.77	20.72±0.78	18.54±0.51	18.81±0.58	26.45±0.56	20.54±0.53
Flower size (cm^2)	4.36	3.64	4.98	4.46	4.59	4.01	3.91	3.11	3.41	5.48	4.20
Pedicel L(cm)	6.46±0.39	4.85±0.24	8.19±0.45	5.16±0.28	5.13±0.17	5.64±0.25	5.29±0.28	6.17±0.28	7.13±0.27	6.99±0.38	6.17±0.29
Ant Sep L (mm)	7.9±0.27	6.45±0.13	7.05±0.11	6.4±0.21	7.05±0.21	6.3±0.2	5.8±0.10	5.9±0.22	6.5±0.14	7.05±0.21	5.75±0.20
Lat Sep L (mm)	6.8±0.31	6.5±0.12	7.2±0.29	7±0.2	6.35±0.12	6.1±0.06	6.4±0.13	6±0.2	5.75±0.08	6.3±0.16	5.9±0.22
Post Sep L (mm)	6.5±0.30	5.75±0.16	5.75±0.24	6±0.15	5.25±0.17	5.4±0.13	4.6±0.15	5.75±0.12	4.9±0.1	5.75±0.14	5.9±0.17
Ant Sep W (mm)	2.14±0.07	1.7±0.07	2±0	1.81±0.1	2.27±0.07	1.86±0.1	1.86±0.07	1.54±0.14	1.77±0.12	2.0±0.13	1.95±0.04
Ant Sep size (mm^2)	16.906	10.96	14.1	11.58	16.1	11.71	10.78	9.1	11.5	14.1	11.21
Lat Sep W (mm)	1.86±0.13	1.36±0.07	1.631±0.1	1.55±0.2	1.45±0.1	1.5±0.13	1.68±0.07	1.36±0.11	1.31±0.07	1.54±0.14	1.95±0.57
Lat Sep size (mm^2)	12.64	8.84	11.74	10.85	8.6	9.15	10.75	8.16	7.53	9.702	11.5
Post Sep W (mm)	1.55±0.17	1.04±0.04	1.13±0.07	1.13±0.07	1.22±0.07	1.41±0.11	1.0±0	1.13±0.09	1.18±0.07	1.18±0.07	1.13±0.08
Post Sep size (mm^2)	10.1	5.98	6.49	6.78	6.41	7.61	4.6	6.49	5.78	6.78	6.66
Total Sep size (mm^2)	69.19	45.58	58.17	51.64	55.81	49.33	47.66	41.01	43.84	54.38	52.08
Ant Pet L (mm)	21.27±0.35	20.18±0.35	19.90±0.36	19.54±0.45	20.45±0.38	18.72±0.48	19.63±0.43	18.72±0.61	17.27±0.52	16.45±1.02	20±0.46
Lat Pet L(mm)	17.09±0.31	18.09±0.39	16.27±0.54	16.81±0.48	15.09±0.96	13.90±0.65	15±0.46	15.54±0.65	15.45±0.45	15.90±0.53	18±0.33
Post Pet L(mm)	16.45±0.34	17.45±0.43	14.27±0.30	16.09±0.47	13.72±0.8	13.36±0.41	13.36±0.54	13.54±0.51	14.36±0.54	17.36±0.45	17.27±0.23
Ant Pet W(mm)	7.91±0.36	10.18±0.29	10.36±0.24	8±0.3	7.9±0.34	10.45±0.49	9.09±0.21	7.54±0.24	10 ± 0.38	10.63±0.33	9.81±0.29
Ant Pet size (mm^2)	168.24	205.43	206.16	156.32	161.55	195.62	178.43	141.14	172.7	174.86	196.2
Lat Pet W(mm)	7±0.13	8.36±0.2	8±0.30	6.72±0.27	6.45±0.47	8.09±0.51	7.18±0.22	6.63±0.15	8.09±0.36	7.09±0.28	7.72±0.30
Lat Pet Size (mm^2)	119.63	151.23	130.16	112.96	97.33	112.45	107.7	103.03	124.99	112.73	138.96
Post Pet W(mm)	6.72±0.14	8.±0.26	7.18±0.37	6.18±0.29	6±0.61	7.81±0.50	6.81±0.18	6.63±0.24	7.63±0.41	7.09±0.25	6.63±0.47
Post Pet size (mm^2)	110.54	139.6	10.45	99.43	82.32	104.34	90.98	89.77	109.56	123.08	114.50
Total Petal size (mm^2)	628.59	787.09	671.40	581.12	520.856	629.21	575.79	526.75	641.81	646.49	703.12
Anther L(mm)	2.63±0.13	2.63±0.11	2.36±0.16	2.72±0.12	2.68±0.12	2.5±0.13	2.63±0.13	2.5±0.13	2.54±0.12	2.68±0.15	2.72±0.12
Anther W(mm)	1.31±0.07	1.18±0.07	1.45±0.10	1.31±0.12	1.13±0.07	1.36±0.13	1.41±0.11	1.36±0.07	1.27±0.07	1.5±0.11	1.18±0.07
Carpel L (mm)	3.83±0.16	3.72±0.14	3.55±0.15	3.94±0.05	3.83±0.11	4.11±0.07	3.72±0.16	4.05±0.05	3.72±0.14	3.77±0.14	3.77±0.14

Ant=Anterior; Lat=lateral; Post=posterior; Sep=Sepal; Pet=Petal; L=length; W=width

Table 7: Matrices of fruit and seed related traits of 11*V. pilosa* populations

Traits	Khellani	Sartingal	Bhairon-ghati	Kapra	Sanasar	Dera-gali	Manyal-gali	Bafliaz	Bhimber-gali	Jai-ghati	Pranoo
Fruit no./ plant	2.63±0.33	2.54±0.24	3.63±0.27	3±0.19	3.45±0.24	3.27±0.30	2.27±0.23	2.81±0.22	2.45±0.15	2.81±0.22	2.90±0.31

Fruit L (mm)	6.45±0.27	6.08±0.16	6.33±0.25	5.75±0.32	5.5±0.13	6.12±0.20	6.04±0.18	5.6±0.27	6.125±0.17	6.37±0.19	6.08±0.14
Fruit W(mm)	6.5±0.27	6.16±0.15	6.79±0.22	6.16±0.20	5.12±0.23	6.20±0.14	6.25±0.14	6.16±0.12	5.91±0.16	6.91±0.21	6.25±0.13
Seed no./fruit	20.63±0.81	18.90±0.74	22.72±0.71	19.90±1.88	21.81±1.17	21.54±0.65	19.63±.0.8	19.54±0.67	22.18±1.37	22±0.63	19.63±0.47
Seed L(mm)	2.04 ±0.08	1.86±0.09	2.18±0.07	2.22±0.07	1.86±0.09	2.13±0.09	1.81±0.07	1.81±0.07	2.18±0.10	2.27±0.12	2±0
Seed W(mm)	1.22±0.07	1.09±0.06	1.31±0.07	1.22±0.07	1.13±0.04	1.27±0.07	1.27±0.02	1.13±0.04	1.13±0.1	1.09±0.05	1.18±0.08

