Std. Doc.: NMHS/PG-FTR

#### National Mission on Himalayan Studies – 2023

## NMHS-Himalayan Institutional Project Grant

# NMHS-REVISED FINAL TECHNICAL REPORT (RFTR)

**Demand-Driven Action Research and Demonstrations** 

NMHS Reference No.: NMHS/2018-19/SG60/60 Date of Submission: 3 0 0 9 2 0 2 2 d D m m y y y y

### **PROJECT TITLE**

# BIOLOGICAL SCREENING, CONSERVATION AND ESTABLISHMENT OF GENE BANK OF *GREWIA OPTIVA* DRUMMOND (BEUL)

Project Duration: from (21.12.2018) to (30.09.2022)

#### **Submitted To:**





Er. Kireet Kumar, Scientist 'G' & Nodal Officer, NMHS-PMU National Mission on Himalayan Studies, GBP NIHE HQs Ministry of Environment, Forest & Climate Change (MoEF&CC), New Delhi Ministry of Environment, Forest & Climate Change, GOI

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# **Submitted By:**



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# PARTA

# PROJECT SUMMARY REPORT

# NMHS-Revised Final Technical Report (RFTR) template

Demand-Driven Action Research Project

DSL: Date of Sanction Letter

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d	d	m	m	у	у	у	У

DPC: Date of Pro	ject Completion
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d	d	m	m	у	у	у	у

## **PART A: PROJECT SUMMARY REPORT**

# 1. Project Description

i.	Project Reference No.	NMHS/201	18-19/SC	<b>360/60</b>				
ii.	Type of Project	Small Grai	nt 🗸	Medium Grant	Large Grant			
iii.	Project Title	_	Biological Screening, Conservation and Establishment of Gene Bank of <i>Grewia optiva</i> Drummond (Beul).					
iv.	State under which Project is Sanctioned	HIMACHAL	PRADE	ESH				
V.	Project Sites (IHR States covered)			imachal Pradesh ur, Kangra and S	n viz., Solan, Bilaspur, Sirmaur.			
	(Maps to be attached)							
vi.	Scale of Project Operation	Local	✓	Regional	Pan-Himalayan			
vii.	Total Budget/ Outlay of the Project	Rs 48,13	,600.00					
viii.	Lead Agency			ersity of Horticul hal Pradesh-173	ture and Forestry, 3230,			
	Principal Investigator (PI)	Dr. HP. Sankhyan Professor and Head Department of Tree Improvement and Genetic Resources, College of Forestry Dr. YS Parmar University of Horticulture and Forestry, Nauni-Solan, Himachal Pradesh-173230, Phone: 98053-65433 E-mail: sankhyanhp@gmail.com						
	Co-Principal Investigator (Co-PI)	Dr. Shikha Thakur Assistant Professor (TIGR) Department of Tree Improvement and Genetic Resources, Dr. YS Parmar University of Horticulture and Forestry, Nauni-Solan, Himachal Pradesh-173230,						
ix.	Project Implementing Partners	State Fores	t Depart	ment, (Himachal	Pradesh Government)			

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#### 2. Project Outcomes

#### 2.1. Abstract

Background: The study on *Grewia optiva* Drummond (Beul) has been undertaken in low and mid hill zones of H.P. Keeping in view the rich genetic diversity of *Grewia optiva* populations, the morphological observations have been recorded of individuals within and among populations. Total thirty-five populations (five in each district) were identified, marked and selected in seven districts of Himachal Pradesh viz. Solan, Bilaspur, Mandi, Una, Hamirpur, Kangra and Sirmaur and further 210 morphologically best genotypes (six from each population) considered with aim to ascertain the response of the different parameters towards the screening of the adopted best genotypes and forage quality and productivity.

With respect to the project entitled "Biological Screening, Conservation and Establishment of Gene Bank of *Grewia optiva* Drummond (Beul) the following broad Objectives/Aim were undertaken:

- 1) Biological screening of the germplasm diversity of the *Grewia optiva* as agroforestry tree species in Himalaya.
- 2) Study the growth parameters for forage quality and productivity of *Grewia optiva*.
- 3) Development of DUS specific descriptors of the selected germplasm of *Grewia optiva*.
- 4) Conservation and Establishment of gene bank of selected genotypes of Grewia optiva.

#### **Methodology and Approach:**

The variation in morphological and phenological characters viz., plant morphology (growth pattern, attitude of branches, branch thickness, bark color, leaf characteristics (leaf length, leaf width, leaf area, leaf blade, 100 leaf fresh and dry weight), fruit characteristics (fruit length, fruit weight, fruit shape, fruit skin colour) and phenological characteristics (leafing, flowering, fruiting, fruit ripening timings) and seed characteristics (seed weight, seed coat colour) were recorded for each 210 selected genotypes and their variation observed between and within the selected populations. The proximate principles (leaf dry matter content %, ether extract (%), crude fiber (%), crude protein (%), total ash (%) and nitrogen free extract (%) were recorded for each selected genotypes during the winter season when mineral nutrients highest in leaves and they are massively lopped for cattle fodder.

The soil physico-chemical characteristics ((soil texture, Bulk density, pH, EC) and other available macronutrients (N, P, K, Ca, Mg and S) were recorded (on the basis of standard methods) for all the 35 soil samples collected from 15 cm–30 cm depth underneath the selected populations of *Grewia optiva* Drummond. Correlation models were developed between the plant morphological characteristics and soil nutrients observed underneath the selected populations.

The seed samples were collected during the month of November-December from all thirty-five populations and their nursery was raised in the Shilly Research Farm of the Department. The variation in seed germination characteristics (per cent germination, germination value and per cent survival) and their seedling growth characteristics viz., seedling height (cm), collar diameter (mm), internodal length, number of branches per plant, branch length (cm), branch angle, number of leaves per plant, leaf characteristics and their biomass (leaf biomass per plant (g) at leaf harvesting stage, root length (cm), shoot length (cm), root and shoot fresh weight, root and shoot dry weight ratio and shoot biomass were recorded for collected seed samples and seedlings raised of all the selected genotypes of thirty five populations.

The first approach was to screen out the best genotypes of the best families selected from the identified populations in all the seven districts of Himachal Pradesh (Hamirpur, Una, Kangra, Mandi, Solan, Sirmaur and Bilaspur). The second approach was to produce genetically superior planting stock of those genotypes and families which are having high crude protein i.e. more than 20 per cent. The over all approach was to find out biologically superior individuals on the basis of morphological and fodder quality scoring index parameters within the populations and among the populations. One superior tree selected in the population is considered as one family in the population, for characterization of parents/ mother trees on the basis of their morphological desirable descriptors.

#### Results:

- Gene banks have been established i.e., 180 genotypes belonging to 60 families have been established in Seedling Seed Orchard. As many as 69 genotypes have been established in Clonal Seed Orchard of 23 best families.
- ii. Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Research Farm of the Department, with total 165 genotypes belonging to 55 families (planted in 3 replication at a spacing of 2x2m).
- iii. On 'Field Demonstration Model' –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Khaltoo Research Farm with total 60 genotypes belonging to 20 families (planted in 3 replications at spacing of 2x2m).

- iv. The Morphological and Morphometrics observations viz., plant height, plant diameter, branch angle, crown spread (E-W and N-S), number of primary and secondary branches, one year old shoot thickness, phenological events, leaf shape, leaf length, leaf width, leaf area, fruit length, fruit weight, fruit shape and seed weight and seed coat colour etc. of all the selected genotypes have been recorded and statistically analysed.
- v. The additional germplasm has been selected/introduced from Chamba block of Tehri Garhwal, Uttarakhand and other agricultural fields of Himachal Pradesh to find out other morpho variants and superior nutritive strains of *Grewia optiva*.
- vi. The fodder quality parameters (leaf fresh weight, leaf dry weight, leaf dry matter, ether extract, crude protein, crude fibre, total ash, nitrogen free extract) have been recorded for all the selected thirty-five populations and their statistical analysis have been completed. The Nine (09) nutritive selections/populations *i.e.* Kothi kanwal (Solan), Unchagaon (Solan), Nerikalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of Bilaspur) have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected and identified populations of *Grewia optiva* Drummond.
- vii. The analysis of soil physicochemical characteristics (soil texture, Bulk density, pH, EC) and other available macronutrients (N, P, K, Ca, Mg and S) of thirty-five soil samples collected beneath *Grewia optiva* populations have been completed. There was significant difference observed in the pH, OC, EC, N, P, K and bulk density within the selected population of each district. Correlation studies were worked out after developing complete screening of the nutritive strains and regression equations, economic and financial models were developed.
- viii. Clonal Seed Orchard of genotypes has 23 genotypes/ families already established has been evaluated for morphometric and fodder Quality Parameters. Ten superior genotypes among different families having high nutritive strains have also been screened out on the basis of morphological and molecular characterization.
- ix. The nursery has been raised at Shilli Farm of the Department from the seeds collected from 35 population of Himachal Pradesh (Solan, Sirmour, Mandi, Hamirpur, Kangra, Una and Bilaspur). The seed germination and seedling growth have been recorded and their statistical analysis has been done.
- x. Raising planting material through cuttings have also been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers replica) material.

- xi. Morphological descriptors of 35 populations of seven districts of Himachal Pradesh have been developed, and 210 superior plants have been identified and screened biologically. Morphological data of 35 populations, which have been earlier recorded and completed on cropland situation, have been analysed statistically. Data base has been generated on 210 genotypes as Example/ Reference Varieties under the project from 35 populations, besides data generated from 23 families maintained in Clonal Seed Orchard (CSO).
- xii. Best 10 families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The morphological descriptors have been prepared for these 10 best screened families.
- xiii. For production of quality planting stock, nursery has been raised at Shilli farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in seven different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.
- xiv. Quality planting material raised of superior nutritive selection of Clonal Seed Orchard has been supplied to 15 Forest Divisions (360 plants to each Forest Division) of H.P. (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh and Mandi) during the year 2020 and 2021, i.e., 5400 plants in each year for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, other 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e., totaling to 12,000 plants supplied for the farmers/stakeholders, identified by the Forest Division for the benefit to the end users, farmers, women groups, self-help group etc.
- xv. This year i.e. 2022, once more 360 plants raised from superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions for field testing and demonstration model (i.e., 5400 plants) and other total 6,000 plants have been supplied to farmers /stakeholders/women groups identified through Forest Divisions. Also, the nursery has been raised in 2021 from seeds collected from thirty-five selected populations of Himachal Pradesh to distribute best planting stock in the year 2022 to farmers, stake holders through the Forest Departments and Animal Husbandry department/Agriculture Department. Total production came out to be 34,200 plants/ saplings, produced and supplied. At present more than 20,000 plants/ saplings are still available with the department, which have been screened out under the project.

#### Conclusion:

- a) The total nineteen (19) superior nutritive selections have been screened from this study i.e., 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies and the Nine (09) best selections/ populations with crude protein i.e. more than 24 % have been identified and screened from thirty-five selected populations on the basis of morphological and fodder quality characteristics.
- b) Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, the economic and financial models have been developed.
- c) Molecular characterization of the 10 best fodder selections made from the Clonal Seed Orchard has been achieved. The molecular characterization of genotypes/ selections helped to determine the genetic characteristics of the plants and establish the identity of the accessions and discern genetic relationship among genotypes. These also help to study genetic diversity and provide basis for conservation of superior strains.
- d) For production of quality planting stock, nursery has also been raised at Shilly Experimental Farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance/ at nursery stage level.
- e) Quality planting of superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions of H.P. for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, quality plants have been supplied for the farmers/stakeholders, identified through choosen Forest Division to end users, farmers, women groups, self-help group etc.
- f) This species is difficult to raise through cuttings but planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers' replica) material under this project.

#### Recommendations:

a) The total nineteen (19) Nutritive selections screened under the project should be used to raise the demonstration block/Seed Orchard and from where further production of quality planting material will be raised and supplied to farmers. The establishment of seed orchards will help in the Mass- multiplication of superior genotypes and transferring of genetically improved material to end-users. We recommend that old material which is existing on the cropland situation on the farmer's field should be gradually replaced with new screened genotypes in phased manner. This will be a good approach for sustainable development of social, economic and environment resources of IHR.

- b) The morphological DUS specific descriptors developed for elected genotypes under the project can be used for the categorization of superior genotypes in other parts of IHR.
- c) The superior seedling raised under the nursery conditions as well as the material that will be produced by mass multiplications from the nineteen (19) fodder selections can be planted in the other parts of IHR. This can be an efficient way to replicate the outcomes of the project in other parts of IHR.
- d) The twenty new populations (five from Uttarakhand and sixteen from the cultivated area of Himachal Pradesh) which have been added/selected to extend the research on *Grewia optiva* should be explored for variations and broader base of the germplasm of the species for further establishment of germplasm bank. This study will also help in comparative analysis among and between the selected populations of H.P and Uttarakhand to screen out the best nutritive selections and to find out the valuable material for future breeding programmes.
- e) The more number of Mass media lectures, workshops, and training should be organized to popularise this species in hilly areas and to popularise and distribute the quality planting material among farmers.
- f) Quality planting of superior nutritive selection of Clonal Seed Orchard which have been supplied to 15 Forest Divisions for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, quality plants that have also been supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc. should be multiplied and supplied in the leftover parts of the IHR.

#### 2.2. Objective-wise Major Achievements

S. No.	Objectives	Major achievements (in bullets points)
1.	Biological Screening of the germplasm diversity of the <i>Grewia optiva</i> as agroforestry tree species in Himalaya.	The gene bank has been established for conservation of Screened genotypes:  A. Different spacing germplasm bank    At spacing 1x1m= 27 Genotypes    At spacing 2x2m= 31 Genotypes    At spacing 3x3m= 07 Genotypes    At spacing 4x4m= 04 Genotypes  B. Seedling Seed Orchard:    180 genotypes belonging to 60 families in Open    Pollinated Seedling Seed Orchard spaced at 2 x 2m

S. No.	Objectives	Major achievements (in bullets points)
		Establishment of Clonal Seed Orchard:     23 genotypes in three replications     i.e. 69 plants are maintained and evaluated for     morphological and fodder quality parameter.
		<ul> <li>morphological and fodder quality parameter</li> <li>Design: RBD for all trials at farm in three replications each</li> <li>D. Established Demonstration Model -cum- Gene Bank of <i>Grewia optiva</i> (Beul) at Naganji Farm of the University with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2mx2m.</li> <li>Absolute management practices have been done/ undertaken on already established spacing trials at Naganji Farm.</li> <li>E. Establishment 'On Field Demonstration Model' -cum-Gene Bank of <i>Grewia optiva</i> (Beul) at Khaltoo Farm of the University with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m.</li> <li>F. As per the objective 35 populations in seven districts of Himachal Pradesh (5 population in each district) were selected as study area under the project. The following morphological and morphometrics observations on plants among and within thirty-five selected populations were recorded and their statistical analysis have been achieved.</li> </ul>
		<ul> <li>Plant growth type: Plant height and trunk diameter</li> <li>Branch angle ()</li> <li>Crown spread (E-W &amp; N-S)</li> <li>No. of primary and secondary branches</li> <li>One year old shoot thickness</li> <li>Phenological events</li> <li>Leaf: Leaf shape, Undulation of margin, colour of adaxial surface, intensity of green colour of abaxial surface</li> <li>Leaf length (cm)</li> <li>Leaf width (cm)</li> </ul>

S. No.	Objectives	Major achievements (in bullets points)
110.		➤ Leaf area (cm²)
		Fruit length (cm)
		Fruit weight (g)
		Fruit: shape, colour of skin, time of beginning of fruit
		ripening
		Seed weight (g)
		➤ Seed coat colour
		<b>G.</b> The additional germplasm has been selected/
		introduced from Chamba block of Tehri Garhwal,
		Uttarakhand and other cropland sources of Himachal
		Pradesh to find out other morphovariants and superior
		nutritive strains of <i>Grewia optiva</i> .
2.	Study the growth parameters	A. The study on the following leaf fodder quality
	for forage quality and	parameters (proximate principles) of thirty-five selected
	productivity of Grewia optiva.	populations have been completed/achieved for the
		second objective.
		Leaf fresh weight (g)
		Leaf dry weight (g)
		➤ Leaf dry matter (%)
		Ether extract (%)
		Crude protein (%)
		Crude fibre (%)
		➤ Total ash (%)
		Nitrogen free extract (%)
		The nine nutritive selections/populations viz., (Kothi kanwal
		(Solan), Uncha gaon (Solan), Neri kalan (Solan), Machair
		(Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari
		(Hamirpur) and Barthi population of Bilaspur district) have
		been identified and screened with crude protein percentage
		more than 24 percent from thirty-five selected populations of
		Grewia optiva.

S.	Objectives	Major achievements (in bullets points)
No.		B. The analyses of the following soil physicochemical characteristics and macronutrients of thirty-five soil samples collected underneath <i>Grewia optiva</i> populations have been completed as per the second objective. Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, economic and financial models have been developed.  > pH  > Electrical conductivity (EC) (dS m <sup>-1</sup> )  > Organic carbon (g kg <sup>-1</sup> )  > Bulk density  > Soil Texture  > Available Nitrogen (kg ha <sup>-1</sup> )  > Available Phosphorus (kg ha <sup>-1</sup> )  > Available Potassium (kg ha <sup>-1</sup> )  C. The study on evaluation of Clonal Seed Orchards established at UHF, Nauni has been completed for Qualitative, Quantitative and Pseudo qualitative characteristics and further for leaf fodder characteristics. The ten best nutritive selections have been identified and screened in the Clonal Seed Orchard under this project study.
		<ul> <li>D. The nursery has been raised at Shilli farm of the department from the seeds collected from 35 population of Himachal Pradesh viz., Solan, Sirmour, Mandi, Hamirpur, Kangra, Una and Bilaspur. The following nursery growth parameters have been recorded and analysed statistically.</li> <li>Leaf biomass per plant(g) at leaf harvesting stage</li> <li>Seedling height (cm)</li> <li>Collar diameter (mm)</li> <li>Root length (cm)</li> <li>Shoot length(cm)</li> </ul>

S. No.	Objectives	Major achievements (in bullets points)
		<ul> <li>Root fresh weight(g)</li> <li>Shoot fresh weight(g)</li> <li>Root dry weight ratio</li> <li>Shoot dry weight ratio</li> <li>Root biomass - (Fresh &amp; Dry)</li> <li>Shoot biomass- (Fresh &amp; Dry)</li> </ul>
		F. Raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/lit) auxin concentration) for true to type (mothers replica) material.
3.	Development of DUS specific descriptors of the selected germplasm of Grewia optiva.	<ul> <li>A. Morphological data of 35 populations, which have been earlier recorded and completed on cropland situation, have been analysed statistically. Data base generated on 210 genotypes as example/ reference varieties under the project from 35 populations, besides data generated from 23 families in Clonal Seed Orchard.</li> <li>B. Best 10 superior tree families have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Morphological descriptors have been developed for these 10 best screened families.</li> <li>C. For production of quality planting stock, nursery has been raised at Shilli farm of the department from the seeds collected from superior families and of superior populations under different ecological locations in seven different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.</li> <li>D. The nine best selections/populations have been identified and screened from thirty-five selected populations on the basis of morphological, molecular and fodder quality characteristics.</li> </ul>

S.	Objectives	Major achievements (in bullets points)
	Conservation and	A As many as 360 plants of superior nutritive selection of
S. No. 4.	Conservation and Establishment of gene bank of selected genotypes of <i>Grewia optiva</i> .	<ul> <li>A. As many as 360 plants of superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during the two year i.e. 2020 and 2021, totalling 5400 plants in each year for conservation and establishment of gene bank in each selected and identified forest division through SFD, for demonstration/ field testing trial/ block plantation.</li> <li>B. Beside this, other lot of 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e. total 12,000 plants supplied for the farmers/ stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc.</li> <li>C. During the current year 2022, again i.e. 360 plants raised from superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions for field testing and demonstration model (i.e., 5400 plants) and other total 6,000 plants have been supplied to farmers /stakeholders/women groups identified through selected Forest Divisions. The nursery has also been raised in 2021 from seeds collected from thirty-five selected populations of Himachal Pradesh and distributed best planting stock in the year 2022 to farmers, stack holders through the Forest Departments and Animal Husbandry department/Agriculture Department. Total production of quality planting material came out to be 34,200 plants/ saplings produced and supplied. At present about more</li> </ul>
		populations of Himachal Pradesh and distributed best planting stock in the year 2022 to farmers, stack holders through the Forest Departments and Animal Husbandry department/Agriculture Department. Total production of quality planting material came out to be 34,200 plants/
		than 20,000 plants/ saplings are available with the Department which have been screened out under the project.

# 2.3. Outputs in terms of Quantifiable Deliverables\*

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
1.	Establishment	Number of	Screened genotypes has been	
	of germplasm	genotypes	conserved in germplasm bank (Nos.)	
	bank of <i>Grewia</i>	conserved in	A. Different spacing germplasm bank;	
	optiva	germplasm bank	At spacing 1x1m = 27 Genotypes	Enclosure-I
	genotypes.	(Nos.)	At spacing 2x2m= 31 Genotypes	Eliciosure-1
			At spacing 3x3m= 07 Genotypes	
			At spacing 4x4m= 04 Genotypes	
			B. Seedling Seed Orchard: 180 genotypes	Enclosure-II
			belonging to 60 families	
			C. Establishment of Clonal Seed Orchard	Englosum III
			23 genotypes in three replications i.e.	Enclosure-III
			69 plants are maintained	
			Design: RBD for all trials at farm in three replications each.	
			D. Demonstration Model -cum- Gene	
			Bank of <i>Grewia optiva</i> (Beul) has been	Enclosure –
			established at Naganji Farm with total	IV
			165 genotypes belonging to 55	
			families, (planted in 3 replication) at a	
			spacing of 2x2m.	
			E. 'On Field Demonstration Model' -cum-	
			Gene Bank of Grewia optiva (Beul)	Enclosure –
			has been established at Khaltoo Farm	V
			with total 60 genotypes belonging to	
			20 families, (planted in 3 replications)	
			at spacing of 2x2m.	
			E Qualitative quantitative and necode	
			F. Qualitative, quantitative and pseudo qualitative morphological	
			characteristics have been recorded for	
			all thirty-five populations.	
			Work done: 100 per cent completed.	
			170.13 dollo : 100 por cent completed.	

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
2.	Introduction of additional germplasm from other institutes or crop land sources	Database generated i.e. morphological, genetical (Nos.)	<ul> <li>A. Introduction of Additional Germplasm from Cropland Situations/ Sources for 35 populations have been completed in the period of 2020-2021.</li> <li>B. The additional germplasm has been selected/ introduced from Chamba block of Tehri Garhwal, Uttarakhand and other cropland sources of Himachal Pradesh during the period of 2021-2022 to find out other morpho variants and superior nutritive strains of <i>Grewia optiva</i>.</li> <li>Work done: 100 per cent completed.</li> </ul>	Enclosure – VI
	Growth parameter for forage quality and productivity		A. The fodder quality analysis has been completed for all the 210 superior families of 35 populations. The following fodder quality characteristics (proximate principles) of thirty-five selected populations have been observed with standard methods and their statistical analysis has been achieved.  > Leaf fresh weight (g)  > Leaf dry weight (g)  > Leaf dry matter (%)  > Ether extract (%)  > Crude protein (%)  > Total ash (%)  > Nitrogen free extract (%)	Enclosure – VII

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
			The nine nutritive selections/populations viz., (Kothi kanwal (Solan), Uncha gaon (Solan), Neri kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur), Bhaleth (Hamirpur) and Barthi population of Bilaspur district) have been identified and screened with crude protein percentage more than 24 per cent from thirty-five selected populations of <i>Grewia optiva</i> .  Work done: 100 per cent completed.  B. The work on soil nutrient analyses has been completed for all 35 selected populations. The following soil physicochemical characteristics, macronutrients and micronutrients of thirty-five soil samples collected underneath <i>Grewia optiva</i> populations were observed and their statistical analysis has been completed as per the quantifiable deliverable.  > pH  > Electrical conductivity (EC) (dS m <sup>-1</sup> )  > Organic carbon (g kg <sup>-1</sup> )  > Bulk density  > Soil Texture  > Available Nitrogen (kg ha <sup>-1</sup> )  > Available Potassium (kg ha-1)  > Zinc (Zn),  > Iron (Fe),  > Copper (Cu),  > Manganese (Mn)	Enclosure – VIII

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
			Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, economic and financial models have been developed.  Work done: 100 per cent completed  C. The study on evaluation of Clonal Seed Orchards established at UHF, Nauni has been completed for Qualitative, Quantitative and pseudo qualitative characteristics and leaf fodder characteristics and ten best nutritive selections have been identified and screened in the Clonal Seed Orchard under this project.  Work done: 100 per cent completed.  D. The nursery has been raised at Shilli farm of the department from the seeds collected from 35 population of Himachal Pradesh viz., Solan, Sirmour, Mandi, Hamirpur, Kangra, Una and Bilaspur. The following nursery growth parameters have been recorded and analysed statistically.  Leaf biomass per plant(g) at leaf harvesting stage  Seedling height (cm)  Collar diameter (mm)  Root length (cm)  Shoot fresh weight(g)  Shoot fresh weight(g)	Enclosure – IX

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
			<ul> <li>Root dry weight ratio</li> <li>Shoot dry weight ratio</li> <li>Root biomass - (Fresh &amp; Dry)</li> <li>Shoot biomass- (Fresh &amp; Dry)</li> <li>Raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers replica) material.</li> <li>Work done: 100 per cent completed.</li> </ul>	Enclosure –X
3.	Morphological characterization based on DUS specific descriptors of Grewia optiva.	Number of region-specific best practices/ models/ technologies developed (Nos.)	<ul> <li>A. Morphological data of 35 populations, which have been earlier recorded and completed on cropland situation, have been analysed statistically. Data base generated on 210 genotypes as example reference varieties under the project from 35 populations and data also have been generated from 23 families in Clonal Seed Orchard.</li> <li>B. The 10 best families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Morphological descriptors have been prepared for these 10 best screened families.</li> </ul>	Enclosure – XII  Enclosure – XIII
			C. For production of quality planting stock, the nursery has been raised at Shilli farm of the department from the seeds collected from superior families and of superior populations under different locations in different districts	Enclosure – XIII

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
4.	Molecular characterization of fast growing individuals of Grewia optiva	No. of Beneficiaries (Nos.)	of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.  D. The nine best selection/populations have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. The morphological DUS specific descriptors have been developed for these nine best selections.  Work done: 100 per cent completed.  A. Ten (10) best families and superior trees have been identified and screened from the clonal seed orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies.  B. The nine best selection/populations (with more than 24 % crude protein) have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. The molecular characterization of these selections have been achieved.  Work done: 100 per cent completed.	thereof:

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
5.	Production and supply of superior planting material for farmers and State Forest Department and also raising of nursery from superior proven genotypes.	Number of Reports/ Research articles/ Policy documents/ Manuals prepared and published (Nos.)	<ul> <li>A. In a first phase, 15 Divisional Forest Officers belonging to seven districts of Himachal Pradesh have been contacted.</li> <li>B. Thirty Five farmers belonging to Seven districts of Himachal Pradesh viz. Solan, Sirmaur, Mandi, Hamirpur, Una, Bilaspur and Kangra have been identified as beneficiaries i.e five farmers in each district i.e. at each population site/ district locality under study.</li> <li>C. Fruits/ Seeds of different 10 families (best proven families) have been supplied to Dr. Sheeraz Saleem Bhat, Senior Scientist (ARS-Forestry),ICAR-Indian Grassland and Fodder Research Institute, Regional Research Station, KD Farm, Rangreth, Srinagar-191 132 (Jammu &amp; Kashmir) out of the state, for raising quality planting stock in their regions and the outcome/results and growth performance data will be shared with us under the project activities for collaborative studies as per agreement.</li> <li>D. A good amount of 5400 plants at the rate of 360 Plants of superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket,</li> </ul>	

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
			Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during the year 2020 and 2021, (5400 plants in each year) for conservation and establishment of gene bank in each selected and identified forest division through SFD, for demonstration/ field testing trial/ block plantation.  E. Beside the above 360 plants other 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e., total 12,000 plants supplied for the farmers/ stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc.  Work done: 100 per cent completed.  The supply of superior quality planting material prepared in nursery in the extended period. i.e., again 360 plants raised from superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions for field testing and demonstration model (i.e., 5400 plants) and total 6,000 plants have been supplied to farmers /stakeholders/women groups identified through selected Forest Divisions.	Enclosure-XIV

S. No.	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations made, if any, & Reason thereof:
			<ul> <li>The nursery has also been raised during the year 2021 of seeds collected from thirty-five selected populations of Himachal Pradesh to distribute best planting stock in the extended period (during year 2022) to farmers, stake holders through the Forest Departments and Animal Husbandry Department/ Agriculture Department.</li> <li>❖ Total production of Quality planting material count to be in three years i.e. 5400 x 3 = 16,200 and 6000 x 3= 18,000, total of 34,200 plants is prepared in the nursery for distribution to the Fifteen Forest Divisions of Himachal Pradesh.</li> <li>❖ Total production of Quality planning material came out to be 34,200 plants/ saplings produced and supplied. At present about more than 20,000 plants/ saplings are available with the Department which have been screened out under the project.</li> <li>❖ Quality raised plants of <i>Grewia optiva</i> have been supplied the Quality Planting Stock of <i>Grewia optiva</i> Drummond (Beul) to DFOs/ stakeholders under the project theme 'Biodiversity Conservation' and Management' under the project.</li> <li>Work done: 100 per cent completed.</li> </ul>	Enclosure – XV  Enclosure – XVI

#### **Monitoring Indicators\***

Number of Reports/ Research articles/ Policy documents/ Manuals prepared and published (Nos.)