Table 8:Matrices of single set of chromosomes of *V. pilosa*

Chromosome No.	**Length (µm)**	**Chromosome No.**	**Length (µm)**
1	3.8±0	6	3.12±0.12
2	3.33±0	7	2.91±0.12
3	3.33±0	8	2.7±0
4	3.33±0	9	2.7±0
5	3.33±0	10	2.7±0
TCL = 62.5 µm, MCL = 3.125µm			

Table 9: PMCs with varying associations and %age pollen viability in *V. pilosa* populations

Population	**Aneuploid cells**	**%age (no.) euploid cells**			**Cells with 10:10 separation**	**% Deviant cells**	**% pollen viability**
		10II	1IV+8II/ 3IV+4II/ 1VIII+6II	1X+1VI+2II	Equal		
Khellani	-	77.4 (24)	16.15 (5)	6.45 (2)	6	29.16	45.1
Pranoo	-	-	-	-	-	-	47.6
Sartingal	-	100 (69)	-	-	11	0	47.6
Jai-ghati	-	100 (28)	-	-	8	0	48
Kapra	-	91.6 (44)	8.4 (4)	-	10	8.33	40.6
Sanasar	-	92.5 (25)	7.5 (2)	-	12	7.4	48.6
Bhairon-ghati	-	93.75 (30)	6.25 (2)	-	7	6.25	41.7
Manyal-gali	-	90 (27)	10 (3)	-	7	10	44.5
Bhimber-gali	1.44(1)	92.7 (64)	5.8 (4)	-	13 (10:10)& 1 (10:9)	7.24	49.9
Dera-gali	2.63 (2)	90.7(69)	6.57 (5)	-	8	9.21	40.4
Bafliaz	-	90.9 (20)	9.1 (2)	-	18	9.1	38.6

Table10: Results of colour tests for phenols, flavonoids and sterols in *V. pilosa*

Phenolic extract (10 mg/ 5ml)	Reagents added	Colour obtained	Indicates presence of
2ml extract	2ml $FeCl_3$	Dark-green	Phenols
2ml extract	2ml NaOH solution	Yellow	Flavonoids
2ml extract	Chloroform (2ml) +2ml conc. H_2SO_4	Red	Sterols

Table 11. Total phenolic content in samples of *V. pilosa* populations

Population	Altitude (masl)	Total Phenolic Content (mg GAE/g)
Khellani	1181	2.16±0.011
Pranoo	1068	3.57±0.016
Sartingal	1902	4.29±0.013
Jai-ghati	2377	1.78±0.019
Kapra	1651	1.66±0.021
Sanasar	2101	2.24±0.024
Bhairon-ghati	1998	5.26±0.028
Manyal-gali	1759	3.45±0.007
Bhimber-gali	1647	1.53±0.012
Dera-gali	2085	2.09±0.027
Bafliaz	1607	2.29±0.009

Table 12. Total flavonoid content in samples of *V. pilosa* populations

Population	Altitude (masl)	Total Flavonoid Content (mg QE/g)
Khellani	1181	5.32±0.023
Pranoo	1068	7.34±0.020
Sartingal	1902	9.71±0.016
Jai-ghati	2377	5.13±0.035
Kapra	1651	4.57±0.019
Sanasar	2101	6.72±0.037
Bhairon-ghati	1998	9.91±0.047
Manyal-gali	1759	8.53±0.016
Bhimber-gali	1647	4.22±0.021
Dera-gali	2085	5.56±0.042
Bafliaz	1607	6.12±0.018

Table 13. IC50 values in samples of various populations of *V. Pilosa*

Population	**Altitude (masl)**	**IC50 Value (µg/ml)**
Khellani	1181	13.22
Pranoo	1068	11.29
Sartingal	1902	10.3
Jai-ghati	2377	24.44
Kapra	1651	16.53
Sanasar	2101	14.81
Bhairon-ghati	1998	9.61
Manyal-gali	1759	11.46
Bhimber-gali	1647	21.21
Dera-gali	2085	20.25
Bafliaz	1607	31.21

Table 14.Retention time, mean under curve and stigmasterol concentration in leaf extracts of *V. pilosa*

Population	Retention time (mins.)	Area (mAU)	Stigmasterol conc. µg/ml
Standard	17.311	7765775	10
		15447292	20
		29787332	40
		60093331	80
Khe-P	17.569	10771	0.014338
Sar-P	17.70	9796	0.01304
Pra-P	17.539	8814	0.011733
Jai-P	17.450	13650	0.018171
Kap-P	17.768	7823	0.010414
San-P	17.48	11793	0.015699
Bha-P	17.827	14819	0.019726
Man-P	17.772	8521	0.011343
Bhi-P	17.554	11659	0.01552
DKG-P	17.77	12193	0.016231
Baf-P	17.530	8035	0.010696

Table 15: Genetic polymorphism analysis using ISSR primers in 11 *V. pilosa* populations

Population	Na	Ne	H	I	PL	PPL
Kapra	1.4286±0.5136	1.2786±0.3803	0.1610	0.2382	12	42.86
Pranoo	1.1466±0.3631	1.1014±0.2568	0.0592	0.0864	4	14.29
Khellani	1.3571±0.4972	1.2155±0.3264	0.1297	0.1939	10	35.71
Sanasar	1.1429±0.3631	1.0803±0.2511	0.0459	0.0694	4	14.29
Bhairon-ghati	1.3352±0.4258	1.197±0.1936	0.0917	0.1755	9	32.13
Jai-ghati	1.1614±0.2673	1.1234±0.0506	0.0423	0.0711	5	17.86
Sartingal	1.1422±0.3631	1.0631±0.1918	0.0409	0.0643	4	14.29
Dera-gali	1.2857±0.4688	1.1813±0.3339	0.1050	0.1558	8	28.57
Bafliaz	1.2143±0.4258	1.1492±0.3138	0.0859	0.1259	6	21.43
Manyal-gali	1.0174±0.2673	1.0505±0.1890	0.0296	0.0432	2	7.14
Bhimber-gali	1.2857±0.4688	1.2196±0.3652	0.1236	0.1782	8	28.57

Table 16: Nei's analysis of gene diversity in populations of *V. pilosa*

Ht	**Hs**	**Gst**	**Nm**
0.2678	0.0768	0.7134	0.2009

Table 17: Relationshipbetween geographic distance (km) (above diagonal) and Nei's genetic distance (below diagonal) of different *V. pilosa*populations