#### Research Papers (Published)

 H.P. Sankhyan\*, Jyoti Dhiman, Neerja Rana, Krishan Chand and Prachi (2022) Physiochemical characteristics of the Soil and their Correlation with Leaf Fodder Quality Parameters of Grewia optiva Drummond of the Himachal Pradesh. Biological Forum - An International Journal 14(2a) 1-8

Enclosure – XVII

- H.P. Sankhyan, Jyoti Dhiman, Krishan Chand, Prachi and Karishma (2021) Physico-Chemical Properties of Soil under *Grewia optiva* (Beul) Populations of Himachal Pradesh. *Indian Journal of Forestry.* 44 (1)193-200
- H.P. Sankhyan, Jyoti Dhiman, Krishan Chand, Prachi, Karishma and Bhupender Negi (2021) Soil Nutrient Analysis Underneath (*Grewia optiva*) Population. International Journal of Bio-resource and Stress Management. 12(6):645-654.
- Hari Paul Sankhyan, Jyoti Dhiman, Prachi and Shanti Swarup Sharma (2021). Seed sources variation in growth traits of *Grewia optiva* (Beul). *Indian Journal of Forestry*. 44 (4), 148-154
- HP Sankhyan, Sanjeev Thakur, Sunil Kumar, Karishma, Prachi and Krishan Chand (2021). Morphological characteristics among different populations for screening of beul trees (*Grewia optiva*) Drummond. *International Journal* of Chemical Studies. 8(4):1845-1848.
- <u>Dr. HP Sankhyan,</u> Dr. Sanjeev Thakur, Sunil Kumar, Karishma and Prachi (2021). Analysis of qualitative characteristics among and between populations of *Grewia* optiva Drummond (Beul). *International Journal of Fauna* and Biological Studies. 8(1): 76-82
- HP Sankhyan., Sanjeev Thakur, Sunil Kumar, Karishma and Prachi (2020). Variation in morphological descriptors among different populations of *Grewia optiva* Drummond (Beul) in Himachal Pradesh. *Journal of Pharmacognosy* and Phytochemistry. 9(5):249-254

#### **Research Papers Accepted for Publication**

- HP Sankhyan, Jyoti Dhiman, Neerja Rana and Krishan Chand (2022). Genetic Variability among different populations of *Grewia optiva* Drummond in low and mid hill zones of Himachal Pradesh. *Indian Journal of Agroforestry*
- HP Sankhyan, Jyoti Dhiman, Krishan Chand, Shikha Thakur, Prachi, and Karishma (2022). Fodder quality evaluation of selected populations of *Grewia optiva* Drummond in Himachal Pradesh. *Chemical Engineering*

#### **Published Technical Bulletin in Hindi**

- HP Sankhyan, Jyoti Dhiman, Prachi, Karishma, Bhupinder Negi and Sunil Kumar (2021), Beul ki Vaigyanik Vriksharopan Takneek, Utpadan Evam Mahatav, Tree Improvement and Genetic Resources, Dr YS Parmar University of Horticulture and Forestry, Nauni-Solan (HP) 173 230; ISBN978-81-927930-0-11: P 20
- Hari Paul Sankhyan, Jyoti Dhiman and Shikha Thakur (2022) Beul (*Grewia optiva*) ke Vividh Upyog, Anusandhan evam Samvardhan, Tree Improvement and Genetic Resources, Dr YS Parmar University of Horticulture and Forestry, Nauni-Solan (HP) 173 230 ISBN978-81-927930-0-12: P 21

#### **Research Papers Submitted for Publication**

- HP Sankhyan, Jyoti Dhiman and Krishan Chand (2022).
   Evaluation of half-sib progenies of Grewia optiva
   Drummond under nursery conditions. Indian Journal of Genetics and Plant Breeding
- HP Sankhyan, Jyoti Dhiman and Shikha Thakur (2022). Evaluation of genotypes of *Grewia optiva* Drummond under different tree spacing and estimation of their genetic parameters. *Journal of Mountain Sciences*

# Research Publication from previous years work on the species by the Principal Investigator of the Project

- Sankhyan H.P., Bhagta Shikha and Thakur Sanjeev. (2019). Evaluation of the half sib progenies to identify prepotency of the mother clones of *Grewia optiva* Drummond. *Journal of Pharmacognosy and Phytochemsitry*. 8(4): 1265-1270
- 2. Bhagta Shikha , <u>Sankhyan H.P.</u>, Thakur, Sanjeev., Bishist Rohit and Gupta R.K (2019). Evaluation of Seedling Seed Orchard of *Grewia optiva* Drummond for Morphometric and Fodder Quality Parameters. *Journal of Non-Timber of Forest Products* . 26(2): 1-5.
- 3. Bhat Sheeraz Saleem, Dand Suheel Ahmad, <u>Sankhyan HP</u>, Mir Nazim Hamid (2019)- *Grewia optiva* Drummod: An autobiography. In Van Sangyan (ISSN 2395 468X) Vol. 6. No. 10. Pages: 21-26 (Published by Tropical Forest Research Institute, Jabalpur, MP, India)
- **4.** Bhagta Shikha, <u>Sankhyan H.P.</u>, Sharma Dushyant and Ashine Tesfaye (2019). Correlation and path coefficient analysis in *Grewia optiva* Drummond. *International Journal of Chemical Studies*. 7 (3):746-749.
- 5. Bhagta Shikha, <u>Sankhyan H.P.</u>, Sharma JP and Kumari Reena. (2019). Assessment of Variability in Half Sib Progenies of *Grewia optiva* Drummond for Various Qualitative and quantitative Traits in North Western Himalayas. *International Journal Current Microbiology Applied Sciences*. 8 (4):1661-1669.

# 2.4. Strategic Steps with respect to Outcomes

S. No.	Particulars	Number/ Brief Details/ Gist	Remarks/ Attachment/
			Enclosure
1.	New Methodology developed	<ul> <li>Best Seed sowing time of <i>Grewia optiva</i> has been standardized. Pre sowing treatment and soil mixture has been standardized.</li> <li>Propagation through cuttings by use of Hormonal treatment has been worked out to produce mother's replica (true to type).</li> </ul>	Enclosure – XVIII
2.	New Models/ Process/ Strategy developed	<ul> <li>Established Demonstration Model -cum- Gene Bank of <i>Grewia optiva</i> (Beul) at Naganji Farm</li> <li>Establishment 'On Field Demonstration Model' - cum- Gene Bank of <i>Grewia optiva</i> (Beul) at Khaltoo Farm</li> <li>After complete screening out of nutritive strains of soil, the Correlation models, regression equations, economic and financial models have been developed for soil nutrients and plant growth and fodder quality characteristics.</li> </ul>	Enclosure – IV Enclosure – V
3.	New Species identified  (19 best fodder selections and one new species identified based on morphological descriptors developed)	<ul> <li>Based on morphological descriptors developed one new species has been identified i.e. <i>Grewia laevigata</i>, locally called as beuli. It is a lesser known tree species found in district Solan in grass land situation.</li> <li>Few plants of <i>Grewia laevigata</i> have been reported as occuring in Kangra district during survey undertaken under this project.</li> <li>Total 19 best fodder selections of <i>Grewia optiva</i> with more than 24 per cent crude protein have been identified and screened out under the project.</li> </ul>	

S.	Particulars	Number/ Brief Details/ Gist	Remarks/
No.			Attachment/ Enclosure
4.	New Database established	<ul> <li>The nine nutritive selections/populations viz., (Kothin Kanwal (Solan), Uncha gaon (Solan), Neri kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthin population of Bilaspur district) have been identified and screened with crude protein percentage of more than 24 percent from thirty-five selected populations of <i>Grewia optiva</i>. The Kothi Kanwal population exhibited average 24.43 % crude protein (CP), Uncha goan population with average 24.96 % crude protein (CP), Neri Kalan with average 25.12 % crude protein, Machair population reported with average 25.02 % crude protein, Jajjer with average 24.56 % crude protein, Balla population with average 25.05 % crude protein, Jhinjkari population with average 24.98 % crude protein, Bhaleth population with average 25.11 percent crude protein and Barthi population showed average 25.15 % crude protein.</li> <li>Analysis work of physicochemical characteristics of soil pH, Electrical Conductivity (EC), Organic Carbon (%), Available Nitrogen (N), Available Phosphorous (P), Available Potassium (K), Soil texture and Bulk density have been completed for all 35 populations. Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, economic and financial models have been developed.</li> <li>Data base generated on 210 genotypes as example reference varieties under the project from 35 populations, besides data have been generated from 23 families in Clonal Seed Orchard.</li> </ul>	

S.	Particulars	Number/ Brief Details/ Gist	Remarks/
No.			Attachment/
			Enclosure
		<ul> <li>The Morphological descriptors have been prepared for the 10 best screened families from Clonal Seed Orchard.</li> </ul>	
		<ul> <li>For production of quality planting stock, nursery has been raised at Shilli farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.</li> </ul>	
		<ul> <li>The nine best selection/populations have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. The morphological DUS specific descriptors have been developed for these nine best selections.</li> </ul>	
5.	New Patent, if any  I. Filed (Indian/		
	International)		
	II. Granted (Indian/ International)  III. Technology Transfer(if any)		
6.	Others (if any)	Only 19 example varieties/ reference varieties developed and being maintained at Farm of University Farm, Nauni-Solan	

# 3. Technological Intervention

S. No.	Type of Intervention	Brief Narration on the interventions	Unit Details (No. of villagers benefited / Area Developed)
1.	Development and deployment of indigenous technology		
2.	Diffusion of High-end Technology in the region		
3.	Induction of New Technology in the region	Raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers replica) material.	
4.	Publication of Technological / Process Manuals	07 (Research Papers Published) 02 (Research Papers Accepted for Publication) 02 (Research Papers Submitted for Publication) 02 (Technical Bulletin Published)	35
	Others (if any)		

# 4. New Data Generated over the Baseline Data

generated for thirty five was generated for obs superior populations already established gen	e variation in tree height erved in new baseline data erated for thirty five superior
generated for thirty five was generated for obs superior populations already established gen	erved in new baseline data
from the seven different districts of the Himachal Pradesh.  Height range recoded for selected genotypes between 3.73-9.54m  Orchards.  The tree height for genotypes of clonal seed orchards was recorded in the range between: 4.52-7.37m height the	ections. Tree height is an ortant parameter required to ntify timber resources and is ential in evaluating the nomic and ecological value of opland situation. In particular, the plays an important role in calculation of individual and I stand volume. Assessing

S.	New Data Details	Status of Existing	Additionality and Utilisation New
No.		Baseline	data
	Diameter –	Diameter –	a site and determining the social
	10.91-33.62cm	5.71-9.97cm	status of an individual tree's
	Leaf area (cm <sup>2</sup> )-	<ul> <li>Leaf area (cm²) -</li> </ul>	ability to access resources.
	44.95-123.49(cm <sup>2</sup> )	38.78-76.12(cm <sup>2</sup> )	The vigorous diameter growth
	Crude protein (%)-	• Crude protein (%) –	observed in new selected
	19.11-25.12%.	18.01-22.01%	families. The bigger the tree's
	The nine best selection/		diameter, the greater the amount
	populations have been		of foliage it has. These superior
	identified and screened		selections can be utilized for the
	with crude protein more		establishment of Seed orchards.
	than 24 % from thirty-five		
	selected populations on		Leaf area growth determines  the light interception conseits of a
	the basis of their		the light interception capacity of a
			crop and is often used as a
	proximate principles		surrogate for plant growth in
	analysis.		height through put phenotyping
			systems.
			Crude protein (CP) content is
			most important criterion for
			judging feed and fodder quality
			The nine nutritive selections/
			populations/ families viz., (Kothi
			Kanwal (Solan), Uncha gaon
			(Solan), Neri Kalan (Solan),
			Machair (Sirmour), Jajjer
			(Sirmour), Balla (Kangra),
			Jhinjkari (Hamirpur), Bhaleth
			(Hamirpur) and Barthi population
			of Bilaspur district) have been
			identified and screened with
			crude protein percentage more
			than 24 percent from thirty-five
			selected populations of Grewia
			optiva whereas the existing crude
			protein percentage was recorded
			less than 22% but in new

S.	New Data Details	Status of Existing	Additionality and Utilisation New
No.		Baseline	screened families/ selections it
			was observed higher than 24%.
			Therefore, the screened families
			with crude percentage more than
			24 % will be used to raise the
			demonstration block/ Seed
			Orchard and from where
			production of quality planting
			material will be raised and
			supplied to farmers. The
			establishment of seed orchards
			will help in the Mass-
			multiplication of superior
			genotypes and transferring of
			genetically improved material to
			end-users.
2.	Correlation and	No existing baseline	The correlation and regression
	regression models developed between	data was generated for soil physico-	Models developed will help to
	physiochemical	for soil physico- chemical	quantify the impact of different soil characteristics on morphological
	characteristics of soil	characteristics.	traits and help in selection and
	samples ((soil texture,	characteriotics.	fertilizers recommendation dose.
	Bulk density, pH, EC)		
	and other available	• Simple linear	Multiple regression models
	macronutrients (N, P, K,	regression D2H was	improved the predictions. DBH
	Ca, Mg and S) collected	found to be highly	alone can be used as most
	underneath the thirty-five	correlated with other	reliable parameters for estimation
	selected populations and	characteristics. The	of total standing biomass, above
	the morphological	allometric	standing biomass and leaf
	characteristics of the	relationships	component part.
	selected populations.	developed with DBH	
		as a predictor variable.	
		In general it gave	
		better values of r2.	

S.	New Data Details	Status of Existing	Additionality and Utilisation New
No.		Baseline	data
3.	The 10 best families	• Baseline data was	• The purpose of molecular
	have been identified and	generated only for	characterization is to determine
	screened from Clonal	morphological and	the genetic characteristics of the
	seed orchards on the	fodder characteristics.	plants and establish the identity of
	basis of the		the accessions and discern
	morphological and		genetic relationship among
	Molecular		genotypes. These also helps to
	characterization.		study genetic diversity and
			provides basis for conservation of
			superior strains.

# 5. Demonstrative Skill Development and Capacity Building/ Manpower Trained

S.	Type of Activities	Details with number	Activity Intended for	Participants/Trained
2.	Workshops On Field	05 Workshops ( local level) Enclosure-XIX	Five local level workshops organized during the year 2019-2020 with the staff and representatives of local bodies/local people  Three practical field	SC ST Woman Total
	Trainings	Enclosure-XX	tailored training/ camps organized for particularly for women.  One practical field tailored training/ camps organized for Pardhans of different Panchayats, Up-pardhans, Nursery Growers and Stake Holders including women, Forest Guards of HFRI and progressive farmers.	

S. No	Type of Activities	Details with number	Activity Intended for	Participants/Trained
	Activities	Enclosure-XXI	<ul> <li>Field Training for Forest         Guards of State Forest         Department of         Himachal Pradesh         Government</li> <li>One more Practical         field training organised         for the small farmers/         nursery growers of         District Solan and         Sirmaur for the         production of quality         planting material of         <i>Grewia optiva</i></li> </ul>	
			(Drummond) Beul during the Year 2022.	
3.	Skill Development		Through Workshops	
4.	Academic Supports		Through Departmental Faculty and project staff engaged under the project	
	Others (if any)			

# 6. Linkages with Regional & National Priorities (SDGs, INDC, etc)/ Collaborations

S. No.	Linkages /collaborations	Details	No. of Publications/	Beneficiaries
			Events Held	
1.	Sustainable Development			
	Goal (SDG)			
2.	Climate Change/INDC			
	targets			
3.	International			
	Commitments			
4.	Bilateral engagements			
5.	National Policies			
6.	Others collaborations			

# 7. Project Stakeholders/ Beneficiaries and Impacts

S. No.	Stakeholders	Support Activities	Impacts
1.	Gram Panchayats	35 Panchayats	The project is implementing in 35 Gram Panchayats in different districts of H.P.
2.	Govt Departments (Agriculture/ Forest)		
3.	Villagers	01	One practical field tailored training/ camps organized for Pardhans of different Panchayats, Up-pardhans, Nursery Growers and Stake Holders including women, Forest Guards of HFRI and progressive farmers
4.	SC Community		SC Community farmers were also involved in the Training/ Workshops programmes accordingly
5.	ST Community		No training organised only for ST Community however, people of ST Community (2-3 people) were involved in Workshops/ Training programmes organised under the project.
6.	Women Group	03	Three practical field tailored training/camps organized particularly for women.
	Others (if any)		

# 8. Financial Summary (Cumulative)

S. No.	Budget head	Amount received	Expenditure	Amount Balance/ excess expenditure
		(INR)	(INR)	(INR)
1.	Salaries	14,20,710.00	14,10,395.00	10,315.00
2.	Travel (Domestic)	4,83,056.00	4,77,012.00	6,044.00
		(4,75,569.00 +7,487.00)		
3.	Expendables &Consumables	7,74,080.00	7,80,870.00	-6,790.00
4.	Contingencies			
5.	Activities & Other Project cost	15,49,428.00	14,48,072.00	1,01,356.00
6.	Institutional Charges/ Over head	4,23,681.00	4,23,681.00	0.00
7.	Equipments			
8.	Total	46,50,955.00	45,40,030.00	1,10,925.00
9.	Accrued Bank Interest	34,030.00	-	34,030.00
		+14,585.00		+14,585.00
		+ 9,289.00		+ 9,289.00
10.	Grand Total	47,08,859.00	45,40,030.00	1,68,829.00

<sup>\*</sup> Bank interest adjusted INR 7,487.00 with carried forward amount during the Financial Year 2020-21 to 2021-22) under Travel Head vide letter no. GBPNI/NMHS-2018-19/SG/174/94/49 Dated: 16.06.2021

## Major Equipment/ Peripherals Procured under the Project

S. No.	Name of Equipments	Cost (INR)	Utilisation of the
			Equipment after project
1.			d V
2.		111 ai	
3.	- a Anne	XCHE	
4.	-please see-Anne		
5			

<sup>\*</sup>Please see Annexure I (the consolidated and audited Utilization Certificate (UC) and Year wise Statement of Expenditure (SE))

## 10. Quantification of Overall Project Progress

S. No.	Parameters	Total (Numeric)	Remarks/ Attachments/ Soft copies of documents		
1.	IHR States Covered	07 Districts	Districts: Una Sirmaur and I	, Hamirpur, Kangra, Mandi, Solan, Bilaspur.	
2.	Project Site/ Field Stations Developed	35 populations covered as project sites/ field stations in 7 districts of Himachal Pradesh  Maps (Enclosure- XXII)	District Una  Hamirpur  Kangra  Mandi Solan  Sirmaur  Bilaspur	Selected sites/ populations Bagana, Nawami, Kant, Lamlehri, Thanakalan at Budhwar. Harbal Neri, Jhinkari, Bhaleth, Janhen, Anu Khurd Katoi, Old Kangra, Balugloa, Balla, Dohran Bagla, Balt, Bharnoi, Gangal, Patta Kothi Kunal, Uncha gaon, Gaddon, Nerikalan, Devera Jajjar, Machher, Neharbag, Bedon, Dharkyari Ghumarwin, Barthin, Kuthera, Nehari, Jukhala	
3.	New Methods/ Modeling Developed	Seed Germination Studies Propagation through cuttings	been star and best to mixture hat Propagation	I sowing time of <i>Grewia optiva</i> has indardized. Pre sowing treatment time of seed sowing along with soil as been standardized. On through cuttings by use of treatment has been worked out to mother's replica (true to type).	
4.	No. of Trainings arranged	06 Enclosure- XXI	organized  One pract organized Panchayar and Stake Guards of Field Train Forest De Governme  One more for the sr District So of quality	for particularly for women.  tical field tailored training/ camps for Pardhans of different ts, Up-pardhans, Nursery Growers Holders including women, Forest HFRI and progressive farmers ning for Forest Guards of State epartment of Himachal Pradesh ent Practical field training organised mall farmers/ nursery growers of lan and Sirmaur for the production planting material of <i>Grewia optiva</i> and) Beul during the Year 2022.	

S. No.	Parameters	Total (Numeric)	Remarks/ Attachments/ Soft copies of documents
5.	No of beneficiaries attended trainings		35 Panchayats
6.	Scientific Manpower Developed (Phd/M.Sc./JRF/SRF/ RA):	02	<ul> <li>One M.Sc student (Sushmita Thakur F-2019-45-M) has completed her research work on the title "Evaluation of Clonal Seed Orchard of <i>Grewia optiva</i> Drummond for morphological and fodder quality parameters " under the project.</li> <li>One Ph.D. student (Jyoti Dhiman F-2019-11-D) is pursuing her research work on the title "Screening of nutritive strains of <i>Grewia optiva</i> Drummond for morphometric and leaf fodder values in Himachal Pradesh and Uttrakhand" under the project.</li> </ul>
7.	SC stakeholders benefited		SC Community farmers were also involved in the Training/ Workshops programmes accordingly.
8.	ST stakeholders benefited		No training organised only for ST Community however, people of ST Community (2-3 people) were involved in Workshops/ Training programmes organised under the project.
9.	Women Empowered	30 Enclosure- XX	<ul> <li>Thirty women (Key lady farmer) were trained and made aware regarding the forage quality and productivity as well as canopy management of <i>Grewia optiva</i> on their crop land situation.</li> <li>One training organized with the staff and representative of local bodies and local people in Shilli Nagar Panchayat and distributed Technical Bulletin to the farmers</li> </ul>

S. No.	Parameters	Total (Numeric)	Remarks/ Attachments/ Soft copies of documents					
10.	No of Workshops Arranged along with level of participation	<ul> <li>Five workshops were organized with and representative of local bodies and I people.</li> <li>During the year 2021-22, one workshop the Expert group was organised as per MOU of the project</li> </ul>						
11.	On field Demonstration Models initiated	02 (Enclosure XXIII (i)) (Enclosure XXIII (ii))	<ul> <li>Naganji experimental farm of the Department</li> <li>Khaltoo experimental farm of the Department</li> </ul>					
12.	Livelihood Options promoted		Skilled trainees					
13.	Technical/ Training Manuals prepared	02						
14.	Processing Units established							
15.	No of Species Collected	35 Seed Samples	One composite sample from each population was selected for the purpose under the study					
16.	New Species identified	01 (LKTS) Grewia laevigata	<ul> <li>Locally called as Beuli. It is a lesser known tree species found in district Solan and Kangra in grass land situation. Efforts are being made to multiply/ propagate this species. About 95% seed of the species are found infertile/ sterile.</li> <li>Few plants of <i>Grewia laevigata</i> have been found occuring in Kangra district during survey being undertaken by the Ph.D Student under this project working on this species.</li> </ul>					
17.	New Database generated (Types):	(07) Research Articles	Enclosure-XIV					
	Others (if any)							

### 11. Knowledge Products and Publications

		Total			
S. No.	Publication/ Knowledge Products		<i>lumber</i> International	Impact Factor	Remarks/ Enclosures
1.	Journal Research Articles/ Special	03	04	racioi	Enclosure-XIV
	Issue: 1) H.P. Sankhyan*, Jyoti Dhiman, Neer and Prachi (2022) Physiochemical cand their Correlation with Leaf Fodd Grewia optiva Drummond of the Hima Forum - An International Journal 14(2)	5.11			
	2) <u>HP Sankhyan,</u> Jyoti Dhiman, Krisl Karishma (2021) Physico-Chemical <i>Grewia optiva</i> (Beul) Populations of F <i>Journal of Forestry.</i> 44 (1)193-200	Properties	of Soil under	3.78	
	3) <u>HP Sankhyan,</u> Jyoti Dhiman, K Karishma and Bhupender Negi (202 Underneath ( <i>Grewia optiva</i> ) Populati of Bioresource and Stress Manageme	1) Soil Nu on. <i>Intern</i> a	trient Analysis ational Journal	5.11	
	4) Hari Paul Sankhyan, Jyoti Dhiman, P Sharma (2021). Seed sources varia Grewia optiva (Beul). Indian Journal 154	ation in g	rowth traits of	3.78	
	5) Sankhyan H.P., Thakur Sanjeev, Prachi and Chand Krishan characteristics among different populeul trees ( <i>Grewia optiva</i> ) Drummond Chemical Studies. 8(4):1845-1848.	(2021). ulations fo	Morphological r screening of	5.31	
	6) Sankhyan H.P., Thakur Sanjeev, Kur Prachi (2021). Analysis of qualitativ and between populations of <i>Grewia a</i> International Journal of Fauna and 76-82	e characte o <i>ptiva</i> Drui	eristics among mmond (Beul).	5.21	
	7) Sankhyan H.P., Thakur Sanjeev, Kur Prachi (2020). Variation in morph among different populations of Gre Journal of Pharmacognosy and Phyto	nological e <i>wia optiv</i>	characteristics a Drummond.	4.00	
2.	Book Chapter(s)/ Books:				
3.	Technical Reports	02			Enclosure-XIV
4.	Training Manual (Skill Development/ Capacity Building)	02			Enclosure-XIV
5.	Papers presented in Conferences/Seminars				
6.	Policy Drafts/Papers	02			
7.	Others:				

## Particulars

#### Recommendations

#### Utility of the Project Findings

- a) The nineteen nutritive selections screened under the project i.e., 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/trial studies and Nine (09) best selections/ populations with crude protein more than 24 % have been identified and screened from thirty-five selected populations on the basis of morphological and fodder quality characteristics, have been used to raise the demonstration block/Seed Orchard and from where production of quality planting material have been raised and supplied to farmers. The establishment of seed orchards will help in the Mass- multiplication of superior genotypes and transferring of genetically improved material to end-users. We recommend that old material which is existing on the cropland situation on the farmer's field should be gradually replaced with new screened superior genotypes in phased manner. The availability of superior nutritive strains with crude protein more than 24 % will reduce the gap between demand and supply of quality fodder for livestock. This will help to raise the socioeconomic status of the hilly farmers. This will be a good approach for sustainable development of social, economic and environment resources of IHR.
- b) The morphological DUS specific descriptors developed for selected genotypes under the project can be used for the categorization of superior genotypes in other parts of IHR.
- c) The superior seedling raised under the nursery conditions as well as the material that will be produced by mass multiplications from the nineteen fodder selections can be planted in the other parts of IHR. This could be an efficient way to replicate the outcomes of the project in other parts of IHR.
- d) The twenty new populations (five from Uttarakhand and sixteen from Himachal Pradesh) which have been added/selected to extend the research on *Grewia optiva* will be explored for variations and broader base of the germplasm of the species for further establishment of germplasm bank. This study will also help in comparative analysis among and between the selected populations of H.P and Uttarakhand to screen out the best nutritive selections and to find out valuable material for future *Grewia optiva* breeding programmes.