Pop.	Kap-P	Khe-P	San-P	Bha-P	Jai-P	Sar-P	Pra-P	Dkg-P	Baf-P	Man-P	Bhi-P
Kap-P	***	27	42	68	7.5	9.5	14	140	141	137	152.4
Khe-P	0.1238	***	16	47	33	35	12	119	120	110	129.6
San-P	0.1235	0.0278	***	31	49	49	29	99	99	96	106.7
Bha-P	0.2009	0.3593	0.3095	***	77	74	59	83	85	81	89.2
Jai-P	0.1312	0.3143	0.2479	0.0808	***	10	20	146	147	145	155.5
Sar-P	0.1191	0.3240	0.2765	0.0893	0.0049	***	22	149	150	147	158.4
Pra-P	0.1052	0.3228	0.2865	0.0958	0.0097	0.0017	***	126	127	124	136.6
Dkg-P	0.0865	0.3188	0.2341	0.2148	0.1158	0.1107	0.1182	***	2.1	3.2	12.1
Baf-P	0.3803	0.4616	0.3894	0.5663	0.4577	0.4465	0.4610	0.2970	***	4.8	12.7
Man-P	0.3950	0.5156	0.3966	0.5899	0.4623	0.4599	0.4739	0.2797	0.0043	***	13.6
Bhi-P	0.3186	0.3568	0.2710	0.4207	0.3083	0.3099	0.3217	0.2245	0.0504	0.0518	***

Table 18: Population-wise matrices of vegetative traits of *V. canescens*

Trait	BGSBU	Channi-Prat	Jammu	Devi-Pindiyan	Natha-top
Leaf number/plant	7.72±0.58	11.63±0.94	6.36±0.45	8.18±0.53	8.90±0.55
Leaf length (cm)	5.15± 0.20	4.47±0.21	3.61±0.21	4.02±0.24	2.59±0.12
Leaf width (cm)	3.8±0.17	3.76±0.15	3.072±0.13	3.3±0.14	2.37±0.14
Leaf size (cm^2)	19.58	16.83	11.12	13.29	6.14
Petiole length (cm)	3.09±0.13	4.48±0.54	4.78±0.48	5.37±0.53	4.23±0.50
Stipule length(cm)	0.79±0.28	1.72±0.63	0.84±0.20	0.99±0.61	0.87±0.74
Stipule width(cm)	0.15±0.12	0.13±0.12	0.17±0.18	0.15±0.11	0.13±0.14

Table 19: Floral morphometric details of *V. canescens* from Jammu province

Characters	BGSBU	Channi-Prat	Jammu (Botanical-garden)	Devi-Pindiyan	Natha-top
Flower number /plant	2.41±0.39	2.41±0.22	2±0.17	2.83±0.32	3.83±0.34
Flower length (mm)	16.53±0.28	14.61±0.62	11.23±0.51	14.38±0.82	15±0.29
Flower width (mm)	22.38±0.70	24.38±0.58	15.23±0.67	20.38±0.79	21.53±0.79
Flower size (cm^2)	3.76	3.51	1.80	2.71	3.09
Pedicel length (mm)	3.5±0.19	3.02±0.18	2.65±0.15	8.83±0.40	5±0.31
AntSep length (mm)	7±0.21	6.3±0.2	6.7±0.26	7.4±0.31	8±0.25
Lat Sep length (mm)	6.3±0.26	5.8±0.25	6.5±0.26	7.4±0.22	6.7±0.21
Post Sep length (mm)	5.7±0.15	5±0.29	5.7±0.3	6.1±0.31	6.1±0.23
Ant Sep width (mm)	2.13±0.07	1.95±0.04	1.63±0.09	1.95±0.08	1.91±0.09
Ant Sep size (mm^2)	14.76	12.34	11.01	14.39	15.27
Lat Sep width (mm)	1.36±0.11	1.31±0.07	1.27±0.10	1.27±0.1	1.45±0.10
Lat Sep size (mm^2)	8.42	7.66	8.33	9.37	9.65
Post Sep width (mm)	1.18±0.07	1.31±0.12	1.09±0.06	1.5±0.11	1.13±0.07
Post Sep size (mm^2)	6.66	6.47	6.24	9.13	6.92
Total Sep size (mm^2)	53.04	46.51	44.92	56.66	56.77
AntPet length (mm)	11.92±0.21	9.92±0.46	9.1±0.34	12.08±0.44	10.15±0.22
Lat Pet length (mm)	13.62±0.38	11.54±0.48	11.08±0.41	12.92±0.48	11.08±0.40
Post Pet length (mm)	13.38±0.47	13.08±0.49	12.23±0.30	13.38±0.62	12.27±0.52
AntPet width (mm)	3.5±0.15	3.33±0.14	3.25±0.13	3.79±0.19	3±0
Ant Pet size (mm^2)	41.76	32.72	29.51	44.93	31.09
Lat Pet width (mm)	5.25±0.13	5.91±0.19	6±0.17	5.58±0.26	4.5±0.13
Lat Pet size (mm^2)	73.33	70.91	68.72	71.73	51.02
Post Pet width (mm)	6.75±0.25	8.25±0.21	7.16±0.11	7.33±0.37	5.25±0.21
Post Pet size (mm^2)	92.97	109.098	87.48	93.88	64.30
Total Pet size (mm^2)	374.39	394.149	341.93	376.16	261.74
Anther length(mm)	1.79±0.07	2.21±0.18	1.85±0.09	2.12±0.10	1.71±0.07
Anther width (mm)	1.12±0.06	1.16±0.07	1.08±0.05	1.21±0.07	1.07±0.05
Carpel length (mm)	3.13±0.05	2.91±0.07	2.77±0.12	3.23±0.06	2.73±0.06

Table 20: Matrices of fruit and seed related traits of *V.canescens*.