Particulars	Recommendations
	e) Quality planting of superior nutritive selection of Clonal Seed Orchard which have been supplied to 15 Forest Divisions for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, quality plants that have been supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc. should be multiplied and supplied in the untouched remaining parts of the IHR.
Replicability of Project	<ul> <li>Total nineteen superior nutritive selections have been screened from this study i.e., 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Nine (09) best selections/ populations with crude protein more than 24 % have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. These nineteen nutritive selections will be used to raise the demonstration block/Seed Orchard and from where production of genetically improved quality planting material will be raised and can be supplied to State Forest divisions, farmers of other parts of IHR.</li> <li>The Forest divisions and institutes of other hilly regions can conserve the quality planting material in Germplasm bank, and Seed Orchards and can establish their own Demonstration models. They can mass multiply quality planting material to produce true to type mothers replica.</li> <li>The methodology and approaches developed under the project can be used by forest institutions of Himalayan regions for screening of superior nutritive strains in other regions of IHR.</li> <li>For production of quality planting stock, nursery has been raised at Shilly Experimental Farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance/ at nursery stage level. This quality planting material can be supplied for cultivation in other areas of hilly regions.</li> </ul>

Particulars	Recommendations					
Exit Strategy	The superior genotypes identified and screened under the project are much					
	superior in fodder quality than the already practiced material. We recommend					
	that old material which is existing on the cropland situation on the farmer's					
	field should be gradually replaced with new screened genotypes in phased					
	manner. To popularize the screened genotypes for the improved plants efforts					
	are going on at all levels like state forest Department Himachal Pradesh, State					
	Agriculture Department/Animal Husbandry and Panchayat level and					
	progressive farmers. We are educating the importance of this valuable					
	germplasm to end users (ultimate beneficiary) for farming of these genotypes					
	developed under the project. This activity will continue for the benefits of end					
	users in the interest of the farmers of the hilly state Himachal Pradesh (in					
	Particular).					

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(Dr. HP Sankhyan)

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Horticulture & Forestry Nauni, Solan-173230

Place: Nauni, Solan.

Date:

# PART B PROJECT DETAILED REPORT

#### PART B: PROJECT DETAILED REPORT

#### 1 EXECUTIVE SUMMARY

The project entitled "Biological Screening, Conservation and Establishment of Gene Bank of *Grewia optiva* Drummond (Beul)" was undertaken with the broad Objectives as Biological Screening of the germplasm diversity of the *Grewia optiva* as agroforestry tree species in Himalaya, Study the growth parameters for forage quality and productivity of *Grewia optiva*, Development of DUS specific descriptors of the selected germplasm of *Grewia optiva* and Conservation and Establishment of gene bank of selected genotypes of *Grewia optiva*.

The study was carried out in low and mid hill zones of H.P. with total 35 populations (5 in each district) in 7 districts viz. Solan, Bilaspur, Mandi, Una, Hamirpur, Kangra and Sirmaur and further 210 morphologically best genotypes (six from each population) considered with an aim to ascertain the response of the different parameters towards the screening of the adopted best genotypes and forage quality and productivity. First approach was to screen out the best genotypes of the best families selected from the identified populations in all the seven districts of Himachal. The second approach was to produce genetically superior planting stock of those genotypes and families which are having high crude protein i.e. more than 20 per cent.

About 180 genotypes belonging to 60 families have been established in Seedling Seed Orchard as Gene bank. As many as 69 genotypes established in Clonal Seed Orchard of 23 best families. Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Research Farm of the Department with total 165 genotypes belonging to 55 families (planted in 3 replication at a spacing of 2x2m). On 'Field Demonstration Model' –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Khaltoo Research Farm with total 60 genotypes belonging to 20 families (planted in 3 replications at spacing of 2x2m). The Morphological and Morphometric observations (plant height, plant diameter, branch angle, crown spread (E-W and N-S), number of primary and secondary branches, one year old shoot thickness, phenological events, leaf shape, leaf length, leaf width, leaf area, fruit length, fruit weight, fruit shape and seed weight and seed coat colour etc.) of all the selected genotypes have been recorded and statistically analysed.

From Chamba block of Tehri Garhwal, Uttarakhand and other agricultural fields of Himachal Pradesh, an additional germplasm has been selected/ introduced to find out other morpho variants and superior nutritive strains of *Grewia optiva*. The fodder quality parameters (leaf fresh weight, leaf dry weight, leaf dry matter, ether extract, crude protein, crude fibre, total ash, nitrogen free extract) have been recorded for all the selected thirty-five populations and analysed statistically. The Nine (09) nutritive fodder selections/populations *i.e.* Kothi kanwal (Solan), Unchagaon (Solan), Nerikalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi

population of Bilaspur) have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected and identified populations of *Grewia optiva* Drummond.

The analysis of soil physicochemical characteristics (soil texture, Bulk density, pH, EC) and other available macronutrients (N, P, K, Ca, Mg and S) of thirty-five soil samples collected under *Grewia optiva* populations has been done. There were significant differences observed in pH, OC, EC, N, P, K and in Bulk density within selected population of each district. Correlation studies were worked out after developing complete screening of the nutritive strains and regression equations, economic and financial models have been developed. Clonal Seed Orchard of genotypes having 23 genotypes/ families already established has been evaluated for morphometric and fodder Quality Parameters. Ten (10) Superior genotypes among different families having high nutritive strains have also been screened out on the basis of morphological and molecular characterization.

The nursery has been raised at Shilli Farm of the Department from the seeds collected from 35 population of Himachal Pradesh, after which the seed germination and seedling growth have been recorded and their statistical analysis has been done. Treatment of (IBA 500 mg/l) auxin concentration has also been standardized for raising planting material through cuttings for true to type (mother's replica) material. Morphological descriptors of 35 populations of seven districts of Himachal Pradesh have been developed, and 210 superior plants have been identified and screened biologically. Data base has been generated on 210 genotypes as Example/ Reference Varieties under the project from 35 populations. Best 10 families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies and their morphological descriptors have been developed.

In Shilli Farm, quality planting stock has been raised from the seeds collected from superior families and of superior populations under different ecological niches in seven different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level. Quality planting material raised of superior nutritive selection of Clonal Seed Orchard has been supplied to 15 Forest Divisions of H.P. (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during the year 2020, 2021 and 2022, i.e., 5,400 plants each year totaling 16,200 plants for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, other 400 plants have been also supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020, 2021 and 2022, i.e., totaling to 18,000 plants supplied for the farmers/stakeholders, identified by the Forest Division for the benefit to the end users, farmers, women groups, self-help group etc.

Total production came out to be 34,200 plants/saplings which have been supplied. At present more than 20,000 plants/saplings are still available with the Department from thirty-five selected populations of Himachal Pradesh to distribute best planting stock to farmers, stake holders

through the Forest Departments and Animal Husbandry Department/Agriculture Department and to the near future in Phased manner. Department has screened and obtained 19 fodder selection of *Grewia optiva* (Buel) with crude protein percentage more than 24 percent under the said adhoc research project.

#### 2 INTRODUCTION

#### 2.1 Background of the Project

The project was started with the aim of identification and screening of superior nutritive selection of *Grewia optiva* to mitigate the gap between demand and availability of quality/nutritive green fodder during winter seasons in IHR, Himachal Pradesh (in particular). *Grewia optiva*, commonly known as Beul/Bihul/Bhimal, belongs to family Tiliaceae, is a very popular and useful tree grown in low and mid-hill regions in agroforestry systems in the western and central Himalaya. It is a moderate sized tree, with a spreading crown, reaching height upto 12 m and a girth of about 80 cm. Bark is smooth and whitish grey, leaves are 5-13 cm x 3.6 cm, ovate, acuminate, sharply serrate, rough and hairy on the upper surface. Petals are yellowish or whitish in colour. The fruit is a fleshy drupe, 2-4 lobed, olive green when immature and black when ripe, edible. It is a tree of sub tropical climate and grows on a variety of soils whereas sandy loam soil with adequate moisture supply supports good growth.

This tree is preferred by the majority of the hill farmers of Himachal Pradesh, Uttarakhand and Nepal, etc., due to its superiority over other fodder tree species for the attributes like palatability, faster growth, ease of propagation and forage yield. It provides fodder during winter months, when no other green fodder is available. Leaves of this tree are excellent source of fodder in mid Himalayan region as they retain appreciable amount of nutrients. It has over 70 per-cent potential DM digestibility and effective degradability (56.7%) which makes it a superior energy source for ruminants. According to earlier study *Grewia optiva* leaves contain 17.4 - 21.0 per cent crude protein, 17.0 - 21.5 per cent crude fiber, 10.4-21.5 per-cent total ash, 4.2 - 6.0 per cent ether extract and 40.4 - 50.2 per cent nitrogen free extract.

The leaf fodder of *Grewia optiva* is almost as nutritious as that of leguminous crops containing high digestibility, good vitamins and mineral contents and it also improves the microbial growth and digestion of cellulosic biomass in the rumen of livestock. Leaves of *Grewia optiva* do not contain tannins which makes it more suitable for fodder. Therefore, this species plays an important role as a supplementary feed to the livestock which enables the livestock to fight against the harsh conditions of winter. Green leaves and fresh tender succulent branches of beul provide fodder of good quality which is comparable with green fodder of barseem, alfa or cowpea in respect to crude protein, crude fiber, calcium and phosphorus contents. Thus, the leaf fodder of *Grewia optiva* play an important role in improving the nutrition of livestock in the hills where the poverty of the farmers neither allows them to purchase concentrated feeds nor their small holdings allow them to resort to cultivation of nutritious leguminous fodder.

The study on *Grewia optiva* Drummond (Beul) under this project has been undertaken in low and mid hill zones of H.P., keeping in view the rich genetic diversity of *Grewia optiva* populations for recording morphological, morphometric variations on plants to among populations and within populations. Total thirty-five populations were identified, marked and selected (five in each district) in seven districts of Himachal Pradesh viz. Solan, Bilaspur, Mandi, Una, Hamirpur, Kangra and Sirmaur.

The list of thirty-five selected populations from seven districts of Himachal Pradesh.

District	Selected site / populations	
Una	Bagana, Nawami, Kant, Lamlehri, Thanakalan at Budhwar.	
Hamirpur	Harbal Neri, Jhinkari, Bhaleth, Janhen, Anu Khurd	
Kangra	Katoi, Old Kangra, Balugloa, Balla, Dohran	
Mandi	Bagla, Balt, Bharnoi, Gangal, Patta	
Solan	Kothi Kunal, Uncha Gaon, Gaddon, Nerikalan, Devera	
Sirmaur	Jajjar, Machher, Neharbag, Bedon, Dharkyari	
Bilaspur	Ghumarvii, Barthin, Kuthera, Nehari, Jukhala	

In thirty-five selected populations, a total of 210 superior genotypes (six in each population) of 20cm–30cm diameter class were identified/marked with aim to ascertain the response of the different parameters towards the screening of the adopted best genotypes and forage productivity.

#### 2.2 Overview of the Major Issues to be Addressed

## a) Need to reduce the gap between demand and availability of quality fodder during lean season :

Livestock rearing with agriculture is a common practice in the hilly areas of the country as it contributes towards economy and improve living standard of the hilly farmers. Livestock production is mainly dependent on the availability of nutritious fodder. But in the Himalayan regions there is a shortage of fuel and fodder species particularly in the lean season (winter season). Availability of the green fodder is very less as compared to the demand for livestock. As per the standing committee report 2016-17 there will increasing demand of 1000 million tones green fodder in the year 2025 but the availability will be only of 600 million tonnes and there will be deficit of 40 %. Therefore to reduce the deficit there is need to reduce the gap between demand and availability of fodder during the lean period. *Grewia optiva* is a multipurpose species which can produce fodder, fuel, supply organic fertilizers to soil fertilizer to maintain the soil productivity and fertility.

#### b) Need to reduce the tremendous pressure on forest for fuel wood, fodder and fibres:

The *Grewia optiva* is a multipurpose species which can be grown in hilly region to produce fodder, fuel wood, fibers etc. during the lean period. Leaf fodder yield from mature trees is reported to be 12-30 kg tree<sup>-1</sup>. Leaves are fairly rich in protein and other nutrients and do not contain tannins. Its leaves contain crude protein, crude fiber, total ash, percent ether extract and nitrogen free extract. The high calorific value (4920 k cal kg<sup>-1</sup>) of the tree wood makes it a very good source of fuel wood and alternate source of energy. It also provides fibre, edible fruits, used for making oar shafts, shoulder poles, cot frames, bows, paddles, tools and axe handles, papermaking, fibre products etc. and has medicinal value. Besides producing valuable product, the tree also provides varieties of ecological functions and associated services.

## c) Need to Popularize the *Grewia optiva* based agroforestry systems in hilly regions to reduce soil erosion, increase soil fertility and production of cash crops:

Grewia-based agroforestry systems in western Himalaya have potential to improve soil quality which can lead to sustainable production and optimum utilization of resources. As the increasing anthropogenic pressure is leading to widespread resource degradation in the fragile Western Himalaya. A more holistic approach is, therefore, required for sustainable production and protecting the natural base from further degradation. Therefore, the use of Grewia optiva as an agroforestry species for land use practices will optimize the environmental as well as economic benefits. This species use nutrients and water from lower soil depths that shallow plant roots cannot access. It checks the soil erosion and runoff, maintain soil organic matter, improve soil physical properties, minimize nutrient loss, promote efficient nutrient cycling, sequester carbon and provide numerous ecosystem services. These Grewia based agroforestry systems are more sustainable than single agriculture systems. The growth and yield of cash crops significantly affected under the influence of canopy of Grewia optiva trees retained on farm bunds by the farmers. The species should be widely adopted in agroforestry specially, on terraces as it is also has capacity to grow and survive in rainfed conditions. The tree also provides raw material for shampoo and hair conditioner.

#### d) Need to increase the employment in hilly regions

There is a scarcity of income sources in the hilly regions. But the cultivation of superior nutritive selections of *Grewia optiva* will increase the overall livestock production i.e., milk, meat etc. The fiber extracted from the bark of this species can be used for making multiple products *e.g.*, hanging baskets, ropes, handloom items etc. The hill farmers can create their own small venture for production of value-added products and can sell them in the market. As the wood of *Grewia optiva* is semi hard which is strong and elastic. Its old twigs can be used for making light farm implements like handles of spades, axes, pick axes,

gulel for bird scaring, wooden oars and other small farm implements like kulta used for weeding. This approach will give them income sources and increase their standard of living.

The nineteen superior nutritive selections have been screened under the project i.e., The Nine (09) nutritive selections/populations viz., Kothi Kanwal (Solan), Unchagaon (Solan), Nerikalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of Bilaspur district have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected and identified populations of *Grewia optiva* Drummond and 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. These selections will be used to raise the demonstration block/Seed Orchard and from where production of quality planting material will be raised through mass multiplications and supplied to farmers. The old stock which is existing on the cropland situation on the farmer's field will be replaced gradually with new screened genotypes in phased manner. This will be a good approach for improving the major fodder issues of hilly regions. As the cultivation of superior nutritive strains will not only provide the good quality fodder but it will also enhance the availability of fuel wood, fibers, wood for production of various wood products.

#### 2.3 Baseline Data and Project Scope

Table: 1. The number of plants saplings to be developed as under:

Sr. No.	Particulars	Numbers	1 <sup>st</sup> Year	2 <sup>nd</sup> Year	3 <sup>rd</sup> Year	Total number of saplings to be developed during the entire project period *
1.	Total number of families	60	60	60	60	
2.	Total number of genotypes in each family	03	03	03	03	
3.	Number of plants of each genotypes	30	30	30	30	
4.	Total plant saplings	60x03x30	5,400	5,400	5,400	16,200
{	Total plant saplings developed from Clonal Seed Orchard Quality planting material raised in nursery from 35 populations					+18,000 +20,700 (more than 20,000 saplings are available with the Department)
	Total plant saplings developed during the project period in (P. bags)					=54,900 Say 55,000 saplings developed/ produced

Design to be employed for planting/field testing trail: RBD

Table: 2. Details of type of passport data with sample size.

Serial Number	COLLSITE	Population	Accession Number (ACCENUM B)	(Important	Collection descriptors (Important morphometric and fodder parameters)		Collecting number (COLLNUMB		MB)	
	Location of collecting site	Family	Unique identifier for accession	Height (m)	Diameter (cm)	Leaf area (cm²)	Fodder quality traits (crude protein %)	Initia collec	otypes co als of the ctor(s) S- 21 cro	
1.	Bilaspur	Bilaspur	UHF-BI-1	6.13	6.97	43.99	21.35	G1	G2	G3
2.	Bilaspur	Kanduar	UHF-BI-2	6.07	8.60	53.77	21.25	G1	G2	G3
3.	Bilaspur	Auhar	UHF-BI-3	7.37	9.43	53.70	21.36	G1	G2	G3
4.	Bilaspur	Kuthira	UHF-BI-4	6.03	8.33	55.04	20.57	G1	G2	G3
5.	Bilaspur	Talvano	UHF-BI-5	5.90	7.77	52.54	21.17	G1	G2	G3
6.	Chamba	Chanad	UHF-CH-1	5.77	6.23	72.25	20.09	G1	G2	G3
7.	Chamba	Shahu	UHF-CH-2	4.52	6.43	52.58	20.66	G1	G2	G3
8.	Chamba	Balu	UHF-CH-3	6.42	7.80	76.12	20.71	G1	G2	G3
9.	Chamba	Audhpur	UHF-CH-4	6.45	9.40	71.26	19.46	G1	G2	G3
10.	Chamba	Rajpura	UHF-CH-5	5.82	8.23	52.97	18.28	G1	G2	G3
11.	Chamba	Saru	UHF-CH-6	6.12	7.63	43.34	19.84	G1	G2	G3
12.	Chamba	Rajnagar	UHF-CH-7	5.99	7.30	41.10	19.81	G1	G2	G3
13.	Hamirpur	Bharari	UHF-HA-1	4.53	6.45	55.55	21.32	G1	G2	G3
14.	Hamirpur	Patta Balakhar	UHF-HA-2	7.58	9.25	73.88	21.35	G1	G2	G3
15.	Hamirpur	Bassi	UHF-HA-3	5.98	6.90	58.64	21.39	G1	G2	G3
16.	Hamirpur	Hamirpur Kanal	UHF-HA-4	7.23	8.27	41.29	21.03	G1	G2	G3
17.	Hamirpur	Ghahar	UHF-HA-5	5.67	7.53	43.10	20.32	G1	G2	G3
18.	Kangra	Dharmshala	UHF-KA-1	6.50	7.27	58.97	21.86	G1	G2	G3
19.	Kangra	Bhalun	UHF-KA-2	6.07	9.33	44.31	20.61	G1	G2	G3
20.	Kangra	Varal	UHF-KA-3	5.90	7.33	52.97	21.16	G1	G2	G3
21.	Mandi	Samaila	UHF-MA-1	6.00	7.40	46.00	20.90	G1	G2	G3
22.	Mandi	Bachhwan	UHF-MA-3	6.40	7.30	59.26	21.39	G1	G2	G3
23.	Mandi	Bambla	UHF-MA-3	5.53	7.90	43.60	21.63	G1	G2	G3
24.	Mandi	Tatahar	UHF-MA-4	5.40	7.03	40.76	20.99	G1	G2	G3
25.	Mandi	Sarkaghat	UHF-MA-5	5.47	6.64	52.09	19.45	G1	G2	G3
26.	Shimla	Daugi	UHF-SH-1	6.47	6.43	67.55	20.91	G1	G2	G3
27.	Shimla	Ninmun	UHF-SH-2	7.25	9.87	61.83	21.2	G1	G2	G3
28.	Shimla	Jeury	UHF-SH-3	6.70	7.87	38.78	21.62	G1	G2	G3
29.	Shimla	Tatapani	UHF-SH-4	5.92	7.73	47.27	21.50	G1	G2	G3
30.	Shimla	Sunni	UHF-SH-5	5.67	6.70	47.27	21.73	G1	G2	G3
31.	Shimla	Palyad	UHF-SH-6	6.17	8.20	54.54	20.44	G1	G2	G3

	Location of collecting site	COLLSITE Population Accession Number (ACCENUM B)		Collection descriptors (Important morphometric and fodder parameters)					Collecting number (COLLNUMB)			
		Family	Unique identifier for accession	Height (m)	Diameter (cm)	Leaf area (cm²)	Fodder quality traits (crude protein %)	Initia collec	otypes co als of the ctor(s) S- 21 cro			
32.	Shimla	Taradevi	UHF-SH-7	6.35	7.90	52.2	20.44	G1	G2	G3		
33.	Sirmour	Narag	UHF-SI-1	6.37	6.60	42.92	20.23	G1	G2	G3		
34.	Sirmour	Nohra	UHF-SI-2	5.57	7.22	67.36	20.27	G1	G2	G3		
35.	Sirmour	Deothal	UHF-SI-3	5.50	7.14	41.91	20.43	G1	G2	G3		
36.	Sirmour	Dilman	UHF-SI-4	4.93	5.71	39.66	20.25	G1	G2	G3		
37.	Sirmour	Deyoltikkeri	UHF-SI-5	5.63	7.48	42.63	20.32	G1	G2	G3		
38.	Sirmour	Kalaghat	UHF-SI-6	7.12	8.40	45.14	20.37	G1	G2	G3		
39.	Sirmour	Nandel	UHF-SI-7	7.57	8.53	69.11	20.65	G1	G2	G3		
40.	Sirmour	Dotliji	UHF-SI-8	6.68	6.70	47.12	22.03	G1	G2	G3		
41.	Sirmour	Maryog	UHF-SI-9	6.43	4.93	61.36	19.47	G1	G2	G3		
42.	Sirmour	Seenaghat	UHF-SI-10	6.57	9.33	57.19	21.40	G1	G2	G3		
43.	Sirmour	Adgu	UHF-SI-11	6.10	9.97	72.58	20.23	G1	G2	G3		
44.	Sirmour	Loyankolta	UHF-SI-12	5.32	6.90	58.42	18.96	G1	G2	G3		
45.	Sirmour	Sarpadol	UHF-SI-13	7.37	9.40	49.44	22.01	G1	G2	G3		
46.	Sirmour	Saraha Chakli	UHF-SI-14	6.50	7.34	54.52	18.69	G1	G2	G3		
47.	Sirmour	Madhobag	UHF-SI-15	6.50	8.17	69.47	20.88	G1	G2	G3		
48.	Sirmour	Nainatikker	UHF-SI-16	6.36	8.60	43.42	19.27	G1	G2	G3		
49.	Solan	Gaura	UHF-SO-1	6.77	8.67	40.10	19.81	G1	G2	G3		
50.	Solan	Nauni	UHF-SO-2	6.53	8.23	48.79	19.54	G1	G2	G3		
51.	Solan	Dharja	UHF-SO-3	6.60	8.57	50.92	19.55	G1	G2	G3		
52.	Solan	Deog	UHF-SO-4	5.39	8.10	49.04	20.09	G1	G2	G3		
53.	Solan	Badhlech	UHF-SO-5	5.03	8.03	49.29	20.09	G1	G2	G3		
54.	Solan	Amberkothi	UHF-SO-6	6.37	7.60	65.37	18.01	G1	G2	G3		
55.	Solan	Oyali	UHF-SO-7	7.10	8.47	48.70	20.10	G1	G2	G3		
56.	Solan	Kailar	UHF-SO-8	6.07	8.03	63.10	20.88	G1	G2	G3		
57.	Solan	Deothi	UHF-SO-9	6.53	8.67	60.07	20.66	G1	G2	G3		
58.	Solan	Jaunaji	UHF-SO-10	5.17	6.67	50.09	19.76	G1	G2	G3		
59.	Solan	Mishuar	UHF-SO-11	6.07	8.40	51.56	18.80	G1	G2	G3		
60.	Solan	Kasholi	UHF-SO-12	7.07	9.87	51.09	19.75	G1	G2	G3		

Table 3. Baseline data generated for thirty-five selected populations from seven districts of Himachal Pradesh.

Serial Number	COLLSI TE	Population	Accession Number (ACCENU MB)		Collection descriptors (Important morphometric and fodder parameters)				Collecting number (COLLNUMB)				
	Location of collecting site	Family	Unique identifier for accession	Height (m)	Diameter (cm)	Leaf area (cm²)	Fodder quality traits (crude protein %)	Ger	notypo	es cod	e		
1.	Solan	Kothi kanwal	HP-SO-1	9.54	22.16	101.02	24.43	G1	G2	G3	G4	G5	G6
2.	Solan	Unchagaon	HP-SO-2	8.54	33.62	123.49	24.96	G1	G2	G3	G4	G5	G6
3.	Solan	Nerikalan	HP-SO-4	4.23	21.74	95.52	25.12	G1	G2	G3	G4	G5	G6
4.	Solan	Gaddo	HP-SO-3	5.08	13.68	76.6	21.98	G1	G2	G3	G4	G5	G6
5.	Solan	Devera	HP-SO-5	8.30	24.02	79.51	20.12	G1	G2	G3	G4	G5	G6
6.	Sirmour	Machair	HP-SI-1	7.84	19.72	84.75	25.02	G1	G2	G3	G4	G5	G6
7.	Sirmour	Jajjar	HP-SI-2	3.85	21.05	94.08	24.56	G1	G2	G3	G4	G5	G6
8.	Sirmour	Neharbag	HP-SI-3	6.99	19.78	74.68	20.43	G1	G2	G3	G4	G5	G6
9.	Sirmour	Badon	HP-SI-4	4.50	12.67	72.19	21.22	G1	G2	G3	G4	G5	G6
10.	Sirmour	Dharkyari	HP-SI-5	4.33	14.47	58.08	20.19	G1	G2	G3	G4	G5	G6
11.	Una	Kant	HP-UN-1	5.50	16.83	64.92	19.23	G1	G2	G3	G4	G5	G6
12.	Una	Navami	HP-UN-2	5.75	20.57	44.95	19.11	G1	G2	G3	G4	G5	G6
13.	Una	Kharuniban gana	HP-UN-3	5.08	15.03	50.18	20.17	G1	G2	G3	G4	G5	G6
14.	Una	Thanakalan	HP-UN-4	5.38	13.52	54.8	19.89	G1	G2	G3	G4	G5	G6
15.	Una	Lamlehri	HP-UN-5	6.00	17.42	71.82	20.33	G1	G2	G3	G4	G5	G6
16.	Kangra	Katoi	HP-KG-1	5.93	19.46	63.81	20.15	G1	G2	G3	G4	G5	G6
17.	Kangra	Balugloa	HP-KG-2	7.10	18.72	65.93	22.34	G1	G2	G3	G4	G5	G6
18.	Kangra	Purana kangra	HP-KG-3	7.10	26.64	67.77	23.43	G1	G2	G3	G4	G5	G6
19.	Kangra	Dohan	HP-KG-4	5.98	21.80	47.06	22.65	G1	G2	G3	G4	G5	G6
20.	Kangra	Balla	HP-KG-5	4.50	15.39	53.8	25.05	G1	G2	G3	G4	G5	G6
21.	Hamirpur	Janhen	HP-HA-1	6.80	29.63	46.42	23.92	G1	G2	G3	G4	G5	G6
22.	Hamirpur	Jhinkari	HP-HA-2	5.75	22.95	55.24	24.98	G1	G2	G3	G4	G5	G6
23.	Hamirpur	Harbalneri	HP-HA-3	5.93	21.00	55.54	23.69	G1	G2	G3	G4	G5	G6
24.	Hamirpur	Anu khurd	HP-HA-4	4.83	17.28	59.58	20.94	G1	G2	G3	G4	G5	G6
25.	Hamirpur	Bhaleth	HP-HA-5	8.57	23.12	54.06	25.11	G1	G2	G3	G4	G5	G6
26.	Mandi	Patta	HP-MA-1	5.67	19.48	57.89	19.34	G1	G2	G3	G4	G5	G6
27.	Mandi	Gangal	HP-MA-2	3.73	12.01	55.49	19.73	G1	G2	G3	G4	G5	G6
28.	Mandi	Bagla	HP-MA-3	5.20	16.97	62.18	20.22	G1	G2	G3	G4	G5	G6
29.	Mandi	Balt	HP-MA-4	6.63	15.03	62.26	20.52	G1	G2	G3	G4	G5	G6
30.	Mandi	Bharnoi	HP-MA-5	6.10	15.92	65.06	19.68	G1	G2	G3	G4	G5	G6
31.	Bilaspur	Ghumarwin	HP-BL-1	6.95	10.91	65.18	23.93	G1			G4	G5	G6
32.	Bilaspur	Barthi	HP-BL-2	7.98	20.32	72.14	25.15	G1		G3		G5	G6
33.	Bilaspur	Kuthera	HP-BL-3	5.91	25.00	67.96	20.98	G1			G4	G5	G6
34.	Bilaspur	Jukhala	HP-BL-4	5.01	21.11	60.71	23.42	G1	G2	G3		G5	G6
35.	Bilaspur	Nehari	HP-BL-5	5.88	23.31	58.94	22.72	G1	G2	G3	G4	G5	G6

Table: 4. Comparative analysis between the existing base line data and new generated base line data

	Existing base line data (Recorded	New base line data generated (Recorded	Utilisation of new data
Height	<b>range)</b> 4.52-	<b>range)</b> 3.73-9.54m	More variation in height observed in new data. Tree height is
(m)	7.37m		an important parameter required to quantify timber resources and is essential in evaluating the economic and ecological value of a cropland situation. In particular, height plays an important role in the calculation of individual and total stand volume. Assessing the overall productive capacity of a site and determining the social status of an individual tree's ability to access resources.
Diameter	5.71-	10.91-	The vigorous diameter growth observed in new selected
(cm)	9.97m	33.62cm	families. The bigger the tree's diameter, the greater the amount of foliage it has. These superior selections can be utilized for the establishment of Clonal orchards.
Leaf area	38.78-	44.95-	Leaf area growth determines the light interception capacity of
(cm²)	76.12(cm <sup>2</sup> )	123.49(cm <sup>2</sup> )	a crop and is often used as a surrogate for plant growth in high-throughput phenotyping systems.
Crude	18.01-	19.11-	Crude protein (CP) content is most important criterion for
protein (%)	22.01%	25.12%	judging feed and fodder quality The nine nutritive selections/populations <i>viz.</i> , Kothi Kanwal (Solan), Uncha gaon (Solan), Neri Kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of Bilaspur district have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected populations of <i>Grewia optiva</i> .  These selections / families will be used to raise the demonstration block/Seed Orchard and from where production of quality planting material will be raised and supplied to farmers. The establishment of seed orchards will help in the Mass- multiplication of superior genotypes and transferring of genetically improved material to end-users.

Table: 5. Details of sample size and description for nursery raising and planting techniques.

Nursery and planting techniques	Description	
Propagules for nursery raising (seeds or cuttings)	200 gram seed from each genotype and 30 cuttings from same genotype/ plant.	
Seed collection time	December to January	
Nursery beds	Sunken beds were prepared in raised beds and seedlings and	
	saplings were raised in poly bags.	
Sowing time	April 15 to May 15.	
Seed	After the collection of berries, seeds were dried under shade for	
	few days and store in cloth or poly bags at room temperature	
	under dry conditions.	
Treatment	Soaking the seeds in cold boiled water for 12 hours and cuttings	
	were treated with different auxin concentrations of Indole-3	
	butyric acid (IBA) and Naphthalene acetic acid (NAA).	
Sowing depth and spacing	Seeds were sown about 2cm deep in lines 15 cm apart. Seed to	
	seed distance was kept 10 cm.	
Seed germination	Normally 60 to 80 per cent germination occurs. We get uniform	
	stock for planting in about 2-1/2 to 3 months or for stump	
	planting in the ensuing winter.	
Irrigation	Nursery beds were irrigated after sowing and regularly then after	
	till germination is over.	
Weeding	Regular weeding is necessary. At the time of weeding the	
	seedlings were spaced about 10cm apart in lines.	
Planting time	July-August after the rains has started.	
Planting	Planting was done in pits of 30cm <sup>3</sup> with the beginning of rainy	
	season.	
Spacing	3x3 meter for block planting and 4-5m for single row planting	
	along the fields.	
Plant protection measures	Seedling and saplings against defoliator's attack through the	
	application of insecticides will be ensured. The plantation areas	
	was protected against grazing and browsing and fire.	
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#### 2.3 Project Scope: The project scope have been well described under the following heading.

1) **Germplasm conservation**: The screened genotypes have been conserved in the germplasm bank and in seed orchards. i.e., the Number of genotypes conserved in germplasm bank (Nos) at spacing 1x1 m = 27 genotypes 2x2m = 31,3x3m=07genotypes 4x4 m=04 genotypes. In Seedling seed orchard 180 genotypes of 60 families and in Clonal Seed

Orchard 23 genotypes in three replications i.e. 69 plants have been conserved. Beside this Established Demonstration Model -cum- Gene Bank of Grewia optiva (Beul) at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replications) at a spacing of 2x2m. Standard management practices have been done/ undertaken on already established spacing trials at Naganji Farm. Established 'On Field Demonstration Model'-cum- Gene Bank of *Grewia optiva* (Beul) at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m. This conserved germplasm can be mass multiplied to produce superior true to type mother's replica. This conserved germplasm can be used for future breeding programs and development of superior hybrids with high fodder quality parameters.

- 2) Superior Nutritive Selections: The 19 Nutritive selections have been screened under the project i.e., 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies and Nine (09) best selections/ populations with crude protein more than 24 % have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. These selections will be used to raise the demonstration block/Seed Orchard and from where production of quality planting material will be raised and supplied to the hilly farmers. The establishment of seed orchards will help in the Mass- multiplication of superior genotypes and transferring of genetically improved material to end-users. The availability of superior nutritive strains will reduce the gap between demand and supply of quality fodder for livestock. This will help to raise the socioeconomic status of the hilly farmers. This will be a good approach for sustainable development of social, economic and environment resources of IHR. The superior seedling raised under the nursery conditions as well as the material that will be produced by mass multiplications from the nineteen nutritive selections can be planted in the other parts of IHR. This can be an efficient way to replicate the outcomes of the project in other parts of IHR.
- 3) Introduction of the additional Germplasm: The twenty new populations (sixteen from the cropland area of Himachal Pradesh and five from Uttarakhand and) which have been added/selected under the to extend the research on *Grewia optiva*. These selected sites will be explored for variations and broader base of the germplasm of the species for further establishment of germplasm bank. This study will also help in comparative analysis among and between the selected populations of H.P and Uttarakhand to screen out the best nutritive selections and to find out more valuable material for future breeding programme.
- 4) Use of DUS specific descriptors developed under the Project: The morphological DUS specific descriptors developed under the project can be used as reference to identification, selection and biological screening of the genotypes in other parts of IHR.

5) Supply of quality planting material: Quality planting of 360 Plants of superior nutritive selection of Clonal Seed Orchard have been supplied to each 15 Forest Divisions (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during the year 2020 and 2021, i.e., 5400 plants in each year for conservation and establishment of gene bank in each selected and identified forest division through SFD, for demonstration/ field testing trial/ block plantation. This quality planting material should be multiplied and supplied in the untouched remaining parts of the IHR. Beside this, 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e., total 12.000 plants supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc. During this year 2022, again 360 plants raised from superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions for field testing and demonstration model (i.e., 5400 plants) and total 6,000 plants were supplied to farmers /stakeholders/women groups identified through selected Forest Divisions. Also, the nursery has been raised in 2021 from seeds collected from thirty-five selected populations of Himachal Pradesh to distribute best planting stock in the year 2022 to farmers, stack holders through the Forest Departments and Animal Husbandry department/Agriculture Department. The cultivation of quality planting material will reduce the problem of fodder deficit in hilly regions particularly during lean season.

#### 2.4 Project Objectives and Target Deliverables (as per the NMHS Sanction Order)

Project Objectives	Target Deliverables	
Biological Screening of the germplasm	Establishment of germplasm bank of Grewia	
diversity of the <i>Grewia optiva</i> as	optiva genotypes.	
agroforestry tree species in Himalaya.		
Study the growth parameters for forage	Introduction of additional germplasm from	
quality and productivity of Grewia optiva.	other institutes or cropland sources.	
Development of DUS specific descriptors of	Morphological characterization based on	
the selected germplasm of Grewia optiva.	DUS specific descriptors of Grewia optiva.	
Conservation and establishment of gene	Molecular characterization of fast growing	
bank of selected genotypes of Grewia optiva	individuals of <i>Grewia optiva</i> .	
on conservation and development.	Production and supply of superior planting	
	material for farmers and State Forest	
	Department and raising of nursery from	
	superior proven genotypes.	

#### 3 METHODOLOGIES, STARTEGY AND APPROACH

#### 3.1 Methodologies used for the study

As per the first objective i.e., "Biological Screening of the germplasm diversity of the *Grewia optiva* as agroforestry tree species in Himalaya" the growth stages of each genotype and their Qualitative, Quantative and Pseudo qualitative characteristics were measured.

## a) Following growth stages of the plants growing in the population have been taken into consideration as under:-

Growth Stages	Stages
Plant: Observations made during the month of October when annual active plant growth	Α
start diminishing	
Shoot: Observations made during active growth period. (March September)	В
<u>Leaf blade</u> : Observations made on mature leaves taken from the middle of the shoot, the	С
third leaf of the current season's growth from the middle part of the plant.	
(July- September)	
Pubescence: Observation made with the help of magnifying glass during the active	D
growth period	
(July – September)	
Fruit: Observation made at the time of fruit maturity.	E
(October to December/ Fortnight of January).	

# b) For determining Qualitative, Quantative and Pseudo Qualitative characteristics following methodology have been adopted.

Sr. No.	Characters	Methodology adopted
1.	Plant: Growth Type	Observed on the basis of Habit and Size of Plant (height and diameter).
2.	Plant: Attitude of Mature Branches/ Spread/ Crown	The Angle of branch from the main stem was measured with the help of a geometric instrument/tool.
3.	Mature stem colour of bark	Recorded on main stem, 25cm above soil level
4.	Plant: Density of Shoots (Primary and Secondary branches)	The number of shoots counted from the main stem in 1m length in the middle part of the plant leaving top and bottom part of the plant.
5.	Plant :Position of Inflorescence	Observed the position of inflorescence on one year -old shoots and further both on one year old and older shoots, respectively. Female Inflorescence small, axillary and male Inflorescence a catkin , borne in spring before emergence of leaves.

Sr. No.	Characters	Methodology adopted		
6.	One -Year old Shoot:	Measured by the vernier caliper on one year old shoot.		
	Thickness			
7.	Time of beginning of	ď		
	flowering	15 weeks. Time of beginning of flowering is when 10 % of		
		flower are fully open.		
	During October	Early : 1 <sup>st</sup> week to 4 <sup>th</sup> week of October		
	During November	Medium: 1 <sup>st</sup> week to 4 <sup>th</sup> week of November		
	During December	Late : 1 <sup>st</sup> week to 4 <sup>th</sup> week of December		
8.	Leaf Blade: Shape	Observation made on mature leaves taken from the		
		middle of the shoot, the third leaf of the current season's		
		growth from the middle part of the plant.		
9.	Leaf length	Recorded as mean length of 10 basal leaves from the		
		secondary fruit bearing branches. Length is measured		
		from the base to the tip of the leaf blade. Total length of		
		leaf blade is recorded with the help of measuring scale		
		and expressed in centimeters(cm).		
10.	Leaf width	Recorded on same leaves used for the measurement of		
		leaf length. Width is measured at the widest portion of the		
		leaf.		
11.	Leaf Area in cm <sup>2</sup>	Measured with the help of Leaf Area meter		
12.	Leaf Blade :Undulation of	Recorded at mature stage of leaf.		
	margin			
13.	Leaf Blade : Colour of adaxial	Recorded at mature stage of leaf.		
	surface			
14.	Leaf Blade: Intensity of	Recorded at mature stage of leaf.		
	Green Colour of abaxial			
	surface			
15.	Fruit length	Recorded mean length of 100 fully mature fruits randomly		
		harvested from a single plant.		
16.	Fruit weight	Recorded the mean weight of 100 randomly collected		
		fruits. Fruits are produced on one year old shoots		
		(unlopped branches) the branches borne a year earlier.		
17.	Fruit: shape	Recorded at full fruit maturity.		
18.	Fruit :colour of skin	Recorded at full ripe stage. Colour is observed with the		
		help of RHS colour chart.		
	<u> </u>			

Sr. No.	Characters	Methodology adopted
19.	Time of beginning of fruit	Time of fruit maturity is when at least 50 % of fruits have
	ripening	achieved the full colour.
		Very early: During September
		Early : During October
		Medium : During November
		Late : During December
		Very late : 1 <sup>st</sup> Fortnight of January
20.	Seed weight	The seed weight is recorded to calculate the amount of seeds:
		1) Seeds/100g
		2) 100 seed weight
		3) 1000 seed weight.
21.	Seed coat colour	Recorded on seeds extracted from fully ripe fruits after
		drying. Seed coat color is observed with the help of RHS
		colour chart.

As per the second objective "Study the growth parameters for forage quality and productivity of *Grewia optiva*." proximate principles, soil physicochemical characteristics and seedling growth characteristics were recorded using following methodology.

i) Proximate principles (leaf dry matter content %, ether extract (%), crude fiber (%), crude protein(%), total ash (%) and nitrogen free extract (%) were recorded for each population during winter season when content of mineral nutrient highest in leaves and heavily lopped for cattle fodder. The following Methodology adopted for estimation of leaf fodder quality parameters.

#### a) Proximate composition (as recommended by AOAC(1995))

S. No	Characters	Methodology adopted as per methods recommended/ suggested	
		by as under	
1.	Leaf fresh weight(g)	Recommended by AOAC(1995)	
2	Leaf dry wieght(g)	Recommended by AOAC(1995)	
3.	Leaf dry matter content(%)	Recommended by AOAC(1995)	
4.	Ether extract(%)	As per procedure outlined by AOAC(1995)and Sankaram(1966)	
5.	Crude fiber(%)	As described by AOAC(1995)and Sankaram(1966)	
6.	Crude protein (%)	Estimated by Microkjedahl method (Sankaram, 1966)	
7.	Total ash(%)	Procedure given by AOAC(1995) and Sankaram(1966)	
8.	Nitrogen free extract(%)	As per method recommended by AOAC(1995) and Sankaram(1966)	

#### b) Mineral nutrients composition (according to methods recommended by AOAC(1995))

Sr.	Characters	Methodology adopted	
No.			
1.	Nitrogen(%)	Estimated by Microkjeldahl, AOAC(1995)	
2.	Phosphorus(%)	Recommended by yellow vanadomolybdate (Gupta, 2000)	
3.	Potassium (%)	Recommended by AOAC(1995) / and by dissolving 1.9069g KCL	
4.	Calcium(%)	Estimated by atomic absorption spectrometer(Gupta,2000)	
5.	Magnesium(%)	Recommended by atomic absorption spectrometer(Gupta,2000)	

ii) The soil physicochemical characteristics ((soil texture, Bulk density, pH, EC) and other available macronutrients (N, P, K, Ca, Mg and S) of all thirty-five populations were measured using following standard methods. The soil samples were collected from 15 cm–30 cm depth underneath the selected populations of *Grewia optiva* Drummond. In each collection 35 composite soil samples (each a composite of six cores) were taken. The soil samples were collected in two consecutive years 2019 and 2020, twice in a year i.e., in the month of May and November. In 2019, first soil sample was collected during the Month of May, when this species was in leafing and flowering stage and second sample was taken at six months interval in the month of November, when the species was in the seed ripening and fodder lopping stage. Similarly, in the year 2020, two consecutive samples were collected in month of May and November from the same selected sites and depth (15 cm–30 cm) respectively, from all thirty-five selected populations.

# a) Standard Methods adopted for Determination of Soil Characteristics and Genetic estimates

Characteristics	Methods	References
рН	Eltop pH meter	AOAC, 1975
Electrical Conductivity (EC)	Conductivity meter method	AOAC, 1975
Organic Carbon (%)	Walkey and Blacks rapid method	Piper, 1966
Available Nitrogen (N)	Alkaline Permanganate method	Subbiah and Asiaja, 1956
Available Phosphorous (P)	Olsen method	Olsen et al ,1954
Available Potassium (K)	Flame photometer method	Merwin and Peach , 1951
Soil texture	Using qualitative method such as texture by feel and quantitative methods such as hydrometer method	Albert Atterberg, 1905

Characteristics	Methods	References
Bulk density	Apparent specific gravity of soil	Singh et. al , 1986
Statistical Analysis ANOVA	Single tree family block design	Panse and Sukhatme, 1967 and Chandel,1984
		Jain, J.P. , 1982
Critical difference (CD)	CD=S.E x t <sub>0.05</sub>	
Correlation coefficient	As per standard statistical method	Karl Pearson correlation coefficient
Variances	Vp=Vg+Me(Phenotypic variance)	
Coefficients of variability	As per standard statistical method	Burton and De Vane, 1953
Heritability	As per standard statistical method	Burton and De Vane, 1953 and Johnson et. al, 1955
Genetic advance	As per standard statistical method	Allard, 1960
Genetic gain	As per standard statistical method	Johnson et. al , 1955
Seed germination traits and	As per international rules of seed	AOAC, 1995
important charaterstics of	testing, TZ test (Bonner 1974)	AOAC,1980
nursery raised seedlings	Czabator method, Djavanshir and Pourbeik method	ISTA, 1966

(iii) The seed samples collected from thirty-five selected populations were sown under nursery conditions and germination parameters, seedling growth characteristics and biomass were measured using following methods.

#### a) Germination and survival percentage were obtained using following methods

Characteristics	Methods	References
- Number of days to initial	As per international rules of seed	AOAC, 1995
germination	testing, TZ test (Bonner 1974)	AOAC,1980
- Number of days to complete	Czabator method, Djavanshir and Pourbeik method	ISTA, 1966
germination	Pourbeik method	1017, 1900
- Per Cent germination		
- Germination Value(GV)		
- Per cent survival (after 9		
months)		

b) Seedling Growth Characteristics were measured using following methods		
Characteristics	Methods	References
- Seedling Height(cm)	As per international rules of seed	AOAC, 1995
- Collar diameter(cm)	testing, TZ test (Bonner 1974)	AOAC,1980
<ul><li>Number of Nodes</li><li>Internodal length(cm)</li></ul>	Czabator method, Djavanshir and	ISTA, 1966
- Number of branches per	Pourbeik method	Bhat , SS, 2010
plant		
- Branch length(cm)		
- Numbers of leaves per plant		
- Leaf shape		
- Leaf length(cm)		
- Leaf breadth(cm)		
- Leaf area(cm <sup>2</sup> )		

#### c) Methods adopted for Biomass study

Characteristics	Methods	References
- Leaf biomass per plant(g) at	As per international rules of seed	AOAC, 1995
leaf harvesting stage	testing, TZ test (Bonner 1974)	AOAC,1980
- Root length(cm)	Czabator method, Djavanshir and	,
- Shoot length(cm)	Pourbeik method	ISTA, 1966
- Root fresh weight(g)	T carbon mounds	
- Shoot fresh weight(g)		
- Root dry weight ratio		
- Shoot dry weight ratio		
- Root biomass-		
(Fresh & Dry)		
- Shoot biomass-		
( Fresh & Dry)		

Methodology, type of molecular method used, kind of analysis of the molecular data.

For determining the Molecular characterization of fast growing individuals of Grewia optiva

The top ranking ten families were selected on the basis of scoring index on desired morphometric and fodder quality characters of *Grewia optiva* Drummond under study as per the standard method of selection followed by Ellstrand and Roose (1987) and Epperson (2003).

The experimental details of material used and methodology adopted for molecular characterization are given under the following headings.

- 1. Source of plant material
- 2. Isolation and Purification of genomic DNA
- 3. Qualitative and quantitative assessment
  - a) Agarose gel electrophoresis
  - b) DNA quantification
- 4. RAPD and ISSR studies for genetic divergence
- 5. Data analysis

#### 1. Source of Plant Material

Top ten families selected based on morphometric and fodder quality parameters for molecular studies

Sr. No.	District	Family	Code
1.	Sirmour	Madhobag	UHF-SI-15
2.	Solan	Dharja	UHF-SO-3
3.	Hamirpur	Patta Balakhar	UHF-HA-2
4.	Hamirpur	Bassi	UHF-HA-3
5.	Hamirpur	Hamirpur Kanal	UHF-HA-4
6.	Solan	Oyali	UHF-SO-7
7.	Shimla	Taradevi	UHF-SH-7
8.	Solan	Deog	UHF-SO-4
9.	Sirmour	Nainatikker	UHF-SI-16
10.	Sirmour	Saraha Chakli	UHF-SI-14

#### 2. Isolation and Purification of genomic DNA

#### a. Collection of leaf material

Fresh, green leaves were separately excised from different plants of ten families. Before plucking, the leaves were wiped off the soil with tissue paper and then wrapped in aluminum foil and brought to the laboratory in ice-box and stored in deep freezer at -80°C till further use.

#### b. Isolation of genomic DNA

Genomic DNA from the collected leaves of top ten families separately isolated using CTAB method of Doyle and Doyle, 1987 with some modification wherever required.

The following reagents were used for DNA isolation

**Reagents:** 10% CTAB, 0.5M EDTA (pH 8.0), 4M Nacl. 1M Tris HCL (pH 8.0), DNA extraction buffer, chloroform: Isoamyl, 70% ethanol and TE buffer.

#### Procedure:

Step 1	About 2 gm of collected leaves were homogenized to fine powder with liquid nitrogen using prechilled pestle and mortar.
Step 2	The leaf powder was transferred to 50 ml centrifuge tube containing 10 ml pre-warmed (at 650C) DNA extraction buffer. Leaf powder should not get moist because under wet condition DNase digests total DNA.
Step 3	Incubated the tubes for one to two hours at 650C in a water bath. During incubation the samples were mixed well by inverting the tubes every five minutes.
Step 4	10 ml of chloroform: isoamyl alcohol (24:1, v/v) was added to each tube followed by mixing gently by hand inversions till the colour in the lower portion of the tube turned dark green.
Step 5	Centrifuged the above suspension at 12000 rpm for ten minutes at room temperature (250C).
Step 6	Transferred the aqueous phase gently without disturbing the inter phase to fresh autoclaved centrifuge tubes.
Step 7	Added equal volume of prechilled isopropanol to the aqueous phase taken, mixed gently by hand inversions and incubated at -200C for 1hour or overnight so that DNA gets precipitated.
Step 8	The precipitated DNA was spooled out with the help of sterilized glass hook or pelleted it by centrifugation at 10000 rpm for ten minutes at 40C.
Step 9	Washed the DNA with 500µl of 70% ethanol and centrifuged at 5000 rpm for five minutes at 40C. Step 10 Supernatant was decanted and dried the pellet overnight to completely evaporate the alcohol. Step 11 Dissolved the DNA pellet in 500 µl TE buffer.

#### c. Purification of genomic DNA

Isolated DNA was purified by successive RNase treatment followed by phenol chloroform extraction.

**Reagents:** RNase (10mg/ml), Phenol: chloroform, Chloroform: isoamyl, 3M sodium acetate, Absolute ethanol (95%)and 70% ethanol.

#### Procedure:

Step 1	0.5 $\mu$ l (10mg/ml) of RNase was added to the isolated DNA samples followed by incubation at 370C for 1 hour.
Step 2	Equal volume of phenol: chloroform was added and mixed thoroughly.
Step 3	Centrifugation was done at 11000 rpm for two minutes at room temperature and aqueous phase was transferred to fresh eppendorf microfuge tubes.
Step 4	Extraction was done twice with equal volume of chloroform: isoamyl alcohol (24:1, v/v) followed by 11000 rpm spin for two minutes.

Step 5	The aqueous phase was separated to which 1/10th volume of 3M sodium acetate and 2.5 volume of absolute ethanol was added. It was mixed properly and incubated at 40C.
Step 6	Pelleted the DNA by centrifugation at 11000 rpm for five minutes.
Step 7	The supernatant was decanted off followed by washing of pellet with 70% ethanol, and the pellet was then air dried.
Step 8	Resuspended the pellet in 100 µl TE buffer.