Characters	BGSBU	Channi-Prat	Jammu (Botanical garden)	Devi-Pindiyan	Natha-top
Fruit number/plant	2.66±0.31	2.16±0.24	2.41±0.25	2.66±0.26	2.92±0.26
Fruit length (mm)	5.33±0.18	4.58±0.19	4.66±0.14	4.91±0.19	4.25±0.13
Fruit width (mm)	5.08±0.15	4.75±0.18	4.5±0.15	4.91±0.14	4±0
Seed number/fruit	21.16±0.60	19.75±0.52	19.33±0.61	21.33±0.69	15.25±0.84
Seed length (mm)	1.34±0.05	1.13±0.03	1.13±0.04	1.77±0.03	1.71±0.08
Seed width (mm)	1.01±0.02	1.02±0.02	1.01±0.01	1.06±0.03	1.1±0.04

Table 21. Population-wise chromosome associations and pollen viability in PMCs of *V. canescens*

Population	10II	9II+2I/8II +4I	1X+1IV+3II/ 6II+2IV/1VI+7II	Aneuploid PMCs	10:10	%deviant cells	% age pollen viability
Natha-top	20	-	-	-	7	-	98
Devi-pindiyan	30	1	2	1	11	11.76 (4/34)	98.8
BGSBU	50	-	6	-	11	10.71 (6/56)	99.3
Channi-prat	54	-	2	-	13	3.57 (2/56)	91.9
Jammu	28	8	2	-	8	27.7 (10/36)	95.6

Table 22: Results of colour tests for phenol, flavonoids and sterols in *V. canescens*

Phenolic extract (10 mg/ 5ml)	Reagents added	Colour obtained	Inference
2ml	2ml $FeCl_3$	Dark green	Phenols present
2ml	Chloroform(2ml) + 2ml conc.H_2SO_4	Red	Sterol present
2ml	2ml NaOH solution	Yellow	Flavonoids

Table 23:Population wise total phenolic, flavonoid content and IC50 values for leaf extracts of *V. canescens*

Population	Altitude (masl)	Total Phenolic Content (mgGAE/g)	Total Flavonoid Content (mg QE/g)	IC50 Value (µg/ml)
Devi-pindiyan	1321	13.87±0.021	19.77±0.032	8.79
Natha-top	2265	2.52±0.012	7.43±0.019	13.54
Jammu	304	1.32±0.009	3.82±0.018	23.56
Channi-prat	535	1.49±0.016	4.65±0.021	24.15
BGSBU	1163	2.88±0.017	8.89±0.028	9.89

Table 24: Retention time, area under curve and stigmasterol concentration in *V. canescens*

Population	Retention time (min.)	Area (mAU)	Stigmasterol conc. (µg/mL)
Standard	17.311	7765775	10
		15447292	20
		29787332	40
		60093331	80
Devi-pindiyan	17.25	4412	0.005874
Natha-top	17.33	3739	0.004978
BGSBU	17.20	3974	0.005291
Channi-prat	17.25	3361	0.004475
Jammu	17.15	2682	0.003571

Table 25: Analysis of genetic polymorphism obtained with ISSR primers in five *V. canescens* populations

Population	Na	Ne	H	I	PL	PPL
Devi-pindiyan	1.6333±0.4901	1.3870±0.3726	0.2280	0.3411	19	63.3
BGSBU	1.4333±0.5040	1.2745±0.3722	0.1592	0.2368	13	43.33
Channi-prat	1.3333±0.4795	1.2920±0.4313	0.1539	0.2177	10	33.33
Natha-top	1.2667±0.4498	1.1842±0.3399	0.1050	0.1544	8	26.27
Jammu (Bot.-garden)	1.1667±0.3790	1.1068±0.2558	0.0637	0.0946	5	16.67

Table 26: Nei's analysis of gene diversity in subdivided populations of *V. canescens*

Ht	Hs	Gst	Nm
0.2252	0.1420	0.3697	0.8525

Table27. Matrices of morphological traits of 14*Viola*× *wittrockiana* cultivars.

Populationidentity	Plant height(cm)	Leaf(cm)			Petiole (cm)		Stipule(cm)				Pedicel (cm)		Bracteole (cm)	
		No.	L*	W**	L	W	L (large)	L (small	W (large)	W (small	L	W	L	W
White-purple	11.78	57.3	4.24	2.89	2.96	0.27	3.98	4.38	1.75	1.63	6.89	0.27	0.25	0.18
Light-Purple	9.8	43.8	4.7	2.76	2.36	0.13	3.5	3.75	1.7	1.95	7.66	0.27	0.24	0.15
White(purpleblotch on3 petals)	9.24	48.4	5.0	3.36	2.45	0.21	3.74	4.11	2.05	1.55	5.25	0.25	0.24	0.18
White(purple blotchon5petals)	8.94	41.4	4.84	2.69	2.36	0.24	3.78	4.83	1.86	1.99	6.85	0.24	0.25	0.19
Yellow with blackstreaks	9.84	40.2	5.6	3.44	3.39	0.25	5.24	4.08	2.04	2.01	7.78	0.3	0.26	0.2
Yellowwithmaroon blotch	11.48	34.4	5.33	3.06	3.22	0.26	4.98	4.4	1.88	1.7	6.76	0.34	0.25	0.18
Yellow	12.28	40.8	4.49	2.69	3.13	0.25	5.02	4.36	2.31	2.22	7.32	0.35	0.27	0.18
Magenta(blueblotch	12.78	41.4	5.67	3.4	2.5	0.23	3.24	3.89	1.96	1.85	7	0.32	0.20	0.22
Royalblue	12.24	58.4	6.18	4.55	3.98	0.25	6.32	5.74	2.84	2.24	8.95	0.33	0.23	0.22
Royal blue withyellowblo tch	10.24	53.4	4.56	3.19	2.26	0.2	3.38	3.82	2.05	1.92	6.25	0.28	0.22	0.23
Lightblue	10.9	46.2	3.13	2.68	2.02	0.26	3.5	3.64	1.38	1.94	8.91	0.38	0.24	0.22
Maroon with purpleblotch	14.98	44.2	4.2	2.98	2.47	0.23	3.6	3.34	1.71	1.62	8.11	0.33	0.25	0.22
Redyellowwith maroonblotch	13.82	56	4.7	2.93	3.08	0.21	3.8	3.46	1.45	1.82	7.8	0.38	0.25	0.22
Maroon	14.66	48	4.8	3.76	2.97	0.23	4.6	3.82	1.39	1.7	7.8	0.38	0.25	0.22

L*=length;W**=width

Table28. Matrices of morphological traits of *V. betonicifolia*.

Leaf length	Leaf width	No. of leaves	Petiole length	No. of fruits	Fruit length	Fruit width	No. of seeds	Seed length	Seed width
2.25±0.38	1.54±0.11	4.75±0.82	3.64±1.33	4.2±0.74	7.75±0.72	4.91±0.27	27.4±4.32	1.84±0.2	1