#### 3. Qualitative and quantitative assessment

#### a) Agarose gel electrophoresis

The isolated genomic DNA was electrophoresed on 0.8 per cent agarose gel (stained with 0.5µg/ml of ethidium bromide) at 80V –100V and 70mA for two hours in 1X TAE buffer (Appendix II) and then observed on UV-transilluminator. Quality of DNA samples was judged on the basis of whether sample DNA formed a single high molecular weight band or smear.

#### b) DNA quantification

The quantity of DNA was assessed by UV/VIS spectrophotometer (Perkin Elmer, Hyderabad, India). Concentration of DNA was quantified using following formula:

DNA (
$$\mu$$
g/ml) = 
$$\frac{(OD(260) \times dilution factor \times 50)}{1000}$$

The ratio of absorbance at 260 nm and at 280 nm was measured to check the contamination of protein.

#### Purity check DNA on the basis of A<sub>260</sub>:A<sub>280</sub> ratio

Sr. No.	A260:A280 ratio (Absorbance ratio)	Indication
1.	Above 1.8	Protein Contamination
2.	1.4 to 1.8	Good quality DNA
3.	Below 1.4	RNA contamination

#### 4. RAPD and ISSR studies for genetic divergence

#### A. Random amplified polymorphic DNA Studies

#### a) DNA amplification

DNA amplification was carried out for RAPD analysis using decamer random primers. A total of twenty decamer primers synthesized by M/S Bangalore Genei, India Limited were used.

#### Nucleotide sequence of 20 decamer random primer used to study polymorphism

Sr. No.	Primer name	Base Sequence
1.	OPC-08	TGG ACC GGT G
2.	OPC-11	AAA GCT GCG G
3.	OPC-12	TGT CAT CCC C
4.	OPC-13	AAG CCT CGT C
5.	OPF-06	GGG AAT TCG G
6.	OPF-07	CCG ATA TCC C
7.	OPF-08	GGG ATA TCG G
8.	OPF-11	TTG GTA CCC C
9.	OPF-12	ACG GTA CCA G
10.	OPF-13	GGC TGC AGA A
11.	OPF-14	TGC TGC AGG T
12.	OPA-01	CAG GCC CTT C
13.	OPA-02	TGC CGA GCT G
14.	OPA-03	AGT CAG CCA C
15.	OPA-04	AAT CGG GCT G
16.	OPA-05	AGG GGT CTT G
17.	OPO-17	GGC TTA TGC C
18.	OPO-18	CTC GTA TCC
19.	OPO-20	ACA CACGCT G
20.	OPO-19	GGT GCA CGT T

#### b) Standardization of concentration of DBNA for RAPD

For standardization the concentration of template DNA, PCR amplification was performed with different DNA concentration using previously standardized master-mix concentration. Five different concentration of DNA were used which were 5 ng, 10 ng, 15ng, 20ng and 25ng, for obtaining the maximum number of amplification products. It was found that maximum number of amplification products was observed at 20 ng of template DNA.

#### Standardization of PCR amplification conditions

Tests were performed for standardizing polymerase chain reaction amplification conditions mainly the annealing temperature. PCR amplification conducted at different annealing temperatures i.e. 36 °C, 37°C, 38°C using standard concentrations of various components of reaction mixture.

Sr. No.	React	ions	Temperature a Specifications	and	Time	Number cycles	of
1.	Initial Denaturation 94°C for 3 minutes 1		1				
2.	Α	Denaturation	92°C for 45 minutes		45		
	B Annealing of Primer 36°C for 1 minutes						
C Primer amplification		72°C for 2 m	ninutes				
3.	Final amplification 72°C for 1 minutes		1				
After co	mpletio	n	4°C till electrophore	esis			

#### Amplification of DNA by PCR

The amplification reactions were performed in a total volume of 25 µl containing.

Sr. No.	Constituents	Quantity
1.	Sterile distilled water	15 μΙ
2.	Taq buffer (10X)	2.5 µl
3.	dNTPs (2.5 mM)	1.25 µl
4.	Primer (10 ng)	1.0 µl
5.	Taq DNA Polymerase (3U/µI)	0.25 μΙ
6.	DNA (5 ng/μl)	4 μΙ
	Total for 1 reaction	25 μΙ

The reagents were mixed thoroughly in a 2 ml Eppendorf tube and vortexed for few seconds. 20  $\mu$ l of mixture was distributed to each PCR tube and 4.0  $\mu$ l of template DNA (5 ng/ $\mu$ l) was added to each tube for each amplification reaction in thermal cycler (Corbett Thermal Cycler) (Williams et al., 1990) programmed as above.

#### **Electrophoresis of amplified DNA**

The amplified DNA was mixed thoroughly with 6X loading dye (Appendix III) and then electrophoresed in 2 per cent agarose gel in 1X TAE buffer. The gel was run at constant voltage at the rate of 5V/cm under submerged conditions for about two hours. Ethidium bromide at the rate of 0.5µg/ml was incorporated in the gel. Stock solution of ethidium bromide @ 10 mg/ml was kept ready. DNA profiles were visualised on UV Transilluminator and photographed on Gel Documentation System (Syngene, Cambridge, UK).

#### B. Inter Simple Sequence Repeat (ISSR) Studies

#### 1. DNA amplification

DNA amplification was carried out for ISSR analysis. A total of eighteen ISSR primers sysnthesized by M/S Bangalore Genei, India Limited were used.

#### **Nucleotide sequences of ISSR primers**

Sr. No.	Primer	Sequences
1.	809	AGAGAGAGAGAGG
2.	810	GAGAGAGAGAGAT
3.	811	GAGAGAGAGAGAC

4.	812	GAGAGAGAGAGAA
5.	825	ACACACACACACCC
6.	830	TGTGTGTGTGTGG
7.	834	AGAGAGAGAGAGT
8.	835	AGAGAGAGAGAGYC
9.	850	GTGTGTGTGTGGTYA
10.	861	ACCACCACCACCACC
11.	864	ATGATGATGATG
12.	881	GGGGTGGGGTC
13.	UBC-807	AGAGAGAGAGAGGT
14.	UBC-826	ACACACACACACAC
15.	UBC-841	GAGAGAGAGAGAYC

#### **Standardization of PCR Amplification Conditions**

Tests were performed for standardization polymerase chain reaction amplification conditions mainly the annealing temperature.

Sr. No.	React	ions	Temperature Specifications	and	Time	Number cycles	of
1.	Initial	Denaturation	94°C for 3	94°C for 3 minutes		1	
2.	Α	Denaturation	92°C for 45 minutes		45		
	В	Annealing of Primer	55°C for 1 minutes				
	С	Primer amplification	72°C for 2 minutes				
3.	Final a	amplification	72°C for 1 minutes		1		
After completion		4°C till elec	trophoresi	S			

#### Amplification of DNA by PCR using ISSR primers

The amplification reactions were performed in a total volume of 24 µl containing

Sr. No.	Constituents	Quantity
1.	Sterile distilled water	15 µl
2.	Taq buffer (10X)	2.5 µl
3.	dNTPs (2.5 mM)	1.25 µl
4.	Primer (10 ng)	1.0 μΙ
5.	Taq DNA Polymerase (3U/µI)	0.25 µl
6.	DNA (5 ng/µl)	4 µl
	Total for 1 reaction	25 μl

The reagents were mixed thoroughly in a 2 ml Eppendorf tube and vortexed for few seconds. 20  $\mu$ l of mixture was distributed to each PCR tube and 4.0  $\mu$ l of template DNA (5NG/  $\mu$ l) was added to each for each amplification reaction in thermal cycle (Corbett Thermal Cycler) Programmed as above.

#### Electrophoresis using agarose gel

The amplified DNA was mixed thoroughly with 6X loading dye (Appendix III) and then electrophoresed in 2 per cent agarose gel in 1X TAE buffer (Appendix II). The gel was run at

constant voltage at the rate of 5V/cm under submerged conditions for about two hours. Ethidium bromide at the rate of 0.5µg/ml was incorporated in the gel. Stock solution of ethidium bromide @ 10 mg/ml was kept ready. DNA profiles were visualised on UV Transilluminator and photographed on Gel Documentation System (Syngene, Cambridge, UK).

#### RAPD and ISSR Fingerprints

a) Viewing of amplified DNA

After the run was over, the gel was viewed under the UV light using Gel Documentation system and was photographed.

b) Scoring of bands

The amplified bands after separation were visualized using Gel Documentation system. Further the bands were scored for percentage polymorphism for each set of primer amplified product using NTSYS 2.2.

#### **Analysis of ISSR Data**

The scored bands were analyzed in the form of binary system to prepare the similarity index. The bands with same molecular weight and mobility were treated as identical fragments. Data matrices were prepared in which the presence of a band was coded as 1 whereas the absence as zero. The data matrices were analyzed by the SIMQUAL Program of NTSYS-PC (Version 2.2) and similarities between Families were estimated using Jaccard similarity coefficient, calculated as J = A / (N-D), where A is the number of positive matches (i.e. presence of band in both samples), D is the number of negative matches (i.e. absence of band in both samples) and N is the total sample size including both the number of matches and unlatches. Dendrogram was produced from the resultant similarity matrices using the UPGMA method.

As per the third objective "Development of DUS specific descriptors of the selected germplasm of *Grewia optiva*", Quantitative and Pseudoqualitative characteristics of all the selected 210 genotypes of 35 populations and of genotypes of already established clonal seed orchard were observed using following methodology i.e., plant morphology (growth types, attitude of branches, branch thickness, bark color, leaf characteristics (leaf length, leaf width, leaf area, leaf blade, 100 leaf fresh weight and 100 leaf dry weight), fruit characteristics (fruit length, fruit weight, fruit shape, fruit skin colour) and phenological characteristics (leafing, flowering, fruiting, fruit ripening timings) and seed characteristics (seed weight, seed coat colour). These characters were recorded for each 210 selected genotypes and 23 selected genotypes of clonal seed orchard. On the basis of results of these characteristics Morphological DUS specific descriptors have been developed for 210 selected genotypes and 23 selected genotypes of clonal seed orchard. DUS specific descriptors were also developed for 10 screened genotypes/ best families from clonal seed orchard and 9 superior selections screened from thirty-five selected populations.

As per fourth objective "Conservation and Establishment of gene bank of selected genotypes of *Grewia optiva*" the clonal seed orchard and seedling seed orchard were established for conservation of superior selected genotypes. Besides this, the superior planting material were produced and supplied to farmers and the State Forest Department and nurseries were raised from superior proven genotypes.

#### 3.2 Preparatory Actions and Agencies Involved

This project has been undertaken with the involvement of State Forest department, Additional PCCF Research and Training, Sundernagar. The Forest Department has the direct linkage with the village farmers and stakeholders. The 15 Forest Divisions viz. Forest Division: Hamirpur, Forest Division: Dehra, Forest Division: Bilaspur, Forest Division: Renuka, Forest Division: Solan, Forest Division: Joginder Nagar, Forest Division: Una, Forest Division: Suket, Forest Division: Karsog, Forest Division: Nachan, Forest Division: Rajgarh, Forest Division: Nahan, Forest Division: Kunihar, Forest Division: Nalagarh, Forest Division: Mandi were contacted and involved to procure the superior planting material from this department and to coordinate activities at the field levels in low and mid hill zone of Himachal Pradesh. The Department is being provided with quality planting stock of *Grewia optiva* by the PI of the project and planting material is being tested in 15 divisions by the DFO's of Himachal Pradesh in the Jurisdictions of different districts of Himachal Pradesh for its field testing, through State Forest Department, Additional PCCF Research & Training, Sundernagar (Himachal Pradesh) and supply to end users/ beneficiaries.

As many as 360 plants of superior nutritive selection of Clonal Seed Orchard supplied to 15 Forest Divisions viz. Forest Division: Hamirpur, Forest Division: Dehra, Forest Division: Bilaspur, Forest Division: Renuka, Forest Division: Solan, Forest Division: Joginder Nagar, Forest Division: Una, Forest Division: Suket, Forest Division: Karsog, Forest Division: Nachan, Forest Division: Rajgarh, Forest Division: Nahan, Forest Division: Kunihar, Forest Division: Nalagarh, Forest Division: Mandi during the year 2020 and 2021, i.e. 5400 plants each year to the above mentioned Forest Divisions for research and demonstration trial in each forest division.

Beside the above supplied material, other lot of 400 plants has been supplied to fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e. total 12,000 plants supplied for the farmers of the stakeholders, identified nodded Forest Division to endorsees farmers, women groups, self-help group etc.

During this year i.e. 2022, 360 more plants of superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions viz. Forest Division: Hamirpur, Forest Division: Dehra, Forest Division: Bilaspur, Forest Division: Renuka, Forest Division: Solan, Forest Division: Joginder Nagar, Forest Division: Una, Forest Division: Suket, Forest Division: Karsog, Forest Division: Nachan, Forest Division: Rajgarh, Forest Division: Nahan, Forest Division: Kunihar, Forest Division: Nalagarh, Forest Division: Mandi i.e. 5400 plants for research and demonstration trial in each Forest Division and also 400 plants to each Forest Division were

supplied to fifteen Forest Division i.e. 6000 plants in total, for the farmers of the stakeholders, identified nodded Forest Division to endorsees farmers, women groups, self-help group etc.

Total production of Quality planting material count to be in three years i.e.,  $5400 \times 3 = 16,200$  and  $6000 \times 3 = 18,000$ , total of 34,200 plants is raised in the nursery for distribution to the Fifteen Forest Divisions of Himachal Pradesh. At present more than 20,000 plants/ saplings are available with the Department which have been screened out under the project.

Besides these activities, Mass media lectures were organized with the involvement of Forest Department, Panchayat Pradhan (35-Gram Panchayat) etc. to spread the awareness about the use of this valuable species. The field demonstration trials were organized to provide practical training about propagation/cultivation of this species through cutting, grafting and seed etc. The farmers also got knowledge about its sowing time, flowering time, fruit ripening time and fruit collection etc.

#### 3.3 Details of Scientific data collected and Equipments Used

#### 1) Morphological and Morphometric Characteristics:

- ➤ Plant height: Measured using Ravimultimeter/4-meter pole
- Plant diameter: Measured with Vernier calliper
- ➤ Branch angle (): Measured with Fixed ruler and semicircular protactor
- > Crown spread (N-S & E-W): Measured with Measuring tape
- ➤ No. of primary and secondary branches per tree: Visual observations
- One year old shoot thickness: Measured with Vernier calliper
- ➤ Leaf length (cm): Measured with Scale
- ➤ Leaf width (cm): Measured with Scale
- Leaf area (cm<sup>2</sup>): Measured with leaf area meter (portal leaf area meter model C-1-203)
- > Fruit length (cm): Measured with Digital vernier calliper
- Fruit weight (g): Measured with Digital weighing balance Machine
- Fruit colour: Measured with help of RHS colour chart
- Seed weight (g): Measured with Digital weighing balance Machine
- Seed colour : Measured with help of RHS colour chart

#### 2) Seed Germination and Seedling Characteristics:

- Germination percentage (%): The number of seeds germinated to the total number of seeds sown were calculated and expressed as germination percentage.
- Germination value: It was calculated by the following formula: Germination value (GV) = Mean daily germination (MDG) x Peak value at mean daily germination (PV).
- Germination energy: The proportion of germination which had occurred upto the time of peak germination was noted.

- Germination index: It was calculated by the following formula: Germination index (GI) = (G/T) where G is the percent of seed germinated per day and T is the germination period.
- Survival percentage (%): The total number of plants survived after six months/ one year of germination was calculated as survival percentage.
- > Seedling height (cm): The height of the one year plants were recorded from the ground level to the apex of the leading shoot by using measuring scale and was expressed in cm.
- Collar diameter (mm): Collar diameter of the one-year plants in the nursery was measured above the ground level at collar region with the help of digital caliper and expressed in mm.
- > Internodal length (cm): The internodal length was measured as distance between two nodes with the help of measuring scale, at mid of the plant height and expressed in cm.
- > Petiole length (mm): The petiole length was measured with the help of measuring scale and expressed in mm.
- ➤ Leaf area (cm²): The full-grown leaves were collected at randomly from the seedling and leaf area was measured with the help of leaf area meter and expressed in cm².
- > Biomass study of seedling was undertaken as per Czabator method, Djavanshir and Pourbeik method
- 3) Physicochemical Properties of Soil samples collected from 15-30 cm depth underneath the thirty-five selected populations.
  - ➤ Soil Colour: It was observed with the help of Munsell colour chart.
  - > Bulk density (BD): It was determined by core method using following formula:

BD (g cm<sup>-3</sup>) = 
$$\frac{\text{wt.of oven dry soll in the core (g)}}{\text{volume of core/soil (cm}^3)}$$

> Particle density (PD): It was determined with the help of Pychnometer method.

PD (g cm<sup>-3</sup>) = 
$$\frac{10}{W_{pw} + 10 - W_{psw}}$$

Where, W<sub>pw</sub> = weight of water filled in pycnometer

W<sub>psw</sub> = weight of Pycnometer + water + soil

Porosity: It was determined by using bulk density and particle density empirical formula:

Porosity = 
$$1 \frac{BD}{PD} \times 100$$

- > Soil pH:It was measured with the help of Digital Eltop pH meter (Jakson, 1973).
- ➤ Electrical conductivity (dSm<sup>-1</sup>): It was measured with the help of digital EC meter (Jakson, 1973).

- Organic carbon (%): It was measured with the help of chromic acid titration method (Walkley and Black, 1934). In titration burets, pipettes, volumetric flasks, and stirrers equipment were used.
- ➤ Available N (kg ha<sup>-1</sup>): It was measured with the help of alkaline permanganate method (Subbiah and Asija, 1956). The equipment used in this method were kjeldahl flask, digestion stand etc.
- ➤ Available P (kg ha<sup>-1</sup>): It was measured with the help of 0.5 M sodium bicarbonate (NaHCO<sub>3</sub>) at 8.5 pH (Olsen *et al.*, 1954). Equipment used: spectrophotometer.
- Available K (kg ha<sup>-1</sup>): It was measured with the help of flame photometric method (1N NH<sub>4</sub>OAC extractable) (Merwin and Peech, 1951). Equipment used : flame photometer, and there are four basic components to flame photometer i.e., flame, nebulizer and mixing chamber, colour filters, and a photo detector.
- Available Ca: Estimated by atomic absorption spectrometer(Gupta, 2000).
- Available Mg: Using atomic absorption spectrometer (Gupta, 2000).

### 4) Fodder quality parameters observed were:

- ➤ Leaf fresh weight (g): It was recorded by weighing 100 fresh leaves in Digital weighing balance machine.
- Leaf dry weight (g): It was recorded by weighing 100 dry leaves.

Equipment used: Digital weighing balance machine.

 $\triangleright$  Leaf dry matter content (%):  $\frac{\text{Weight of dried sample}}{\text{Weight of fresh sample}} \times 100$ 

Equipment used: Digital weighing balance machine.

➤ Crude protein (%): It was estimated following Sankaram (1966). For the crude protein estimation, nitrogen content of leaves will be estimated by Microkjeldhal method and estimated Nitrogen content was multiplied by standard factor of 6.25.

Crude protein (%) = N (%)  $\times$  6.25

Equipment used: Kjeldahl flask, digestion stand etc.

Ether extract (%): It was extracted by using petroleum ether (AOAC, 1 995).

Ether extract = 
$$\frac{\text{Wt of fat}}{\text{original weight of Sample}} \times 100$$

Equipment used: Digital weighing balance machine, Soxhlet extractor etc.

Where; Weight of fat = (wt. of thimble + sample)-(weight of thimble + sample after extraction)

Crude fibre (%): It was estimated by acid alkali digestion (ashing) method described by AOAC (1995).

Equipment used: Analytical balance, 4 decimals • Glass Crucibles P2, 6pcs (A00000140), a weighing balance.

Crude fiber (%) = 
$$\frac{\text{weight of crude fiber}}{\text{original weight of sample}} \times 100$$

> Total ash (%): It was estimated by using the procedure given by AOAC (1995).

Total ash (%) = 
$$\frac{\text{weight of ash}}{\text{original weight of sample}} \times 100$$

Equipment used: a weighing balance, high temperature muffle furnace

➤ Nitrogen free extract (%): It was determined by substracting the sum of crude protein, crude fiber, ether extract and total ash content from 100 (AOAC, 1995).

# 3.4 Primary Data Collected

Our study is purely based on the primary data. The data on plant growth characteristics, soil nutrients, fodder quality, and seedling growth characteristics have been collected either under field conditions or lab conditions. As per the first objective of the project, 35 populations in seven districts of Himachal Pradesh (5 population in each district) were selected. The following morphological and morphometric observations on plants among and within thirty-five selected populations were collected in the field and under lab conditions.

- Plant growth type: Plant height and Plant diameter
- Branch angle ()
- > Crown spread (N-S & E-W)
- ➤ No. of primary and secondary branches
- One year old shoot thickness
- Phenological events
- ➤ Leaf: Leaf shape, Undulation of margin, colour of adaxial surface, intensity of green colour of abaxial surface
- Leaf length (cm)
- ➤ Leaf width (cm)
- Leaf area (cm²)
- Fruit length (cm)
- Fruit weight (gm)
- Fruit: shape, colour of skin, time of beginning of fruit ripening
- Seed weight (gm)
- Seed dimensions
- > Seed coat colour

As data on plant height, plant diameter, crown spread, no. of primary and secondary branches, One year old shoot thickness, phenological events were collected under natural growing field condition of

the selected genotypes whereas, data on leaf characteristics (leaf shape, margin, color of leaf, leaf length, width, leaf area) and fruit length, fruit width, fruit shape, colour of fruit skin, 100 seed weight, seed dimensions, seed coat colour were recorded on collected samples under lab conditions using leaf area meter, scale, weighing machine, digital vernier calliper and RHS colour chart etc.

The primary data on the following leaf fodder quality parameters (proximate principles) of thirty-five selected populations (as per 2<sup>nd</sup> objective) were recorded under lab conditions. The leaf samples were collected during winter season (October – November) from the different parts of crown of 210 selected genotypes of thirty -five populations.

- > 100 Leaf fresh weight (g)
- > 100 Leaf dry weight (g)
- Leaf dry matter (%)
- Ether extract (%)
- Crude protein (%)
- Crude fibre (%)
- > Total ash (%)
- Nitrogen free extract (%)

The primary data on the following soil physicochemical characteristics and macronutrients of thirty-five soil samples were recorded under lab condition using standard methods. The soil samples were collected at depth of 15-30 cm underneath selected populations of *Grewia optiva*.

- ▶ pH
- > Electrical conductivity (EC) (dS m<sup>-1</sup>)
- Organic carbon (g kg<sup>-1</sup>)
- ➤ Bulk density
- ➤ Soil Texture
- > Available Nitrogen (kg ha<sup>-1</sup>)
- > Available Phosphorus (kg ha<sup>-1</sup>)
- ➤ Available Potassium (kg ha<sup>-1</sup>)

The nursery has been raised at Shilli farm of the department from the seeds collected from 35 population of Himachal Pradesh *viz.*, Solan, Sirmour, Mandi, Hamirpur, Kangra, Una and Bilaspur. The primary data on seedling growth parameters were recorded under nursery conditions.

- Leaf biomass per plant(g) at leaf harvesting stage
- Seedling height (cm)
- Collar diameter (mm)
- Root length (cm)
- Shoot length(cm)

- Root fresh weight(g)
- Shoot fresh weight(g)
- Root dry weight ratio
- Shoot dry weight ratio
- Root biomass (Fresh & Dry)
- Shoot biomass- (Fresh & Dry)

# 3.5 Details of Field Survey arranged

The survey was carried out in the low -mid Himalayan regions of Himachal Pradesh to identify different populations of *Grewia optiva*. On the basis of field survey initially six districts of H.P. viz., Solan, Bilaspur, Mandi, Una, Hamirpur, Kangra were selected keeping in view species abundance, rich genetic diversity and phenotypically superior plant populations of *Grewia optiva*. For the comprehensive study district Sirmaur was also included, whereas it was not earlier mentioned in the project proposal. As rich genetic diversity of *Grewia optiva* populations was also reported in this district (Sirmaur). Therefore, total seven districts were selected and in each district 5 populations *i.e.*, total thirty-five populations (7 district X 5 populations in each district) of superior genotypes were identified.

The List of thirty-five populations of seven districts in Himachal Pradesh:

District	Selected sites/ populations
Una	Bagana, Nawami, Kant, Lamlehri, Thanakalan at Budhwar.
Hamirpur	Harbal Neri, Jhinkari, Bhaleth, Janhen, Anu Khurd
Kangra	Katoi, Old Kangra, Balugloa, Balla, Dohran
Mandi	Bagla, Balt, Bharnoi, Gangal, Patta
Solan	Kothi Kunal, Uncha gaon, Gaddon, Nerikalan, Devera
Sirmaur	Jajjar, Machher, Neharbag, Bedon, Dharkyari
Bilaspur	Ghumarvii, Barthin, Kuthera, Nehari, Jukhala

In each population six superior trees of 20 cm–30 cm diameter class were marked for recording morphological observations on plants to among populations and within populations. In the second field survey observations on morphological and morphometric characters were observed for each 210 genotypes of thirty-five population.

In second field survey leaf samples were collected during winter season (when species heavily lopped for fodder) for analysis of fodder quality [(leaf dry matter content %, ether extract (%), crude fiber (%), crude protein (%), total ash (%) and nitrogen free extract (%)] parameters.

Initially fourteen populations were selected for fodder quality parameters and soil nutrient analysis. The details of the populations/ Sources Identified and Selected is as under:

Name of the Districts	Populations/ Sources Identified and Selected for collection of leaf and soil samples (15-30 cm depth)	
Sirmour	Jajjar , Neharbag	
Solan	Kothikunal, Gaddon	
Kangra	Balla, Katoi	
Una	Kant, Lamlehri	
Hamirpur	Jhinkari, Harbal Neri	
Mandi	Patta, Bagla	
Bilaspur	Ghumarwin, Barthin	

In next field survey leaf samples and soil samples were collected of remaining 21 populations for fodder quality analysis. The details of the populations/ Sources Identified and Selected is as under:

District	Selected sites/ populations	
Una	Bagana, Nawami, Thanakalan at Budhwar.	
Hamirpur	Bhaleth, Janhen, Anu Khurd	
Kangra	Old Kangra, Balugloa, Dohran	
Mandi	Balt, Bharnoi, Gangal	
Solan	Uncha gaon, Nerikalan, Devera	
Sirmaur	Machher, Bedon, Dharkyari	
Bilaspur	Kuthera, Nehari, Jukhala	

# 3.6 Strategic Planning for each Activities

The basic aim of the study was to screen out biologically superior individuals within the populations and among the populations based on morphological and fodder quality scoring index parameters and to produce genetically superior planting stock of those genotypes and families which are having high crude protein i.e. more than 20 per cent and above. Therefore, our first activity was started with the selection of superior populations with high genetic diversity of *Grewia optiva* after those further activities were made with following strategies.