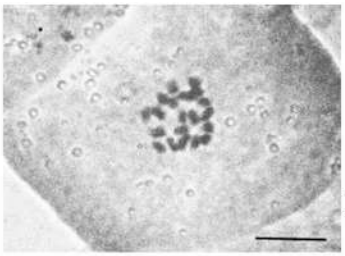

Fig. 2a: Somatic complement of *V. pilosa* showing 20 chromosomes

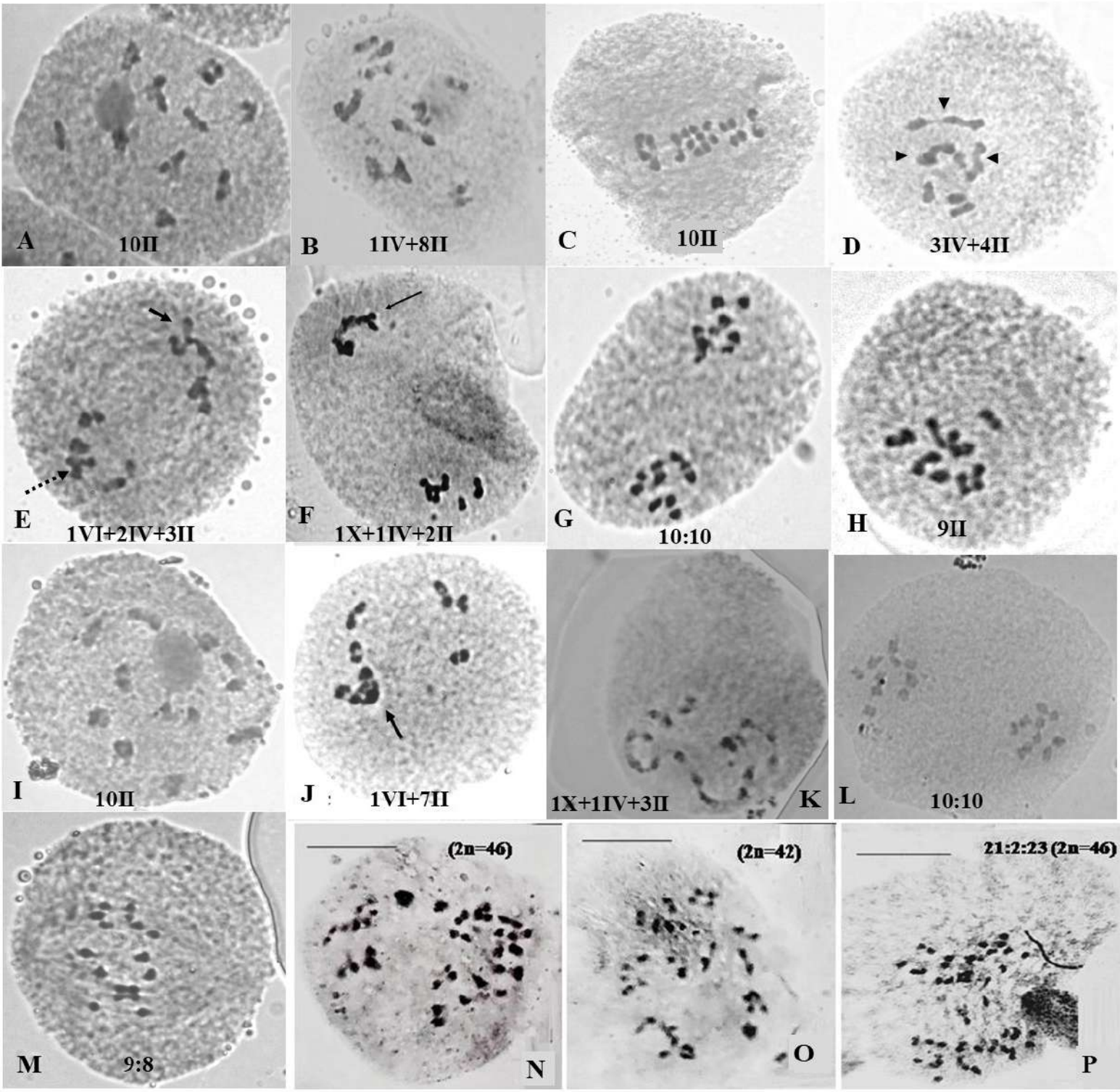

Fig.2b. PMCs at diakinesis and metaphase-I depicting 10II (A,C) 1IV+8II (B) 3IV+4II (D) 1VI+2IV+3II (E) 1X+1IV+2II (F) and at anaphase-I showing 10:10 segregation in *V. pilosa*.
An aneuploid PMC in *V. pilosa* at metaphase-I showing 9II.
PMCs at diakinesis and metaphase-I showing 10II (I), 1VI+7II (J), 1X+1IV+3II (K) and at anaphase-I showing 10:10 segregation in *V. canescens*.
An aneuploid PMC at anaphase-I sin *V. canescens* showing 9:8 segregation
PMCs of *V. wittrockiana* at metaphase-I depicting 2n=46, 42 and at anaphase-I depicting 21:2:23 segregation.