# A) Selection of sites having rich genetic diversity of Grewia optiva

The survey was carried out in the low -mid Himalayan regions of Himachal Pradesh to identify different populations of *Grewia optiva*. On the basis of field survey initially six districts of H.P. viz., Solan, Bilaspur, Mandi, Una, Hamirpur, Kangra were selected keeping in view species abundance, rich genetic diversity and phenotypically superior plant populations of *Grewia optiva*. For the comprehensive study, district Sirmaur was also included, whereas it was not earlier mentioned in the project proposal. As in rich genetic diversity of *Grewia optiva* populations was also reported in this district (Sirmaur). Therefore, total seven districts were selected and in each district 5 populations *i.e.*, total thirty-five populations (7 district X 5 populations in each district) of superior genotypes were identified, marked and selected.

## Detail of thirty-five selected sites.

District	Selected sites/ populations
Una	Bagana, Nawami, Kant, Lamlehri, Thanakalan at Budhwar.
Hamirpur	Harbal Neri, Jhinkari, Bhaleth, Janhen, Anu Khurd
Kangra	Katoi, Old Kangra, Balugloa, Balla, Dohran
Mandi	Bagla, Balt, Bharnoi, Gangal, Patta
Solan	Kothi Kunal, Uncha gaon, Gaddon, Nerikalan, Devera
Sirmaur	Jajjar, Machher, Neharbag, Bedon, Dharkyari
Bilaspur	Ghumarwin, Barthin, Kuthera, Nehari, Jukhala

### B) Selection of superior genotypes of 20-30 cm diameter class

In each thirty-five selected populations, six superior trees of 20 cm—30 cm diameter class (i.e., total 210 genotypes) were marked for recording different observations on growth stages, morphological, Qualitative, Quantitative and Pseudo Qualitative characteristics. The 20-30 cm diameter class was considered best for the study, based on the previous reports, which have shown that trees of this diameter class found to be more suitable for fodder purpose and for agroforestry systems and other plantation programs. The tree of this diameter class was measured with the help of vernier caliper.

# C) Growth stages of plants growing taking into consideration for the collection of different morphological and morphometric data.

Growth Stages	Stages
Plant: Observations made during the month of October when annual active plant	Α
growth start diminishing	<b>D</b>
Shoot: Observations made during active growth period.	В
(March September)	
<u>Leaf blade</u> : Observations made on mature leaves taken from the middle of the shoot,	С
the third leaf of the current season's growth from the middle part of the	
plant. (July- September)	
Pubescence: Observation made with the help of magnifying glass during the active	
growth period	_
(July – September)	D
Fruit: Observation made at the time of fruit maturity.	E
(October to December/ Fortnight of January).	

# D) Determination of Qualitative, Quantative and Pseudoqualitative characteristics following methodology/ strategy were adopted.

Sr.	Characters	Methodology adopted		
1.	Plant: Growth Type	Observed on the basis of Habit and Size of Plant (height and		
		diameter).		

Sr. No	Characters	Methodology adopted
2.	Plant: Attitude of	The Angle of branch from the main stem was measured with the help
	Mature Branches/	of a geometric instrument/tool.
	Spread/ Crown	
3.	Mature stem	Recorded on main stem, 25cm above soil level
	colour of bark	
4.	Plant: Density of	The number of shoots counted from the main stem in 1m length in the
	Shoots (Primary	middle part of the plant leaving top and bottom part of the plant.
	and Secondary	
	branches)	
5.	Plant :Position of	,
	Inflorescence	further both on one year old and older shoots, respectively. Female
		Inflorescence small, axillary and male Inflorescence a catkin, borne in
		spring before emergence of leaves.
6.	One-Year old	Measured by the vernier caliper on one year old shoot.
	Shoot: Thickness	
7.	Time of beginning	The Time taken from flowerig of fruit maturation is 12 to 15 weeks.
	of flowering	Time of beginning of flowering is when 10 % of flower are fully open.
	During October	Early : 1 <sup>st</sup> week to 4 <sup>th</sup> week of October
	During November	Medium: 1st week to 4th week of November
	During December	Late : 1 <sup>st</sup> week to 4 <sup>th</sup> week of December
8.	Leaf Blade: Shape	Observation made on mature leaves taken from the middle of the
		shoot, the third leaf of the current season's growth from the middle part
		of the plant.
9.	Leaf length	Recorded as mean length of 10 basal leaves from the secondary fruit
		bearing branches. Length is measured from the base to the tip of the
		leaf blade. Total length of leaf blade is recorded with the help of
		measuring scale and expressed in centimeters(cm).
10.	Leaf width	Recorded on same leaves used for the measurement of leaf length.
		Width is measured at the widest portion of the leaf.
11.	Leaf Area in cm <sup>2</sup>	Measured with the help of Leaf Area meter
12.	Leaf Blade: Undulation of margin	Recorded at mature stage of leaf.

Sr.	Characters	Methodology adopted		
<b>No</b> 13.	Leaf Blade : Colour	Recorded at mature stage of leaf.		
	of adaxial surface			
14.	Leaf Blade: Intensity of Green Colour of abaxial surface	Recorded at mature stage of leaf.		
15.	Fruit length	Recorded mean length of 100 fully mature fruits randomly harvested from a single plant.		
16.	Fruit weight	Recorded the mean weight of 100 randomly collected fruits. Fruits are		
		produced on one year old shoots (unlopped branches) the branches		
		borne a year earlier.		
17.	Fruit: shape	Recorded at full fruit maturity.		
18.	Fruit :colour of skin	Recorded at full ripe stage. Colour is observed with the help of RHS		
		colour chart.		
19.	Time of beginning	Time of fruit maturity is when at least 50 % of fruits have achieved the		
	of fruit ripening	full colour.		
		Very early: During September		
		Early : During October		
		Medium : During November		
		Late : During December		
		Very late : 1 <sup>st</sup> Fortnight of January		
20.	Seed weight	The seed weight is recorded to calculate the amount of seeds:		
		1) Seeds/100g		
		2) 100 seed weight		
		3) 1000 seed weight.		
21.	Seed coat colour	Recorded on seeds extracted from fully ripe fruits after drying. Seed		
		coat color is observed with the help of RHS colour chart.		

The superior genotypes were screened among and within the selected populations on the basis of their Qualitative, Quantitative and Pseudo Qualitative characteristics. These screened genotypes were conserved in the germplasm bank, seed orchard and in demonstration models.

## E) Fodder quality and Productivity measurement:

- i) The selected thirty-five populations were evaluated on the basis of following leaf fodder quality parameters (proximate principles). The main aim of analysis of fodder quality parameters evaluation to find or screen superior genotypes with more than 20 percent crude protein percentage. The leaf samples were collected from different parts of the crown. These samples were collected during the winter season (during month of October- November) when leaves are rich in mineral nutrients and heavily lopped for fodder.
  - > 100 Leaf fresh weight (g)
  - > 100 Leaf dry weight (g)
  - Leaf dry matter (%)
  - Ether extract (%)
  - Crude protein (%)
  - Crude fibre (%)
  - > Total ash (%)
  - Nitrogen free extract (%)
- ii) The analyses of following soil physicochemical characteristics and macronutrients of thirtyfive soil samples collected underneath Grewia optiva populations were completed with the aim of development of correlation and regression models. The soil samples were collected from 15 cm-30 cm depth underneath the selected populations of Grewia optiva Drummond. In each collection 35 composite soil samples (each a composite of six cores) were taken. The soil samples were collected in two consecutive years 2019 and 2020, twice in year i.e., in the month of May and November. In 2019, first soil sample was collected during the Month of May, when this species was in leafing and flowering stage and second sample was taken at six months interval in the month of November, when the species was in the seed ripening and fodder lopping stage. Similarly, in the year 2020, two consecutive samples were collected in the month of May and November from the same selected sites and depth (15 cm-30 cm) respectively, from all thirty-five populations. The collected soil samples were air dried, lightly grounded and sieved to remove coarse particles (2mm) to ensure homogeneity. Mineral soil <2mm was subsampled in the field (approx. 150 g) and transported to laboratory of Soil- science, University of Horticulture and Forestry, Nauni, Solan (HP) for further analysis of morphological (soil texture), physiochemical (Bulk density, pH, EC) and other available macronutrients (N, P, K) on the basis of standard methods.
  - ➤ pH
  - Electrical conductivity (EC) (dS m<sup>-1</sup>)
  - Organic carbon (g kg<sup>-1</sup>)
  - Bulk density

- Soil Texture
- Available Nitrogen (kg ha<sup>-1</sup>)
- Available Phosphorus (kg ha<sup>-1</sup>)
- > Available Potassium (kg ha<sup>-1</sup>)

### F) Germplasm conservation

The selected genotypes were screened out on the basis of their Qualitative, Quantitative, and Pseudoqualitative characteristics and fodder quality characteristics. The screened genotypes have been conserved in germplasm banks (Clonal seed orchard, seedling seed orchard, and demonstration blocks). These conserved genotypes will be mass multiplied to produce true to type mother replica and supplied to farmers and stakeholders through the Forest departments, Animal husbandry department.

- 180 genotypes belonging to 60 families have been established in Seedling seed orchard, 23 genotypes established in Clonal Seed Orchard.
- ii) Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2x2m.
- iii) 'On Field Demonstration Model' –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m.

#### **G)** Distribution of Quality Planting Materials:

The quality planting material prepared in the nursery of the Department (under the project) were distributed to farmers, stakeholders, village panchayat with the following strategy:

This project has been taken with the involvement of State Forest Department, Additional PCCF Research and Training, Sundernagar. The forest Department has the direct linkage with the village farmers, stakeholders. The 15 Forest Divisions viz. Forest Division: Hamirpur, Forest Division: Dehra, Forest Division: Bilaspur, Forest Division: Renuka, Forest Division: Solan, Forest Division: Joginder Nagar, Forest Division: Una, Forest Division: Suket, Forest Division: Karsog, Forest Division: Nachan, Forest Division: Rajgarh, Forest Division: Nahan, Forest Division: Kunihar, Forest Division: Nalagarh, Forest Division: Mandi were contacted and involved to procure the superior planting material from this department and to coordinate activities at the field levels in low and mid zone of Himachal Pradesh.

# 3.7 Activity wise Time frame followed

Biological Screening of the germplasm diversity of the Grewia optiva a gortorestry tree species in Himalaya.  Establishment of germplasm bank; 27 Genotypes  - At spacing 2x2m= 31 Genotypes - At spacing 3x3m= 07 Genotypes  - At spacing 2x2m= 3x3m= 07 Genotypes  - At spacing 3x3m= 07 Genotypes  - A	Objectives	Year 2019-2020	Year 2020-2021	Year 2021-2022
optiva Drummond.	Screening of the germplasm diversity of the Grewia optiva as agroforestry tree species in Himalaya.  Establishment of germplasm bank of Grewia optiva genotypes.  Introduction of additional germplasm from other institutes or cropland	germplasm bank;-  - At spacing 1x1m= 27 Genotypes  - At spacing 2x2m= 31 Genotypes  - At spacing 3x3m= 07 Genotypes  - At spacing 4x4m= 04 Genotypes  - Establishment of Seedling Seed Orchard: 180 genotypes belonging to 60 families  - Establishment of Clonal Seed Orchard 23 genotypes in three replications i.e. 69 plants are	Demonstration Model — cum- Gene Bank of Grewia optiva (Beul) at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2x2m.  Absolute management practices have been done/ undertaken on already established spacing trials at Naganji Farm.  Establishment 'On Field Demonstration Model' —cum- Gene Bank of Grewia optiva (Beul) at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing	germplasm has been selected/ introduced from Chamba block of Tehri Garhwal, Uttarakhand and other cropland sources of Himachal Pradesh to find out another morpho variants and superior nutritive strains of Grewia optiva.  The nine (09) nutritive selections/populations viz., Kothi Kanwal (Solan), Unchagaon (Solan), Nerikalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of Bilaspur district have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected and identified

Objectives	Year 2019-2020	Year 2020-2021	Year 2021-2022
Study the growth parameters for forage quality and productivity of Grewia optiva	■ Soil and leaf were collected from 30 selected populations (05 samples from each district) of 6 districts of Himachal Pradesh viz. Solan, Sirmaur, Una, Kangra, Hamirpur and Mandi, for studying the fodder quality parameters and productivity of <i>Grewia optiva</i>	■ Analysed 30 soil samples from six districts of Himachal Pradesh for soil physicochemical characteristics namely viz. pH, Electrical Conductivity (EC), Organic Carbon (%), Available Nitrogen (N), Available Phosphorous (P), Available Phosphorous (P), Available Potassium (K), Soil Texture and Bulk density etc., in soil testing Department under the University of Horticulture and Forestry.  ■ Soil samples were collected from the remaining five	■ The analysis on physicochemical characteristics was completed for remaining five selected populations.  ■ Analysis on micronutrients of soil samples collected underneath nine best nutritive selections/ populations have been completed  ■ The analysis on leaf fodder quality parameters (proximate principles) of thirty-five selected populations have been completed.
Development of DUS specific descriptors of the selected germplasm of Grewia optiva.  Morphological characterization based on DUS specific descriptors of Grewia optiva	■ Thirty (30) populations were identified, in crop land situations in six Districts of Himachal Pradesh namely Hamirpur, Una, Kangra, Mandi, Solan and Bilaspur	populations.  Five populations from the district Sirmaur of Himachal Pradesh were identified and their morphological observations on leaf, fruit and plant characteristics were recorded.	<ul> <li>Morphological data of 35 populations, which was earlier recorded and completed on cropland situation, have been analysed statistically.</li> <li>Data base generated on 210 as Example/Reference Varieties under the project from 35 populations, besides data</li> </ul>

Objectives	Year 2019-2020	Year 2020-2021	Year 2021-2022
Molecular characterization of fast growing individuals of Grewia optiva.	■ Morphological observations on parameters i.e. plant height, plant diameter, crown spread, number of primary branches, number of secondary branches, branch angle, leaf length, leaf width, petiole length, leaf area , leaf colour, leaf shape, leaf margin, leaf apex, fresh weight of 100 leaves, dry weight of 100 leaves and bark	<ul> <li>Morphological data of total 35 populations (30+5) , which were earlier recorded and completed on cropland situation, analysed statistically for conclusion.</li> <li>The seed samples were collected from seventeen selected seed sources (populations) of seven districts of Himachal Pradesh and the cuttings were collected from five populations of district Kangra.</li> </ul>	generated from 23 families in Clonal Seed Orchard.  Ten (10) best families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/trial studies. The Morphological descriptors have been prepared for these 10 best screened families of Clonal Seed
	characteristics were recorded for all selected genotypes (180 genotypes) of thirty populations.  Morphological observations have been recorded on the established Clonal Seed Orchard of Grewia optiva at the farms of the Department in the University Campus (69 plants).	<ul> <li>Morphological descriptors were prepared for 10 best families of <i>Grewia optiva</i>, which were screened on the basis of molecular characterization.</li> <li>Fresh Seeds Collected during the month of November and December 2020-2021 of <i>Grewia optiva</i> from the Seven districts of Himachal Pradesh of</li> </ul>	■ For production of quality planting stock, nursery has been raised at Shilly Experimental Farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS

Objectives	Year 2019-2020	Year 2020-2021	Year 2021-2022
	<ul> <li>100 fruits collected in three replications each from identified populations of Grewia optiva in selected sites of Himachal Pradesh.</li> <li>Morphological observations on fruits i.e fresh weight of 100 fruits, dry weight of 100 fruits and seed features were recorded.</li> <li>Best 10 families were identified and screened on the basis of morphological and molecular characterization.</li> <li>Standardized best seed sowing time and pre sowing seed treatment of Grewia optiva, while raising quality planning stock .</li> </ul>	identified superior families on the basis of morphological characteristics.  Standardized techniques were developed to produce genetically superior plants through cuttings with hormonal treatments. The Collected cuttings from identified superior populations of district Kangra were planted in Majgaon Nursery in Mist Chamber treating with growth hormones (IBA 500 mg/lit) auxim concentration was found suitable treatment for raising cuttings of Grewia optiva as replica of mother's trees (true to type).	specific descriptors of the selected germplasm have been developed at population level as well as progeny performance/ at nursery stage level.  The Nine (09) best selections/ populations have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics, but the work on Molecular characterization of these Nine (09) best selections have been completed.

Objectives	Year 2019-2020	Year 2020-2021	Year 2021-2022
Conservation and Establishment of gene bank of selected genotypes of Grewia optiva.  Production and supply of superior planting material for farmers and State Forest Department and also raising of nursery from superior proven genotypes.	<ul> <li>In a first phase, 15         Divisional Forest         Officers belonging         to six districts of         Himachal Pradesh         were selected and         identified.</li> <li>30 farmers         belonging to six         districts of Himachal         Pradesh viz. Solan,         Sirmaur, Mandi,         Hamirpur, Una and         Kangra were         identified as         beneficiaries i.e.         five farmers in each         district, at each         population site/         locality under study.</li> <li>Fruits/ Seeds of         different 10 families         (best proven         families) were         supplied out of state         to Dr. Sheeraz         Saleem Bhat,         Scientist Sr. Scale         (ARS-Forestry),         ICAR-Indian         Grassland and         Fodder Research         Institute, Regional         Research         Station, KD Farm,</li> </ul>	<ul> <li>Number of Beneficiaries added (125 i.e [35= 7 Districts x 5 progressive farmer from each district] + [75= 15 Divisions x 5 farmer from each division] + [15=15 Forest Divisions through DFO's])</li> <li>Quality planting materials of best families of <i>Grewia optiva</i> were provided to 110 farmers [35= 7 Districts x 5 progressive farmer from each district] + [75= 15 Divisions x 5 farmer from each division].</li> <li>Sowing of 7,200 seeds of <i>Grewia optiva</i> in polybags in the Shilli Nursery Observation were recorded on germination related parameters i.e. Number of days to initial germination, Number of days to complete germination, Germination percentage, Germination value and Percent survival.</li> </ul>	<ul> <li>400 plants have been supplied to each forest division in different Fifteen (15) Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e., total 12,000 plants supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc.</li> <li>During 2022, again 360 plants raised from superior nutritive selection of Clonal Seed Orchard were supplied to 15 Forest Divisions for field testing and demonstration model (i.e., 5400 plants) and other 6,000 plants were supplied to farmers/stakeholders/women groups identified through selected Forest Divisions.</li> </ul>

Objectives	Year 2019-2020	Year 2020-2021	Year 2021-2022
	Rangreth, Srinagar- 191 132 (Jammu & Kashmir), for raising quality planting stock in that region and the outcome/ results and growth performance data was shared with us under the project activities.	■ Quality planting material raised of superior nutritive selection of Clonal Seed Orchard has been supplied to 15 Forest Divisions of H.P.) during the year 2020 and 2021, i.e., 5400 plants in each year for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, other 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e., totaling to 12,000 plants supplied for the farmers/ stakeholder, identified by the Forest Division for the benefit to the end users, farmers, women groups, self-help group etc.	■ Also, the nursery has been raised in 2021 from seeds collected from thirty-five selected populations of Himachal Pradesh to distribute best planting stock in the year 2022 to farmers, stake holders through the State Forest Departments. Animal Husbandry Department/ Agriculture Department as per the requirement for cattle/ cow sanctuary in the respective Districts of the state Himachal Pradesh are also be the beneficiaries of the project.

# 4 KEY FINDINGS AND RESULTS

### 4.1 Major Research Findings

- 1. Germplasm bank of Grewia optiva has been established i.e.,
  - a) 180 genotypes belonging to 60 families have been established in Seedling seed orchard, 23 genotypes established in Clonal Seed Orchard.
  - b) Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2x2m.
  - c) 'On Field Demonstration Model' –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m.
  - 2. A total of nineteen superior nutritive selections have been identified and screened under the project. The nine nutritive selections/populations viz., Kothi Kanwal (Solan), Uncha gaon (Solan), Neri Kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur), Bhaleth (Hamirpur) and Barthi population of Bilaspur district have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected populations of Grewia optiva. Ten best nutritive selections have been identified and screened in the Clonal Seed Orchard under this project on the basis of Qualitative, Quantitative and pseudoqualitative characteristics and leaf fodder characteristics.
  - 3. The analyses on soil physicochemical characteristics ((soil texture, Bulk density, pH, EC) and other available macronutrients (N, P, K, Ca, Mg and S of thirty-five soil samples collected underneath *Grewia optiva* populations have been completed. There was significant difference observed in pH, OC, EC, N, P, K and in Bulk density within selected population of each district. pH range of moderately acidic to moderately alkaline (according to standard soil classification) founded best for growth of this species. There was highly positive correlation observed between Nitrogen and leaf area (0.33), Phosphorus and leaf area (0.45). 100 leaf fresh weight showed highly positive correlation with Nitrogen (0.37) and organic carbon (0.39). 100 leaf dry weight showed highly positive correlation with Nitrogen and Organic Carbon. Crown spread showed highly positive correlation with Organic carbon (0.29), Nitrogen (0.38) and with Phosphorus (0.30). Moderately Positive correlation observed between Potassium and 100 fruit dry weight (0.15). 50 Per cent of soil texture observed as sandy loamy and sandy clay loamy. Correlation studies were worked out after developing complete screening out of the nutritive strains and regression equations, economic and financial models have been developed.

- 4. The additional germplasm has been selected/introduced from Chamba block of Tehri Garhwal, Uttarakhand and other cropland sources of Himachal Pradesh to find out another morpho variants and superior nutritive strains of *Grewia optiva*.
- 5. Raising planting material through cuttings has been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mother's replica) material. As this plant is difficult to raise through cutting.
- Morphological DUS specific descriptors have been developed for the 210 genotypes of selected thirty-five populations and 23 families of clonal seed orchards.
- 7. The best 10 families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Morphological descriptors has been prepared for these 10 best screened families.
- 8. For production of quality planting stock, nursery has been raised at Shilly farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.

#### 4.2 Key Results

- Superior germplasm material has been conserved in the seed orchard and demonstration model have been developed at Naganji and Khaltoo farm of the department. This conserved material will be Mass-muliplied and supplied to farmers under exit sustainable strategy:
  - a) 180 genotypes belonging to 60 families have been established in Seedling seed orchard,
     23 genotypes established in Clonal Seed Orchard.
  - b) Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2x2m.
  - c) 'On Field Demonstration Model' –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m.
- 2) The total nineteen superior nutritive selection have been identified and screened under the project. The nine nutritive selections/populations *viz.*, Kothi Kanwal (Solan), Uncha gaon

(Solan), Neri kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur), Bhaleth (Hamirpur) and Barthi population of Bilaspur district have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected populations of Grewia optiva. The Kothi Kanwal population exhibited average 24.43 % crude protein, Uncha goan population with average 24.96 % crude protein, Neri Kalan with average 25.12 % crude protein, Machair population reported with average 25.02 % crude protein, Jajjer with average 24.56 % crude protein, Balla population with average 25.05 % crude protein, Jhinjkari population with average 24.98 % crude protein, Bhaleth population with average 25.11 percent crude protein and Barthi population showed average 25.15 % crude protein. All these nine populations showed crude protein percentage more than 24 percent which considered above highest range as per value given by Sankhyan and Bhagta (2016) for crude protein i.e.,17.35 -20.99 % in their study on Grewia optiva. The study on evaluation of Clonal Seed Orchards established at UHF, Nauni has been completed for Qualitative, Quantitative and Pseudoqualitative characteristics and leaf fodder characteristics and ten best nutritive selections have been identified and screened in the Clonal Seed Orchard under this project. Therefore, total nineteen superior nutritive selections have been identified and screened under the project.

- 3) The analyses on soil physicochemical characteristics and macronutrients of thirty-five soil samples collected underneath *Grewia optiva* populations have been completed. Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, economic and financial models have been developed.
- 4) Raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mother's replica) material.
- 5) Morphological data of 35 populations, which have been earlier recorded and completed on cropland situation, have been analysed statistically. Data base generated on 210 genotypes as example reference varieties under the project from 35 populations, besides data generated from 23 families in Clonal Seed Orchard.
- 6) The best 10 families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The mean values recorded for plant height ranged between 6.96 10.45 m and 6.34-8.68 cm for basal diameter. The mean values 4.41-10.33 for number of branches per plant and 40.5-120.12 () for branch angle whereas 67.47-90.12 for number of leaves per branch and 3.75-4.97 for straightness score and 73.66-128.49 cm² for leaf area. The morphological descriptors have been prepared for these 10 best screened families.

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- 7) For production of quality planting stock, the nursery has been raised at Shilli farm of the department from the seeds collected from superior families and populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.
- 8) The nine best selection/populations have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics and Molecular characterization.
- 9) A stock of 360 Plants of superior nutritive selection of Clonal Seed Orchard have been supplied to each of the 15 Forest Divisions (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during both the year 2020 and 2021, i.e., 5400 plants in each year for conservation and establishment of gene bank in each selected and identified forest division through SFD, for demonstration/ field testing trial/ block plantation.
- 10) Beside this, 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 and 2021, i.e., therefore, total 12,000 plants supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc.
- 11) During the current year 2022, again 360 plants raised from superior nutritive selection of Clonal Seed Orchard supplied to 15 Forest Divisions for field testing and demonstration model (i.e., 5400 plants) and total 6,000 plants were supplied to farmers /stakeholders/women groups identified through selected Forest Divisions. Also, the nursery has been raised in 2021 from seeds collected from thirty-five selected populations of Himachal Pradesh to distribute best planting stock in the year 2022 to farmers, stackholders through the Forest Departments and Animal Husbandry department/Agriculture Department.
- 12) Investigations were carried out with a view to determine genetic divergence between selected families using molecular markers (RAPD, ISSR). In order to suggest breeding procedure for improving yield and other contributing characters, collection and computation of data were done accordingly. The data collected on different characters were subjected to statistical and biometrical analysis covering all the proposed objectives. The results obtained are presented separately for laboratory and field studies under the following headings.

Isolation, quantitative and qualitative assessment of genomic DNA for subsequent use in molecular studies

Survey of polymorphism

RAPD (Random Amplified polymorphic DNA) studies

Data analysis

Dendrogram based on RAPD banding pattern

ISSR (Inter Simple Sequence Repeat) studies

Data analysis

Dendrogram based on ISSR banding pattern

#### Isolation, Quantitative and Qualitative Assessment of Genomic DNA

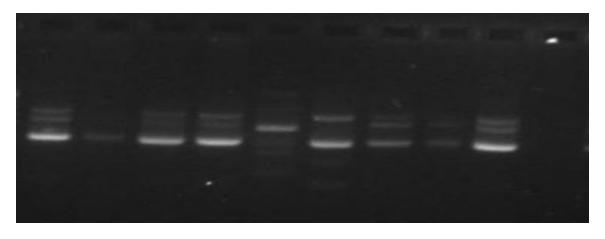
Genomic DNA from the collected leaves of all families used in the present study was isolated by employing the CTAB method of Doyle and Doyle (1987). Agarose gel electrophoresis on 0.8 per cent gel was carried out to check the quality of the isolated DNA. A single high molecular weight band was observed in all the samples, indicating good quality DNA. Purity of DNA was assessed by ratio of absorbance at 260 nm by using UV/VIS Spectrophotometer. The ratio was found to lie between 1.4 and 1.8, indicating good quality of DNA.

Quantity of DNA was evaluated using picodrop spectrophotometer and all the samples were found to contain weight range of 14µg/ml to 18 µg/ml.

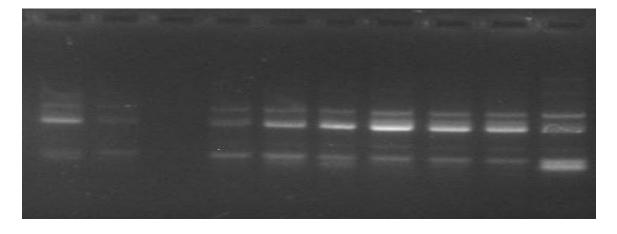
# Survey of polymorphism

# RAPD (Random Amplified Polymorphic DNA) studies

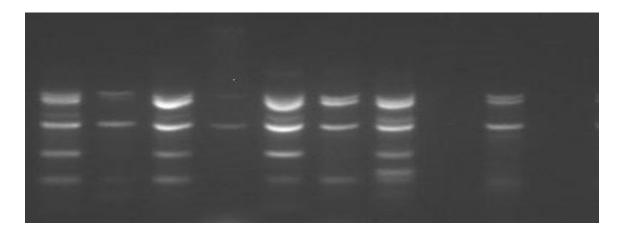
Genetic variation among ten families of *Grewia optiva* collected from different districts of Himachal Pradesh was investigated using RAPD primers. Initial screening of 15 RAPD primers out of which 9 RAPD primers produced profiles with intense banding pattern, which detected polymorphism among 10 accessions were used in the study.



Primer OPC-11 (AAA GCT GCG G)



Primer OPC-13 (AAGCCTCGTC)



Primer OPF-08 (GGGATATCGG)

Plate 1 RAPD fingerprints of 10 Families generated by OPC-11, OPC-13 and OPF-08

Total of 29 amplified bands were scored with 9 primers in 10 families of *Grewia optiva*. Six polymorphic and 4 monomorphic bands were observed. Detail of data for amplification pattern of the primers maximum number of amplified bands, i.e., five were produced by primers 'P1'and 'P4' whereas minimum number of bands, i.e., one was produced by primer P9. Out of the total 29 scorable bands, 20 showed polymorphism and 9 bands exhibited monomorphism resulting in 68.96 per cent polymorphism among ten families. Five of nine primers exhibited 100 per cent polymorphism (Table). The banding pattern generated by each RAPD primer for 10 families is presented in Table.

Table: Total number of amplified and polymorphic fragments generated by PCR using RAPD primers

S1. No	Primer name	Base Sequences (5'-3')	Total no. of scorable bands (y)	Total no. of polymorphi c bands	Total no. of monomorphic bands	Polymorphism (%) $\frac{x}{y} X 100$
p1	OPC-08	TGG ACC GGT G	5	0	5	0
p2	OPC-11	AAA GCT GCG G	3	3	0	100
р3	OPC-13	AAG CCT CGT C	3	3	0	100
p4	OPF-08	GGG ATA TCG G	5	5	0	100
p5	OPF-11	TTG GTA CCC C	4	3	1	75
р6	OPA-01	CAG GCC CTT C	3	3	0	100
p7	OPA-04	AAT CGG GCT G	3	3	0	100
p8	OPO-17	GGC TTA TGC C	1	0	1	0
p9	OPO-18	CTC GTA TCC	2	0	2	0
		TOTAL	29	20	9	68.96

Table: Summary of RAPD amplified products obtained from 10 families of *Grewia optiva* 

Sr. No.	Parameters	
1.	Total number of primers examined	15
2.	Total number of primer amplified	9
4.	Total number of polymorphic primer	5
5.	Total number of bands amplified from polymorphic primers	29
6.	Total number of polymorphic bands identified	20
7.	Total number of monomorphic bands	9
8.	Per cent of total polymorphic bands	68.96
9.	Number of primers exhibited 100 per cent polymorphism	5

The RAPD banding pattern of OPC-08 produced a total of 5 bands and all were monomorphic. The total number of bands amplified with OPC-11 primer was found to be 3

and per cent polymorphism revealed was 100 per cent as all bands exhibited polymorphism. RAPD analysis with primer OPC-13 yielded 3 bands, all were found to be polymorphic.

The total number of bands amplified with OPF-11 primer were 4 and 3 bands revealed polymorphism and 1 was monomorphic. The RAPD banding pattern of OPA-01 produced a total of 3 bands and all were polymorphic. Per cent polymorphism revealed was 100 per cent

With OPA-04 primer, total number of amplified products was 3, and per cent polymorphism revealed was 100 per cent as all bands exhibited polymorphism. RAPD analysis with primer OPO-17 yielded scorable 1 band which is momomorphic in nature. All 2 bands amplified with OPO-18 primer were monomorphic.

Table: Banding pattern of 9 RAPD markers in 10 families of Grewia optiva

Primers						Fa	milies				
	SI-15	SO-3	HA-2	SI-14	HA-4	SO-7	НА-3	SO-4	SI-16	SH-7	Total no. of alleles or bands
OPC-08	5	5	5	5	5	5	5	5	5	5	50
OPC-11	3	1	3	3	1	2	3	2	3	0	21
OPC-13	3	3	0	3	3	3	3	3	3	3	27
OPF-08	5	2	5	2	5	3	5	0	2	0	29
OPF-11	4	2	2	4	3	1	1	4	4	4	29
OPA-01	3	3	3	2	3	3	3	0	3	0	23
OPA-04	3	3	2	3	2	3	0	4	0	3	23
OPO-17	1	1	1	1	1	1	1	1	1	1	10
OPO-18	2	2	2	2	2	2	2	2	2	2	20
Total	29	22	23	25	25	23	23	21	23	18	232

# **RAPD** data analysis

The RAPD fragments obtained after the amplification of genomic DNA from 10 families were subjected to analysis. The scoring of bands for presence as 1 (band present) and 0 (band absent) for each family. The data matrix so obtained was analysed with NTSYS-2.2 software to obtain the Jaccard's similarity correlation coefficient. The matrix obtained has been presented in Table.

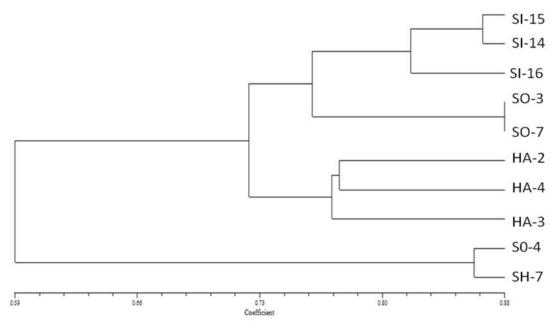


Fig: Dendrogram based on UPGMA analysis among 10 families of *Grewia optiva* using RAPD markers

### Dendrogram based on RAPD banding pattern

Dendrogram showing different clusters was based on NTSYSpc ver. 2.02h. Similarity coefficient obtained by Jaccard coefficient was used for UPGMA analyses of NTSYS. SAHN module of NTSYS used the pairwise obtained coefficient to construct dendrogram.

In the dendrogram (Fig. ), the 10 families separated into two main clusters, 'l' and 'll', at 59 per cent similarity. Cluster 'l' was found to contain only two families i.e. 'SO-4' and 'SH-7' and Cluster'll' accommodated rest 8 families namely SO-3, HA-2, HA-3, HA-4,SO-7, SI-16,SI-14 and SI-15. This revealed less similarity between cluster I and cluster 'll'. Cluster 'll' was further subdivided into two clusters i.e. Ila and Ilb at similarity value of 70 percent (Fig.). It was concluded that 'SO-3' and 'SO-7' were closely related as they showed 88 per cent similarity.

The advent of molecular markers is one of the significant developments in the field of biological sciences. Molecular markers, in particular, DNA based markers offer numerous advantages over conventional phenotype based alternatives as they are stable, highly heritable, and relatively easy to assay and are not affected by environment. With the help of molecular markers, it has become possible to decipher the genomes of plants, animals and microbes. There are various types of markers which have been used to detect and exploit DNA polymorphism. Most commonly used markers for analyzing genetic diversity and linkage map construction are RFLP, RAPD, SSR, ISSR and AFLP.

In the present investigation RAPD markers has been used for assay in genetic variation at molecular level of ten families using twenty five decamer primers designed from

Genei Bangalore, India limited. Results showed that RAPDs were highly informative for revealing relationship based upon similarity with in reference set of families.

# Inter Simple Sequence Repeat (ISSR) studies

Molecular characterization of 10 families of *G. optiva* collected from different districts were investigated using an initial screening of 20 ISSR primers. 12 primers produced ISSR profiles with intense banding pattern, which showed polymorphism between 10 accessions used in the study. ISSR analysis revealed high levels of genetic diversity within the reference set of *G. optiva* families. Out of the total 74 scorable bands, 57 showed polymorphism and 17 bands exhibited monomorphism. Total number of amplified and polymorphic fragments generated per ISSR primer revealed 71.25 per cent polymorphism among families (Table).

Table: Total number of amplified and polymorphic fragments generated by PCR using ISSR primers in *Grewia optiva* 

S. No	Prime r name	Base sequences (5'-3')	Total no. of scorable bands	Total no. of polymorphi c bands	Total no. of monomorphic bands	Polymorphism (%) $\frac{x}{y} X 100$
			(y)	(x)		
1	809	AGAGAGAGAGAGAGAG	7	7	0	100.0
2	810	GAGAGAGAGAGAGAT	8	8	0	100.0
3	811	GAGAGAGAGAGAGAC	5	2	3	40.0
4	812	GAGAGAGAGAGAA	5	4	1	80.0
5	830	TGTGTGTGTGTGTGG	6	6	0	100.0
6	834	AGAGAGAGAGAGAGY T	6	6	0	100.0
7	850	GTGTGTGTGTGTYA	6	6	0	100.0
8	861	ACCACCACCACCACC	6	4	2	66.7
9	862	AGCAGCAGCAGCAGC	9	3	6	33.3
10	UBC- 807	AGAGAGAGAGAGAGT	5	5	0	100.0
11	UBC- 826	ACACACACACACACAC	4	2	2	50.0
12	UBC-	GAGAGAGAGAGAGAY	7	4	3	57.1
	841	TOTAL C	74	57	17	71,25
		IUIAL	/4	5/	1/	/1.25

Table: Summary of ISSR amplified products obtained from 10 families of Grewia optiva

Sr. No.	Parameters	
1.	Total number of primers examined	20
2.	Total number of primer show amplification	12
3.	Total number of polymorphic primers	6
4.	Total number of bands amplified from polymorphic primers	74
5.	Total number of polymorphic bands identified	57
6.	Total number of monomorphic bands	17
7.	Per cent of total polymorphic bands	71.25
8.	Number of primers exhibited 100 per cent polymorphism	6

ISSR analysis with primer 809 yielded a total of 7 bands and all the bands exhibited polymorphism. Primer 810 yielded a total of 8 bands and all were found to be polymorphic in nature. With primer 811, a total of 5 scorable bands of which 2 were found to be polymorphic and 3 were monomorphic. Per cent polymorphism was 40 percent.

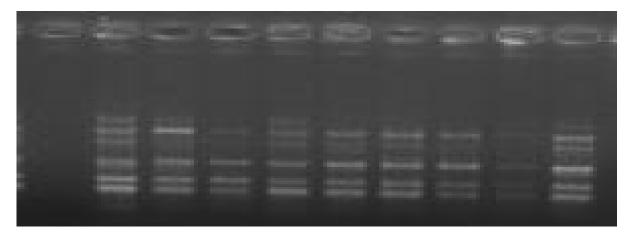
ISSR analysis with primer 812 yielded 5 bands. One band exhibited monomorphism and rest of the bands showed polymorphism revealing 80 per cent polymorphism among ten families.

ISSR analysis with primer 830, primer 834and primer 850 yielded 6 bands and 100 per cent polymorphism was recorded.

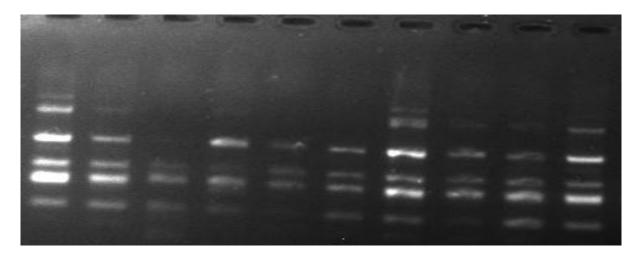
Primer 861 also recorded 6 bands and 4 were polymorphic and 2 were monomorpic. ISSR analysis with primer 862 yielded highest number of bands (9). Six band exhibited monomorphic and rest of the bands were polymorphism revealing 33.3 per cent polymorphism.

Table: Banding pattern of 12 ISSR markers in 10 families of *Grewia optiva* 

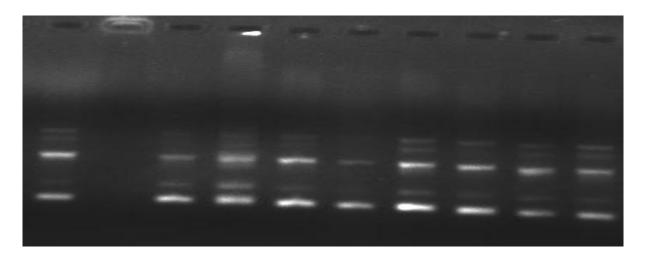
Primer					Fan	nilies					
name	SH-7	SO-4	HA-3	SI-16	SI-14	SO-7	SI-15	SO-3	HA-2	HA-4	Total
809	1	4	3	4	1	3	2	2	2	1	23
810	7	5	0	3	1	2	3	3	6	3	33
811	3	3	3	5	3	3	3	3	3	3	32
812	4	5	5	4	4	4	4	4	4	4	42
830	3	0	1	1	0	0	2	0	2	3	12
834	5	2	0	1	4	0	5	3	5	1	26
850	0	6	6	4	5	5	5	5	4	6	46
861	6	5	4	4	3	3	6	5	5	5	46
862	7	8	8	9	8	9	9	8	7	8	81
UBC-	5	0	4	5	5	3	5	4	5	5	41
807											
UBC-	4	4	4	4	4	4	4	2	2	2	34
826											
UBC-	5	6	5	5	5	5	5	5	6	6	53
841											
Total	50	48	43	49	43	41	53	44	51	47	



Primer 850 (GTGTGTGTGTGTYA)



Primer 861 (ACCACCACCACCACC)



Primer UBC-807 (AGAGAGAGAGAGAGT)

Plate 2 ISSR fingerprints of 10 Families generated by 850, 861 and UBC-807

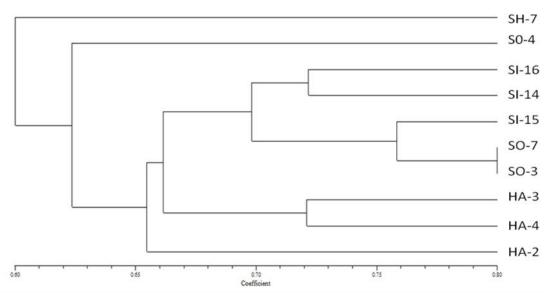


Fig: Dendrogram based on UPGMA analysis among 10 families of *Grewia optiva* using ISSR markers

Primer UBC 807 also recorded 5 bands and all were monomorphic. A total of 4 bands were obtained by primer UBC 826 which exhibited 2 and 2 monomorphic band. With primer UBC 841 a total of 7 bands were amplified and 4 bands were polymorphic in nature and rest 3 were monomorphic.

# ISSR data analysis

ISSR fragments obtained after the amplification of genomic DNA from 10 were subjected to analysis. The scoring of bands for presence as 1 (band present) and 0 (band absent) for each family. The data matrix so obtained was analysed with NTSYS- 2.2 software to obtain the Jaccard's similarity correlation coefficient. The matrix so obtained has been presented in Table.

The mean coefficient value of any families or accession gave an idea about its overall relatedness with all other families or accessions in the study. The coefficient values ranged from 0.52 to 0.80. This indicated a fair range of variability in the similarity coefficient values suggesting a broad genetic base of thirty accessions included in the experiment. The highest value (0.80) was found between SH-7 and SO-4. The lowest value of 0.52 was exhibited between SI-15 and SO-7, SI-15 and HA-2 depicting that the families were more diverse respectively.

#### Dendrogram based on ISSR banding pattern

Dendrogram showing different clusters was based on NTSYSpc ver. 2.02h. Similarity coefficients obtained by Jaccard coefficient were used for UPGMA analyses of NTSYS. SAHN module of NTSYS used the pair wise obtained coefficient to construct dendrogram.

In the dendrogram (Fig.), the 10 families separated into two main clusters, 'I' and 'II', at 60 per cent similarity. Cluster 'I' was found to contain only one family i.e. SH-7 'and Cluster'II' accommodated rest 9 families namely 'SO-4, SO-3, HA-2, HA-3, HA-4, SO-7, SI-16, SI-15 and SI-14. This revealed less similarity between cluster I and cluster 'II'. Cluster 'II' was further subdivided into two clusters i.e. IIa and IIb at similarity value of 63 percent (Fig.). It was concluded that 'SO-3' and 'SO-7' were closely related as they showed 80 per cent similarity.

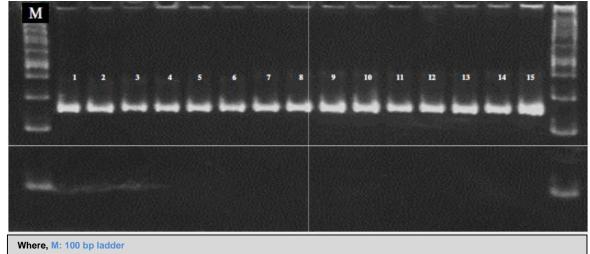
# Molecular characterization on the basis of nutritive selections and seedling growth characteristics

Molecular characterization of the nine best nutritive selections made from the thirty-five selected populations has been completed. On the basis of seedling growth characteristics, six best identified populations were used for molecular marker based analysis viz. HP-SO-1, HP-SO-2, HP-SO-4, HP-SI-1, HP-SI-2, HP-KG-5, HP-HA-5, HP-HA-2, HP-BL-2, HP-SO-5, HP-KG-2, HP-KG-3, HP-SI-3, HP-BL-1, HP-HA-1

Out of a total of ten ISSR primers used in this investigation, only two of them (UBC 809 and UBC 820) amplified and produced a banding pattern mentioned in the table given below. Primers UBC 809 and UBC 820 produced different types of bands, with the former producing monomorphic bands and the latter producing polymorphic bands. The maximum variations were observed between the populations of HP-SO-2 and HP-SO-5 genotypes which was developed based on seedling growth and nutritive selections.

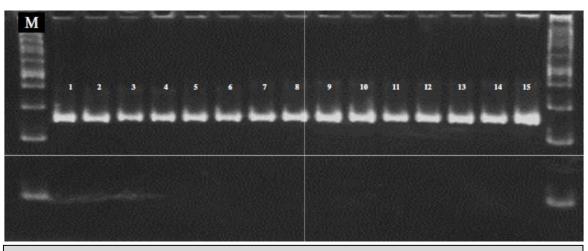
Table Total number of amplified and polymorphic fragments generated by PCR using ISSR primers in *Grewia optiva* 

Primer name (ISSR)	Base sequence (5'-3')	Total number of scorable bands	No. of polymorphic bands	No. of monomorphic bands		Percentage of monomorphism	0
UBC -809	AGAGAGAGAGAGAGAG	1	-	1	250-300bp	100%	-
UBC -820	GAGAGAGAGAGAGAT	2	2	-	200bp, 200-250bp	-	100%



 $HP-SO-1,\ HP-SO-2,\ HP-SO-4,\ HP-SI-1,\ HP-SI-2,\ HP-HA-5,\ HP-HA-2,\ HP-BL-2,\ HP-SO-5,\ HP-KG-2,\ HP-KG-3,\ HP-SI-3,\ HP-BL-1,\ HP-HA-1$ 

# **UBC 809**



Where, M: 100 bp ladder

HP-SO-1, HP-SO-2, HP-SO-4, HP-SI-1, HP-SI-2, HP-KG-5, HP-HA-5, HP-HA-2, HP-BL-2, HP-SO-5, HP-KG-2, HP-KG-3, HP-SI-3, HP-BL-1, HP-HA-1

# **UBC 820**

Fig. ISSR fingerprints of half sib progeny of 15 genotypes generated by UBC-820 and UBC-809

## 4.3 Conclusion of the study

- a) The total nineteen superior quality fodder selections have been screened from present study i.e., 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Nine (09) best selections/ populations with crude protein more than 24 % have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. Crude protein (CP) content is most important criterion for judging feed and fodder quality.
- b) The nineteen best nutritive selections identified under this project will be used to raise the demonstration block/Seed Orchard from where production of quality planting material will be raised and supplied to farmers. The establishment of seed orchards will help in the Massmultiplication of superior genotypes and transferring of genetically improved material to endusers.
- c) The genotypes identified and screened and conserved in the germplasm bank are much superior in fodder quality than the already practiced material. This superior material will be multiplied and supplied to farmers, end users so that the old material which is existing on the cultivated situation on the farmer's field should be gradually replaced with new screened genotypes in phased manner. For the production of quality planting stock, nursery has been raised at Shilly from the seeds collected from the selected thirty-five populations of H.P.
- d) Both the qualitative and quantitative observations on superior genotypes of *Grewia optiva* in selected sites of seven districts in Himachal Pradesh have been recorded (35 populations i.e., 210 superior plants).
- e) Morphological DUS specific descriptors which have been developed under the project will be utilized further for identification of superior nutritive selection in another IHR regions.
- f) The study on soil physicochemical characteristics and macronutrients have been completed for all thirty soil samples collected beneath the selected population. The knowledge of physicochemical properties viz; organic carbon, available Nitrogen (N), Phosphorus (P<sub>2</sub>O<sub>5</sub>), Potassium (K<sub>2</sub>O), pH, electrical conductivity, soil texture and bulk density of soil is also important to determine the available nutrient status in soil and to develop specific fertilizer recommendations.
- g) Correlation studies were worked out after developing complete screening out of the nutritive strains and regression equations, economic and financial models have been developed.
- h) Quality planting of superior nutritive selection of Clonal Seed Orchard has been supplied to 15 Forest Divisions for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, quality plants have been supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc.

- i) This species is difficult to raise vegetatively but raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers' replica) material under this project.
- j) The twenty new populations (five from Uttarakhand and sixteen from the cropland area of Himachal Pradesh) have been added/ selected to extend the research material on *Grewia optiva*. These selected sites of Uttarakhand and Himachal Pradesh will be explored for variations and broader base of the germplasm of the species for further establishment of germplasm bank. This study will also help in comparative analysis among and between the selected populations of H.P and Uttarakhand to screen out the best nutritive selections and to find out valuable material for future breeding programme.
- k) Diversity analysis amongst 10 different selected families of Grewia optiva was carried out using 15 RAPD and 20 ISSR primers and 9 RAPD and 12 ISSR primers show amplification respectively. Nine RAPD primers showed 68.96 per cent polymorphism and 12 ISSR primers showed 71.25 per cent polymorphism. Total scorable bands obtained were 29 in RAPD and 74 in ISSRs. Similarity matrices and dendrograms were generated using SAHN module of NTSYSpc ver.2.02h. Jaccard's similarity matrices revealed maximum similarity coefficient 0.88 between 'SO-7' and 'SO-3' with RAPD primers. For ISSRs, coefficient values ranged from 0.52 to 0.80. The highest value of similarity was 0.80 between 'SH-7' and 'SO-4'. Minimum similarity of 0.52 was obtained between 'SI-15' and 'SO-7', SI-15 and HA-4. The dendrograms also revealed to larger extent similar results and maximum similarity found among the 10 families of Grewia optiva collections was 88 per cent between 'SO-7' and 'SO-3'with RAPD primers and 80 per cent between 'SH-7' and 'SO-4' with ISSRs. In the present study 15 RAPD and 20 ISSRs primers were used to study genetic diversity of 10 families of Grewia optiva, leading to the conclusion that these marker systems are very efficient to carryout diversity study among families. Hence, these markers can also be used for fingerprinting studies.

# 5 OVERALL ACHIEVEMENTS

### 5.1 Achievement on Project Objectives

Project Objective 1: Biological Screening of the germplasm diversity of the *Grewia* optiva as agroforestry tree species in Himalaya.

Quantifiable Deliverable: Establishment of germplasm bank of *Grewia optiva* and introduction of germplasm:

- a) Under this deliverable germplasm bank of superior selections has been established. i.e.,
  - 180 genotypes belonging to 60 families have been established in Seedling seed orchard and 23 genotypes established in Clonal Seed Orchard.

- II. Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2x2m.
- III. 'On Field Demonstration Model' –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m.
- b) Morphological and morphometrics observations [ (growth types, attitude of branches, branch thickness, bark color, leaf characteristics (leaf length, leaf width, leaf area, leaf blade, 100 leaf fresh weight and 100 leaf dry weight), fruit characteristics (fruit length, fruit weight, fruit shape, skin colour) and phenological characteristics (leafing, flowering, fruiting, fruit ripening timings) and seed characteristics (seed weight, seed coat colour)] recorded for each selected genotype. The variation on these characteristics also has been observed between and among the selected thirty five populations and have been analysed statistically.

# Quantifiable Deliverable: Introduction of additional germplasm from other institutes or cropland sources

The additional germplasm has been selected/ introduced from Chamba block of Tehri Garhwal, Uttarakhand and other cropland sources of Himachal Pradesh to find out another morpho variants and superior nutritive strains of *Grewia optiva*.

# Project Objective 2: Study the growth parameters for forage quality and productivity of *Grewia optiva*.

#### Quantifiable Deliverable: Growth parameter for forage quality and productivity

The following results have been achieved under this objective and quantifiable deliverables:

- i) The study on following leaf fodder quality parameters (proximate principles) of thirty-five selected populations has been completed and statistical analysis of these parameters has been achieved.
  - Leaf fresh weight (g)
  - Leaf dry weight (g)
  - Leaf dry matter (%)
  - Ether extract (%)
  - Crude protein (%)
  - Crude fibre (%)
  - > Total ash (%)
  - Nitrogen free extract (%)

The contribution of study of Growth parameter for forage quality is that the nine nutritive selections/populations *viz.*, Kothi Kanwal (Solan), Uncha gaon (Solan), Neri Kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of

Bilaspur district have been identified and screened with crude protein percentage showing more than 24 percent from thirty-five selected populations of *Grewia optiva*.

- ii) The analyses of following soil physicochemical characteristics and macronutrients of thirty-five soil samples collected underneath *Grewia optiva* populations have been completed as per the 2<sup>nd</sup> objective and growth parameters for soil productivity deliverable. Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, economic and financial models have been developed.
  - **>** pH
  - ➤ Electrical conductivity (EC) (dS m<sup>-1</sup>)
  - > Organic carbon (g kg<sup>-1</sup>)
  - ➤ Bulk density
  - ➤ Soil Texture
  - ➤ Available Nitrogen (kg ha<sup>-1</sup>)
  - Available Phosphorus (kg ha<sup>-1</sup>)
  - > Available Potassium (kg ha<sup>-1</sup>)
- iii) The study on evaluation of Clonal Seed Orchards established at UHF, Nauni has been completed for Qualitative, Quantitative and pseudoqualitative characteristics and leaf fodder characteristics and ten best nutritive selections have been identified and screened in the Clonal Seed Orchard under this project.
- iv) The nursery has been raised at Shilly farm of the department from the seeds collected from 35 population of Himachal Pradesh *viz.*, Solan, Sirmour, Mandi, Hamirpur, Kangra, Una and Bilaspur. The following nursery growth parameters (productivity parameters) have been recorded and analysed statistically.
  - Leaf biomass per plant(g) at leaf harvesting stage
  - Seedling height (cm)
  - Collar diameter (mm)
  - Root length (cm)
  - Shoot length(cm)
  - Root fresh weight(g)
  - Shoot fresh weight(g)
  - Root dry weight ratio
  - Shoot dry weight ratio
  - Root biomass (Fresh & Dry)
  - Shoot biomass- (Fresh & Dry)

iii) Raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers replica) material.