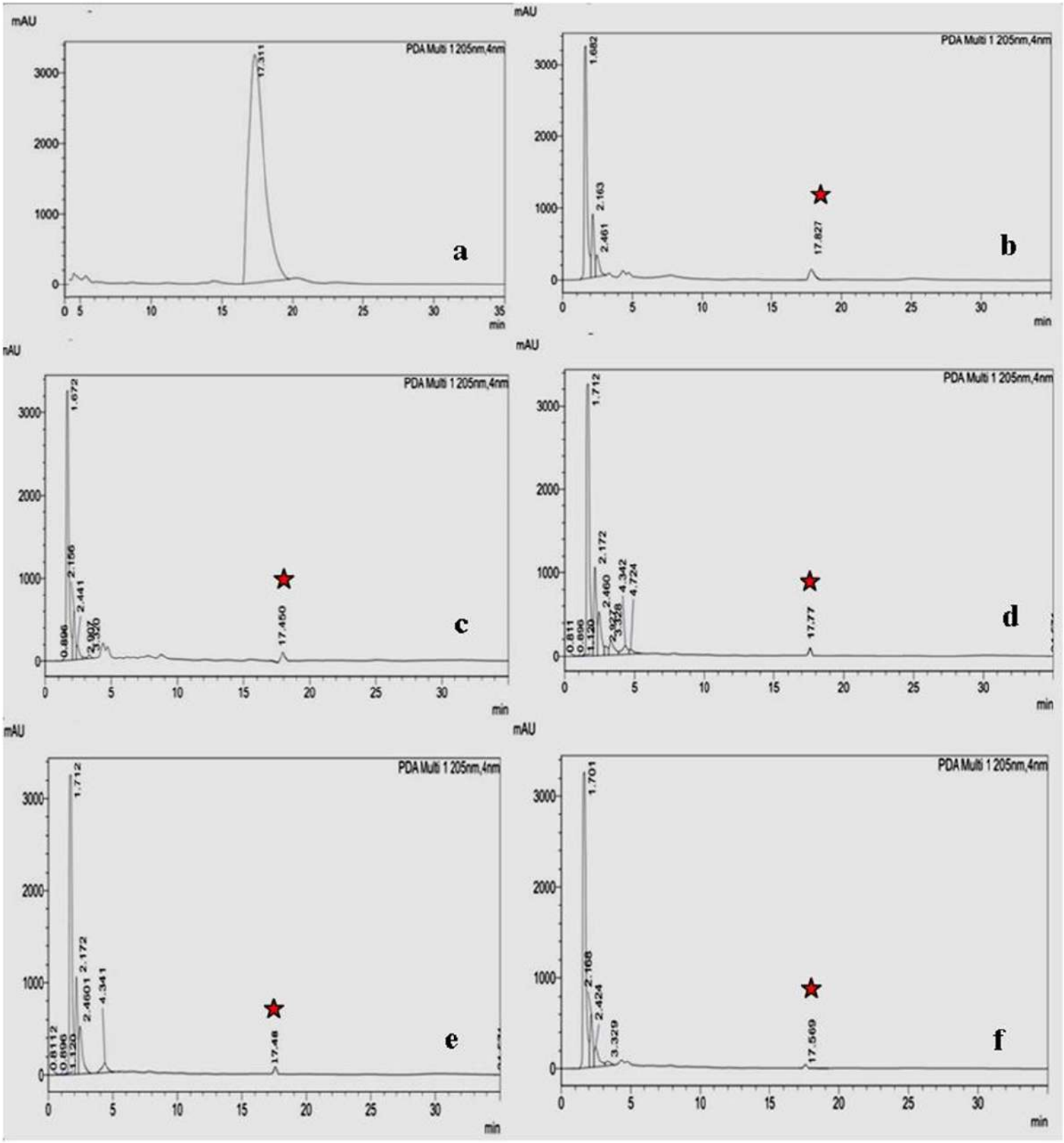

Fig.3a : Chromatograms showing retention time and area peaks of samples of *V. pilosa* populations indicating quantity of stigmasterol (asterisk) (a= Standard, b= Bhairon-ghati, c= Jai-ghati, d= Dera-gali, e= Sanasar, f= Khellani)

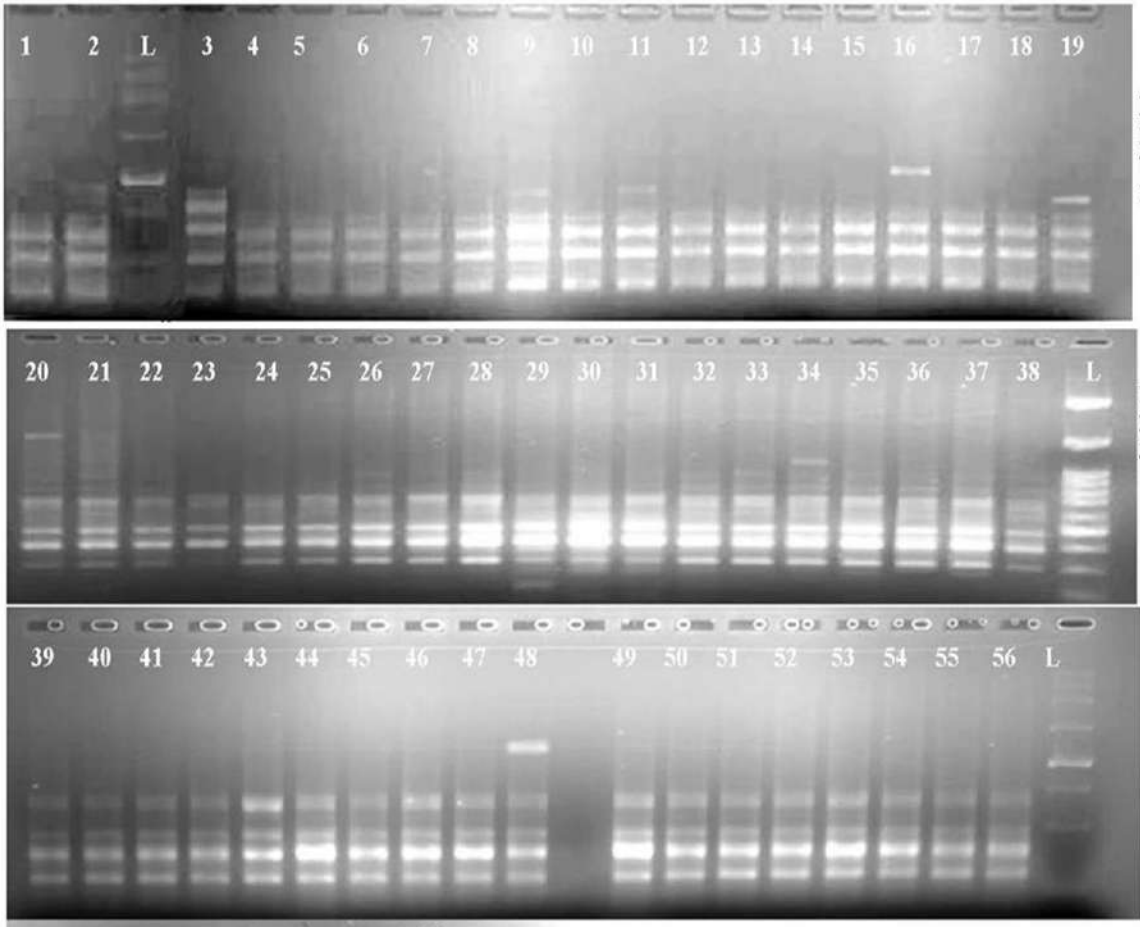

Fig.4 : ISSR profile of *V. pilosa* populations using ISSR 17 primer

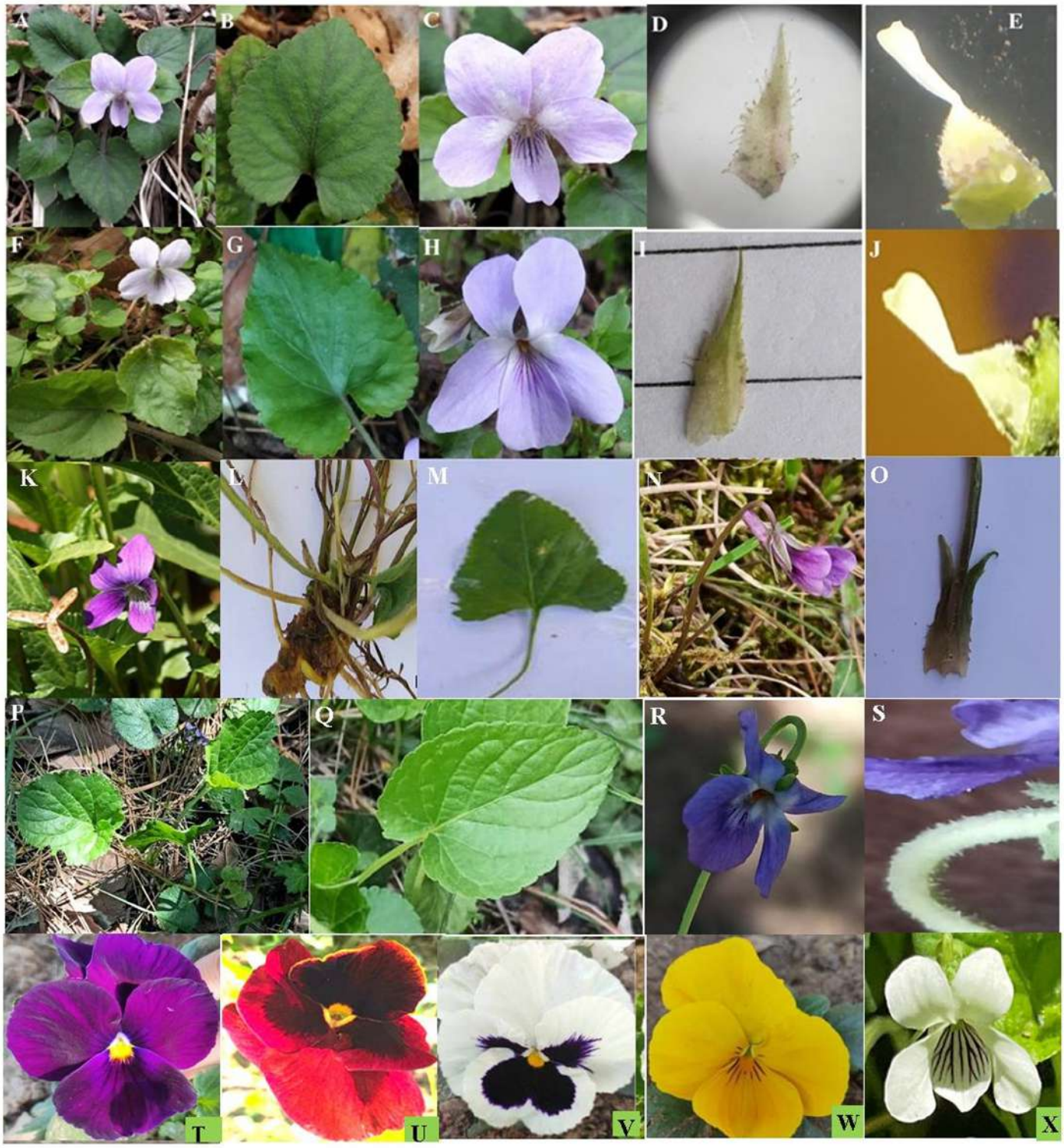

Fig.1: Figs A-E : Plants, leaf, flower, stipule & stigma of *V. canescens*
Figs F-J : Plants, leaf, flower, stipule & stigma of *V. pilosa*
Figs K-O : Plants, root stock, leaf, flower & stipule of *V. betonicifolia*
Figs P-S: Plants, leaf, flower & hair on pedicel of *V. odorata*
Figs T-W : Flowers of *V. wittrockiana*

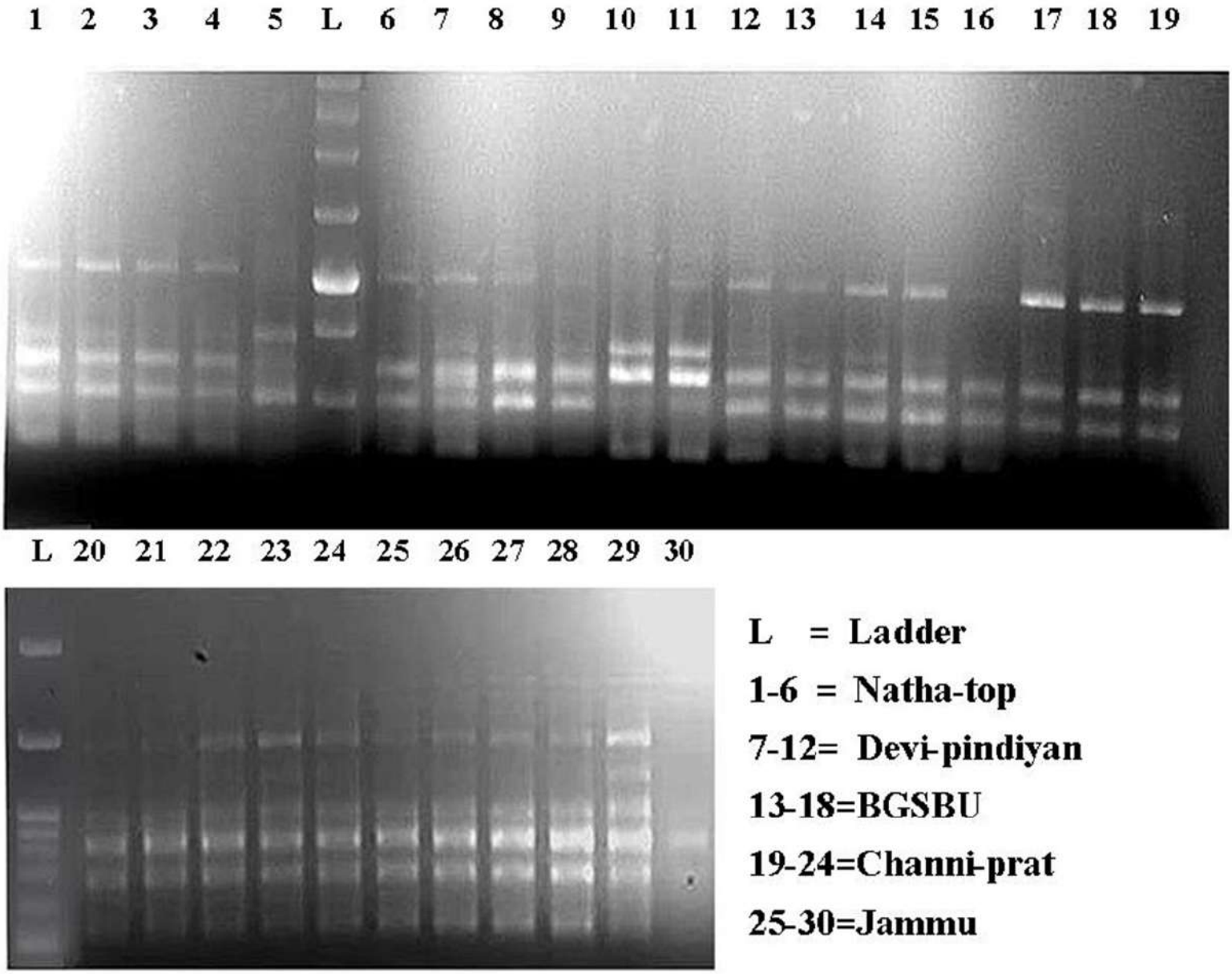

Fig. 6 : ISSR profile of *V. canescens* populations using UBC812 primer

GFR 12 – A [(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23

in respect of *NON-RECURRING*

as on 19th May, 2022 to be submitted toSERB

1. Name of the grant receiving Organization : University of Jammu, Jammu
2. Name of Principal Investigator(PI) : Dr. Geeta
3. SERB Sanction order no. & date: CRG/2018/003919 dated: 13-May-2019
4. Title of the Project : **Assessment of cytological, biochemical and molecular variability in *Viola* species from Jammu and Kashmir**
5. Name of the SERB Scheme :CRG
6. Whether recurring or non-recurring grants : ***NON-RECURRING***

i. Grants position at the beginning of the Financialyear : Nil

ii. Carry forward from previousfinancialyear : **1,49,698**

iii. Others,Ifany : Nil

iv. Total : 1,49,698

Details of grants received, expenditure incurred and closing balances:(Actuals)

Unspent Balance of Grants received previous years [figure as at Sl.No. 7(iii)]	Interest Earned thereon	Interest deposited back to the SERB	Grants received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date(ii)	Amount (iii)			
1,49,698	**2,117**	-	**CRG/2018/003919**	**Nil**	**Nil**	**1,51,815**	**Nil**	**1,51,815/-**

Component wise utilization of grants:

Grants-in-aid- General	Grant-in-aid-creation for capital assets	Total
Nil	Nil	Nil

Details of grants position at the end of the year

Balance available at end of financial year : **1,51,815/-**
Unspent balance refunded to SERB (If any) : **1,51,815/-**
Balance (Carried forward to next financial year) ifapplicable: Nil

Dr. Geeta Principal Investigator SERB Funded Project Assessment.....Kashmir Univ. of Jammu, Jammu 26/7/2024 **Signature of PI :**	**Signature with Seal:** ..... **Name:** ..... **Chief Finance Officer (Head of Finance)**	**Signature withSeal**..... **Name:** ..... **Head of Organisation**

For RC Gupta & Co. CHARTERED ACCOUNTANT Lalit Kumar Gupta (Partner) UDIN:-24504307BKEXIF3345 GUPTA & Co. Chartered Accountants FRN-001498N Jammu

GFR 12 – A [(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23

in respect of *NON-RECURRING* as on 19th May, 2022 to be submitted to SERB

Certified that I have satisfied that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

i. The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

ii. There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.

iii. To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.

iv. The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.

v. The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.

vi. The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.

vii. It has been ensured that the physical and financial performance under CRG (Name of the scheme has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure– I duly enclosed.

viii. The utilization of the fund resulted in outcomes given at Annexure–II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

ix. Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure –II (to be formulated by the Ministry/Department concerned as per their requirements /specifications).

Date: 26/7/2024

Place: Jammu

Dr. Geeta Principal Investigator SERB Funded Project "Assessment.....Kashmir" Univ. of Jammu, Jammu Geeta 26/7/2024 **Signature of PI :**	**Signature with Seal:** ..... **Name:** ..... **Chief Finance Officer (Head of Finance)**	**Signature withSeal**..... **Name:** ..... **Head of Organisation**

Finance Officer
University of Jammu

Registrar
University of Jammu

29/7/2024

For R C Gupta & Co.
CHARTERED ACCOUNTANT
Lalit Kumar Gupta
(Partner)

R.C. GUPTA & Co. CA FRN-001198 N Jammu Chartered Accountants

UDIN:- 24504307BKEXIF3345

Date:- 05/08/2024

GFR 12 – A [(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23

in respect of *RECURRING*

as on19th of May 2022to be submitted toSERB

1. Name of the grant receiving Organization: University of Jammu, Jammu
2. Name of Principal Investigator(PI) : Dr. Geeta
3. SERB Sanction order no. & date : CRG/2018/003919 dated: 13-May-2019
4. Title of the Project : **Assessment of cytological, biochemical and molecular variability in *Viola* species from Jammu and Kashmir**
5. Name of the SERB Scheme :CRG
6. Whether recurring or non-recurring grants : ***RECURRING***
   - i. Grants position at the beginning of the Financialyear : Nil
   - ii. Carry forward from previousfinancialyear :3,05,031-
   - iii. Others,Ifany: Nil
   - iv. Total : 3,05,031/-

7. Details of grants received, expenditure incurred and closing balances:(Actuals)

Unspent Balance of Grants received previous years [figure as at Sl. No. 7(iii)]	Interest Earned thereon	Interest deposited back to the SERB	Grants received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date(ii)	Amount(iii)			
3,05,031	**4234**	**Nil**	**Nil**	**Nil**	**Nil**	**3,09,265**	**29,218**	**2,80,047**

Component wise utilization of grants:

Grants-in-aid- General	Grant-in-aid-creation for capital assets	Total
29,218	**Nil**	**29,218**

Details of grants position at the end of the year

Balance available at end of financial year :**2,80,047/-**
Unspent balance refunded to SERB (If any) : **2,80,047/-**
Balance (Carried forward to next financial year) if applicable: Nil/-

Signature of PI : ..... Dr. Geeta	Signature with Seal : ..... Name: ..... Chief Finance Officer (Head of Finance)	Signature with Seal..... Name: ..... Head of Organisation

Geeta 26/7/2024

Principal Investigator
SERB Funded Project
"Assessment.....Kashmir"
Univ. of Jammu, Jammu

Finance Officer University of Jammu

29/7 29/7

Registrar University of Jammu

For R C Gupta & Co.
CHARTERED ACCOUNTANT
Lalit Kumar Gupta
UDIN:- 24504301BKEXIF3345
Date:- 05/08/2024

R.C. GUPTA & Co. FRN-001198 N Jammu Chartered Accountants

GFR 12 – A [(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23

in respect of *RECURRING*

as on 19th May, 2022 to be submitted to SERB

Certified that I have satisfied that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

i. The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

ii. There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.

iii. To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.

iv. The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.

v. The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.

vi. The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.

vii. It has been ensured that the physical and financial performance under CRG (Name of the scheme has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure– I duly enclosed.

viii. The utilization of the fund resulted in outcomes given at Annexure–II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

ix. Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure –II (to be formulated by the Ministry/Department concerned as per their requirements /specifications).

Date: 26/7/2024

Place: Jammu

Dr. Geeta Principal Investigator SERB Funded Project Assessment.....Kashmir of Jammu, Jammu Geeta 26/7/2024 **Signature of PI :**	**Signature with Seal :** ..... **Name:** ..... **Chief Finance Officer (Head of Finance)**	Finance Officer University of Jammu **Signature withSeal**..... **Name:** ..... **Head of Organisation** Registrar University of Jammu

For R C Gupta & Co.
CHARTERED ACCOUNTANT
Lalit Kumar Gupta
(Partner)
UDIN:-24504307BKEXIF3345
Date:- 05/08/2024

R. C. GUPTA & Co. FRN-001198 N Jammu Chartered Accountants