# Project Objective 3: Development of DUS specific descriptors of the selected germplasm of *Grewia optiva*.

# Quantifiable Deliverable: Morphological characterization based on DUS specific descriptors of *Grewia optiva*.

- i) Morphological data of 35 populations, which have been earlier recorded and completed on cropland situation, have been analysed statistically. Morphological DUS specific descriptors have been developed and useful grouping characteristics finalized. It will help to develop DUS guidelines of this particular species.
- ii) Data base generated on 210 genotypes as example reference varieties under the project from 35 populations, besides data generated from 23 families in Clonal Seed Orchard.
- iii) The best 10 families and superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Morphological descriptors has been prepared for these 10 best screened families.
- iv) For production of quality planting stock, the nursery has been raised at Shilly farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.

# Quantifiable Deliverable: Molecular characterization of nutritive selections of *Grewia* optiva.

Under this deliverables molecular characterization of the 10 screened families from clonal seed orchard have been achieved. As the purpose of molecular characterization is to determines the genetic characteristics of the plants and establish the identity of the accessions and discern genetic relationship among genotypes. These also helps to study genetic diversity and provides basis for conservation of superior strains. Molecular characterization of the nine best nutritive selections made from the thirty-five selected populations has been completed. On the basis of seedling growth characteristics, six best identified populations were used for molecular marker based analysis viz. HP-SO-1, HP-SO-2, HP-SO-4, HP-SI-1, HP-SI-2, HP-KG-5, HP-HA-5, HP-HA-2, HP-BL-2, HP-SO-5, HP-KG-2, HP-KG-3, HP-SI-3, HP-BL-1, HP-HA-1

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# Project Objective 4: Conservation and Establishment of gene bank of selected genotypes of *Grewia optiva*

Quantifiable Deliverable: Production and supply of superior planting material for farmers and State Forest Department and also raising of nursery from superior proven genotypes.

To achieve this objective and quantifiable deliverables, quality planting have been raised under nursery conditions and supplied to forest divisions and farmers, stakeholders, villagers through forest department and animal husbandry departments. The results achieved under this objective are:

- i) 360 Plants of superior nutritive selection of Clonal Seed Orchard have been supplied to 15 Forest Divisions (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during the year 2020, 2021 and 2022 i.e., 5400 plants in each year and totaling 16,200 plants for conservation and establishment of gene bank in each selected and identified forest division through SFD, for demonstration/ field testing trial/ block plantation.
- ii) Besides this, 400 plants have been supplied to each forest division in different fifteen Forest Division in each year i.e., 6000 plants in each year during 2020 ,2021 and 2022, i.e., total 18,000 plants supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc.
- iii) Total production of Quality planting material count in three years i.e.  $5400 \times 3 = 16,200$  and  $6000 \times 3 = 18,000$ , Grand Total of 34,200 plants are developed in the nursery/ Experimental Farm for distribution to the 15 Forest Divisions of Himachal Pradesh.
- iv) Till now, the total production of Quality planting material in nursery/ experimental farm is more than 55,000 plants out of which 34,200 plants (2 years old) have been distributed for research purpose and farmers, stakeholders, self help groups through 15 Forest Divisions of Himachal Pradesh through State Forest Department as partner under this project.

### 5.2 Establishing New Database/Appending new data over the Baseline Data

The nine nutritive selections/populations *viz.*, (Kothi Kanwal (Solan), Uncha gaon (Solan), Neri kalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of Bilaspur district) have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected populations of *Grewia optiva*. The Kothi Kanwal population exhibited average 24.43 % crude protein (CP), Uncha goan population with average 24.96 % crude protein (CP), Neri Kalan with average 25.12 % crude protein, Machair population reported with average 25.02 % crude protein, Jajjer with average 24.56 % crude protein, Balla population with average 25.05 % crude protein,

- Jhinjkari population with average 24.98 % crude protein, Bhaleth population with average 25.11 percent crude protein and Barthi population showed average 25.15 % crude protein.
- Analysis work of physicochemical characteristics of soil pH, Electrical Conductivity (EC), Organic Carbon (%), Available Nitrogen (N), Available Phosphorous (P), Available Potassium (K), Soil texture and Bulk density have been completed for all 35 populations. Correlation studies were worked out after developing complete screening out the nutritive strains and regression equations, economic and financial models have been developed.
- Data base generated on 210 genotypes as example reference varieties under the project from 35 populations, besides data have been generated from 23 families in Clonal Seed Orchard.
- The Morphological descriptors have been prepared for the 10 best screened families from Clonal Seed Orchard.

### **5.3** Generating Model Predictions for different variables

Correlation models have been developed between soil nutrients and plant morphological characteristics after complete screening out the nutritive strains. This model depicts either the positive or negative effects of different soil nutrients on the plant's morphological characteristics. In this model, there was a highly positive correlation observed between Nitrogen and leaf area (0.33), Phosphorus and leaf area (0.45). 100 leaf fresh weight showed a highly positive correlation with Nitrogen (0.37) and organic carbon (0.39). 100 leaf dry weight showed a highly positive correlation with Nitrogen and Organic Carbon. Crown spread showed a highly positive correlation with Organic carbon (0.29), Nitrogen (0.38), and with Phosphorus (0.30). A moderately Positive correlation was observed between Potassium and 100 fruit dry weight (0.15).

### 5.4 Technological Intervention

- This plant species is difficult to raise through cuttings but efforts made under the project for vegetative propagation is quite successful and tongue grafting is also successful to develop VMG (Vegetative Multiplication Garden).
- Best Seed sowing time of *Grewia optiva* has been standardized. Pre sowing treatment and best time of seed sowing along with soil mixture has been standardized.
- This species is difficult to raise through cutting but raising planting material through cuttings
  have been standardized to develop with hormonal treatment (IBA 500 mg/lit) auxin
  concentration) for true to type (mothers' replica) material under this project.

### 5.5 On field Demonstration and Value-addition of Products

1) The leaf fodder of *Grewia optiva* is valuable fodder particularly during winter months when green fodder scarcely available in quantity and quality. People live in hilly areas used to

travel several kilometers daily for harvesting fodder from nearby forest area for satisfying the livestock requirement. At the same time, in hilly areas, due to rain fed conditions and small land holdings of the farmers, they have limited scope of producing green fodder on their land. So, there is a dire need to have fast growing tree species with maximum nutritive value. Therefore, the supply of nutritive selection in these areas will enhance the livestock production, reduce the physical pressure for fodder and will increase the income source for poor farmers.

- 2) The wood obtained from this tree can be used for making axe handles, cat frames, oar-shafts, bows, shoulder poles, etc., where a resultant product requires elasticity and strength. The wood is also said to be very suitable for paper making. Baskets can be made from elastic branches. The bark yields the fiber used for ropes/cordage making.
- 3) Beul bark can be used to make Shampoo for washing hair. It has saponin and local people can use it as an alternative to soap in villages of the Himalayan regions. An herbal shampoo product can be developed by mixing it with Amla and Shikakai. The bark of the tree is dried in sunlight or in dryer and ground to a powder, this is then mixed with Amla and Shikakai powder in the proportion of 35 % Beul bark, 40 % Shikakai powder and 25 % Amla powder and packed. This product is a good treatment for dandruff and graying hair. Liquid shampoo can also be prepared with sap.
- 4) Medicinal products from *Grewia optiva*: Products from *Grewia optiva* can be used as to make different types of medicines for treating various diseases like cough, dysentery, diarrhea, smallpox, malaria, typhoid, intestine and bladder with irritable conditions, rheumatism, and eczema. The main medicinal action appears to come from the mucilage that is found in the leaves, stems, and roots, which has been shown to have soothing and healing properties. Taken internally, it is often used as a remedy for diarrhea and dysentery, whilst externally, it is applied to wounds, cuts, ulcers, irritations, etc. The plant can be taken as a simple infusion or decoction, or it can be applied topically as a poultice of the plant, or the mucilage can be extracted from the plant, if required, by maceration followed by decoction.

The outer bark and fruit, when soaked in water, yield a gelatinous material which after drying, is used to make tablets that are used as aphrodisiac.

Leaves extract can be applied to eruptions. Crushed bark extract is taken for indigestion and gastric problems, also used as a lubricant during difficult childbirth. Fruits for fever. Veterinary medicine, bark paste as plaster, and fresh bark as a bandage on fracture of cattle.

- 5) The tender shoots left after the collection of leaf fodder can be used to extract fiber. These tender shoots and bark are dipped in the water more than fortnight to extract fibers from them. This is old traditional method of extraction of fiber used by hilly farmers. The fiber can be used to make wall hangings, paper making, baskets etc.
- 6) Used as a Biofertilizer: The leaves of *Grewia optiva* can be used to make biofertilizers. As the Beul leaves are proteinaceous and good for mulching of several vegetable crops. Large quantity of Organic matter adds to the soil due to the litter (leaves) which falls in winter season. Best response of growing bell peeper using organic manures under widely spaced *G. optiva* can offer economically more benefit better than sole.
- 7) When used with a mordant, the leaves yield a dye that is a fine bright clean shade of dark fawn, approaching to yellow.
- 8) Use of fruits: Raw or Ripe fruits have a pleasant acid taste and can be used to make edible products.

### 5.6 Promoting Entrepreneurship in IHR

- 1) The people of low and mid-Himalayan regions can start a new business/venture with the use of superior nutritive selections provided by our department to different forest division and village panchayats. They can raise their own nursery with superior planting material and can sell it to land-holding farmers and can fetch a good price. This will give hilly farmers a more stable and high quality of community life.
- 2) Small industries can be opened for making value-added products with the use *Grewia optiva* leaves, fiber, and wood i.e., the fiber obtained from the bark can be used for cloth and cordage making. Fibers can also be used for making handloom items. Beul bark can be used to make Shampoo by mixing it with Amla and Shikakai. These small-scale industries will provide employment and income to local people. This will increase the standard of living of the hilly farmers.
- 3) The wood obtained from this tree can be used for making axe handles, cat frames, oar-shafts, bows, shoulder poles also at other places where resultant product requires elasticity and strength. The wood is also said to be very suitable for paper making. Baskets can be made from elastic branches. The bark yields a fiber used for cordage making.
- 4) The production of superior nutritive selection with crude protein of more than 24 % will reduce the gap between demand and availability of nutritious fodder to livestock. As Livestock is an important factor in the rural economy because milk, meat, wool, and various dairy products have been an appreciable source of income for the rural community. Closer scrutiny of the sector however reveals that the contribution to the GDP by the livestock

sector is far too low for such a large size of livestock population. This low productivity of the sector is as much attributable to underfeeding of the livestock. The available fodder is not only insufficient but also poor in nutritive value. This gap in fodder deficit can be filled by increasing the cultivation of superior nutritive selections (screened under the project) of *Grewia optiva* in the cropland situation of hilly regions.

5) The production of cash crops can be increased (in hilly regions) under the canopy of widely spaced *Grewia optiva* trees and income can be generated from the marketing of these crops. like growing bell peeper using organic manures under widely spaced *G. optiva* can offer economically more benefit better than sole. As the Beul leaves are proteinaceous and can be used for mulching of several vegetable crops. A large quantity of Organic matter adds to the soil due to the litter (leaves) which falls in the winter season.

### 5.7 Developing Green Skills in IHR

The green skills refer to "the knowledge, abilities, values and attitudes needed to live in, develop and support a sustainable and resource-efficient society". The outcomes of this project will help to develop green skills in the village community of IHR. As the nineteen superior selections have been developed under this project i.e., ten superior strains have been identified and screened from the Clonal Seed Orchard on the basis of their morphological, molecular, and progeny testing and other nine superior selections with crude protein more than 24 % have been screened from the thirty-five selected populations of Himachal Pradesh. The production of these superior selections will be enhanced by the mass multiplication under the field conditions of the department and will be supplied to the farmers, and stakeholders through the forest department and agriculture department. The cultivation of superior selections in cropland situations will give farmers superior nutritive fodder. This will reduce the dependency of hill farmers on forest resources for fodder. This will also decrease the rate of deforestation and destruction of natural forests. This will promote the sustainable development of society, economy, and environment.

*Grewia optiva* is a multipurpose species that gives multiples benefits to hilly farmers with efficient use of scarce resources of the environment. As it is a good source of fuelwood, fibers, fodder, etc., its wood is also used to make many valuable products. This species also enriches the soil with organic matter which helps to increase the production of cash crops and maintains the fertility status of soil. This species can grow in rainfed conditions and on the slopy terrain which helps in the conservation of the catchment/watershed areas.

In the Himalayan states, this species can be grown for checking soil erosion, farmers can grow them in their field periphery and widely used for checking soil erosions, since it has a deep tap root system which provides support and hence it checks the soil erosion. It can be easily seen in 90-degree slopes where most of trees don't grow.

The farmers can make ecofriendly value-added products from this species e.g., ropes, handloom items, baskets etc. and can start their own small venture.

The mass media lecture, and workshops have been organized in IHR to render practical skill of raising the nursery through cutting, seeds, grafting etc. therefore the low and marginal land holding hilly farmers can raise their own nursery and sell them at good price to large land holding farmers and NGOs for cultivations and conservation.

### 5.8 Addressing Cross-cutting Issues

We have identified nineteen superior nutritive selections under the project which will be mass multiplied to produce true to type quality material. Besides this, our project findings will help to address the problems associated with IHR. A few cross cutting issues of IHR have been discussed below:

- 1) Global warming and Climate change: The climate change in IHR is increasing the incidence of flooding, drought etc. The Climate Change poses a risk to range and composition of forest biomass (upward shifting in range and composition of forest biomass (e.g., upward shift in Himalayan pines., affecting productivity and ecological health. Increase in temperature will reduce the amount of snowfall, reducing the water flow in snowed rivers during the summer month, water recharge decreased, affecting agriculture production and livelihood of the mountain regions. All four dimensions of food security are predicted to be affected by climate change: food availability, food accessibility, food utilization and food systems stability. Inhabitants of IHR are exposed to many natural hazards such as floods and flash floods, landslides, and earthquake. The cultivation of superior nutritive selections in the cropland situations of IHR will help to mitigate the effects of global warming by sinking atmospheric CO<sub>2</sub> etc.
- 2) Migration of Peoples to Urban areas: The peoples of hilly regions are migrating toward the urban areas for employment and livelihoods. But the availability of superior nutritive selections and training on value added products (ropes, handloom items, wall hanging etc.) will give them local employment and livelihood sources. They can start their small ventures like can develop nursery of superior selection and sell them at good price in the markets.
- 3) Forests and Ecosystem Conservations: The people of the IHR, like elsewhere in other mountain ecosystems, are heavily dependent for their livelihood on their surrounding natural resources and production from primary sectors such as agriculture, forestry and animal

husbandry, etc. The dependence of the continually growing population on finite resources, lack of viable technologies to mitigate the mountain specificities and enhance production to meet the ever-increasing demands are depleting the natural resources with concomitant increase in marginality of farmers, poverty and out migration. The hill farmers are highly dependent on the forests for fodder and manure prepared from forest leaf litter and livestock excreta. The extraction of leaf litter from forest area baring the soil and increases the incidence of soil erosions. Forests are not only the providers of a variety of ecosystem goods and services but also provide a sink for atmospheric  $CO_2$ , which is on the rise due to various anthropogenic activities. The hydrological regulation by mountain forest ecosystem in the lowlands, connected with the river systems, is yet another service that needs to be understood fully and its value appreciated. Himalayan forests help maintain soil fertility and essential atmospheric moisture in the adjacent Gangetic plains through rivers originating from the region. The production of multipurpose species of *Grewia optiva* on the cropland situations will decrease the dependency of the farmers on the forest for fodder, fuel wood and fiber etc. and will reduce the pressure on the forests.

- 4) Human and wildlife conflict: The hill farmers depend on the livestock for their livelihood requirement i.e., meat, wool, milk etc. but the scarcity of valuable and nutritious fodder in hilly regions increase the dependence of hill people on the wildlife animals. The availability of superior nutritive selections will help to reduce the gap between demand and supply of the quality fodder and reduce the human wildlife conflict.
- 5) Loss of biodiversity of medicinal and aromatic plants: Since valuable medicinal plants are declining in the hilly regions, cultivation of superior nutritive sections of *Grewia optiva* can be enhanced as a medicinal tree.
- 6) Groundwater recharge: The cultivation of *Grewia optiva* increases the organic matter content of the soil and increases the porosity of soil which helps in runoff reduction and ground water recharge.
- 7) Other issues: Sustainable management of land and water resources, Study/ Inventory of Springs/ River systems in the IHR, remoteness and isolation, long distance from the market limits the livelihood options, prone to natural disaster etc.

### 6 PROJECT'S IMPACTS IN IHR

### 6.1 Socio-Economic Development

 We have reached 35-gram panchayats of different districts of the H.P. The quality planting material has been distributed to these selected panchayats with the involvement of the Forest and Animal husbandry department. The 15 forest Divisions of Himachal Pradesh

- were contacted and linked to procure the planting material from this department and to coordinate activities at the field levels in low and mid hill zone of Himachal Pradesh.
- 2) The people of low and mid-Himalayan regions can start a new business/venture with the use of superior nutritive selections provided by our department to different forest division and village panchayats. They can raise their own nursery with superior planting material and can sell it to large land-holding farmers and can fetch a good price. This will give hilly farmers a more stable and high quality of community life.
- 3) Small industries can be opened for making value-added products with the use *Grewia optiva* leaves, fiber, and wood i.e., the fiber obtained from the bark can be used for cloth and cordage making. Fibers can also be used for making handloom items. Bhimal bark can be used to make Shampoo by mixing it with Amla and Shikakai. These small-scale industries will provide employment and income to local people. This will increase the standard of living of the hilly farmers.
- 4) The wood obtained from this tree can be used for making axe handles, cat frames, oar-shafts, bows, shoulder poles also at other places where resultant product requires elasticity and strength. The wood is also said to be very suitable for paper making. Baskets can be made from elastic branches. The bark yields fiber which is used for cordage making.
- 5) The production of superior nutritive selection with crude protein of more than 24 % will reduce the gap between demand and availability of nutritious fodder to livestock. Livestock is an important factor in the rural economy because milk, meat, wool, and various dairy products have been an appreciable source of income for the rural community. Closer scrutiny of the sector however reveals that the contribution to the GDP by the livestock sector is far too low for such a large size of livestock population. This low productivity of the sector is as much attributable to underfeeding of the livestock. The available fodder is not only insufficient but also poor in nutritive value. This gap in fodder deficit can be filled by increasing the cultivation of superior nutritive selections (screened under the project) of *Grewia optiva* in the cultivated situation of hilly regions.
- 6) The leaf fodder of *Grewia optiva* is valuable particularly during winter months when green fodder is scarcely available in quantity and quality. People living in hilly areas have to travel several kilometers daily for harvesting fodder from nearby forest area for satisfying the livestock requirement. At the same time, in hilly areas, due to rain fed conditions and small land holdings of the farmers, they have limited scope of producing green fodder on their land. So, there is a vital need to have fast growing tree species with maximum nutritive value. Therefore, the supply of nutritive selection in this area will enhance the livestock production, reduce the physical pressure for fodder and will increase the income source for poor farmers.

7) The production of agricultural crops can be increased (in hilly regions) under the canopy of widely spaced *Grewia optiva* trees and income can be generated from the marketing of these crops like growing bell peper using organic manures under widely spaced *G. optiva* can offer economically more benefit better than sole as the Beul leaves are proteinaceous and can be used for mulching of several vegetable crops. A large quantity of organic matter is added to the soil due to the litter (leaves) which falls in the winter season.

### 6.2 Scientific Management of Natural Resources In IHR

- This plant species is difficult to raise through cuttings but the efforts undertaken under the
  project for vegetative propagation are quite successful and tongue grafting is also successful
  to develop VMG. With the use of this new technological intervention, we can raise quality
  planting material.
- Pre-sowing treatment and best time of seed sowing along with soil mixture has been standardized under the project. The best seed sowing time of *Grewia optiva* have been standardized. This approach will help in the production of quality material through seeds.
- We can interbreed individuals from genetically distinct populations to produce a hybrid and can produce superior nutritive strains with crude protein more than the existing material /highly nutritive strains developed under the project.
- We can use micropropagation techniques (tissue culture) for clonal propagation of superior nutritive selections, for germplasm storage and the protection of superior selections.

### 6.3 Conservation of Biodiversity in IHR

- 1) We have developed gene conservation banks for the conservation of superior germplasm material. For example: the Number of genotypes conserved in germplasm bank (Nos) at spacing 1x1 m = 27 genotypes 2x2m = 31genotypes,3x3m=07genotypes 4x4 m=04 genotypes.
  - a) 180 genotypes belonging to 60 families have been established in Seedling seed orchard, 23 genotypes established in Clonal Seed Orchard. This conserved germplasm can be used for future breeding programs and development of superior hybrids with higher percentage of crude protein.
- 2) The superior material collected from different selected locations of Himachal Pradesh has been conserved in demonstration models. This conserved material is a large source of quality planting material for future production and for application of future breeding programs. For example: Demonstration Model –cum- Gene Bank of *Grewia optiva* (Beul) has been established at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2x2m. 'On Field Demonstration Model' –cum- Gene

Bank of *Grewia optiva* (Beul) has been established at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m.

- 3) The cultivation of superior nutritive selection in the cropland situation will reduce the pressure on the forest resources. This will help in the conservation of genetic resources of rare, endemic, threatened, and globally significant flora and fauna species present in the forest area.
- 4) Grewia optiva is a multipurpose species that gives multiple benefits to hill farmers with efficient use of scarce resources of the environment, as it is a good source of fuelwood, fibres, fodder, etc., its wood is also used to make many valuable products. It provides supplementary livelihood options to IHR which also reduces the pressure on the forest flora for fuel wood, fiber extraction etc.
- 5) The leaves of *Grewia optiva* enrich the soil with organic matter. The soil organic matter enhances the fertility and productivity status of the soil, which increase the production of agricultural crops under the canopy of *Grewia optiva*. The improved soil status also helps in the conservation of micro soil flora and fauna.
- 6) The Grewia optiva can grow in rainfed conditions and on the slopy terrain which helps in the conservation of the catchment/watershed areas. It has a deep root system that holds soil particles tightly and prevents soil erosions, reduces the siltation in rivers, and conserves the unique flora and fauna of IHR.
- 7) The bare lands of small and marginal land holding farmers which is not in use for the production of agricultural crops can raise their nursery of multiple nutrient selections and this quality planting material can be used effectively in the rehabilitation of degraded lands through community engagement. Additional carbon sinks will be developed through degraded land rehabilitation for mitigating the effects of global warming.
- 8) The network developed under the project between villagers, the Forest department, and Animal Husbandry department will help in the conservation of rare flora and fauna of the Himalayan regions. The awareness workshops have been organized at panchayat levels with the involvement of SHGs for the production of value-added products from wood, fiber, and leaves of *Grewia optiva*. This approach will enhance the cultivation and conservation of *Grewia optiva* in hilly regions.

#### 6.4 Protection of Environment

- 1) The hill farmers depend on the livestock for their livelihood requirements i.e., meat, wool, milk, etc. but the scarcity of valuable and nutritious fodder in the hilly regions increases the dependence of hill people on the wildlife. The availability of superior nutritive selections will reduce the gap between demand and supply of the quality fodders in cropland situations and reduce the human-wildlife conflict. The cultivation of superior selections on the cropland situation will also reduce the competition between livestock and wild ungulate/ herbivore species for grazing.
- 2) Grewia-based agroforestry systems in the western Himalayas have the potential to improve the soil quality which can lead to sustainable production with optimum utilization of resources. As the Increasing anthropogenic pressure is leading to widespread resource degradation in the fragile western Himalayas. A more holistic approach is therefore required for sustainable production and protecting the natural base from further degradation. Therefore, the use of Grewia optiva as an agroforestry species for land-use practices will optimize the use of environmental resources as well as economic benefits. This species uses nutrients and water from lower soil depths that shallow plant roots cannot access. It checks soil erosion and runoff, maintains soil organic matter, improves soil physical properties, minimizes nutrient loss, promotes efficient nutrient cycling, sequesters carbon, and provides numerous ecosystem services. These grewia-based agroforestry systems are more sustainable than single agriculture system.
- 3) In Himalayan states, this species can be grown for checking soil erosion since it has a very deep taproot system which provides it support and hence it checks the soil. It can be easily seen on 90-degree slopes where most of the trees don't grow.
- 4) The farmers can make eco-friendly value-added products from this species e.g., ropes, handloom items, baskets, etc., and can start their own small venture and can control and prevent pollution in the region.
- 5) This provides greenery and green fodder in the hilly areas during the winter season when no green fodder is available.
- 6) The leaves of *Grewia optiva* can be used to make biofertilizers. The Beul leaves are proteinaceous and good for mulching of several vegetable crops. A large quantity of Organic matter adds to the soil due to the litter (leaves) which falls in the winter season. This improves the soil fertility status and decreases the dependence on fertilizers. The reduction in the use of chemical fertilizer protects our environment.
- 7) Grewia optiva is a multipurpose species that enhances supplementary and/or alternative livelihood options (for fuelwood, fiber, timber, etc.) and the overall economic well-being of the region. The use of this species reduces pressure on forest areas for livelihood resources. The hill farmers are highly dependent on the forests for fodder and manure prepared from forest leaf litter and livestock excreta. Forests not only provide ecosystem

- goods and services but also provide a sink for atmospheric CO<sub>2</sub>, which is on the rise due to various anthropogenic activities.
- 8) The cultivation of *Grewia optiva* increases the organic matter content of the soil and increases the porosity of soil which helps in decreasing runoff and increasing ground water recharge.
- 9) The cultivation of *Grewia optiva* will help in the improvement of the microclimate and bioclimate of the hilly regions.

### 6.5 Developing Mountain Infrastructures

- 1) The leaves are rated as good fodder and trees are heavily lopped for this purpose in the winter months when usually no other green fodder is available. The green leaves constitute about 70 % of the total green weight of branches. Leaf fodder yield is reported to be 11 ton/ha from 2-year-old plants, green fodder yield from mature trees is reported to be 12-30 kg. Leaves are fairly rich in protein and other nutrients and do not contain tannins. Crude protein is highest in young leaves and in winter leaves but decreases during the rainy season. The species provides protein rich green fodder during the winter season when no other green fodder is available. Its protein content is very high ranges between 19-22%. Our present study developed highly nutritive fodder selection having more than 24 per cent crude protein. In this regard nineteen fodder selection have been developed and screened under the project.
- 2) The palatability and digestibility of the species is very high. It is highly preferred by the livestock even branches are eaten by the animals.
- 3) Specific gravity is also very high and stem is used for making charcoal.
- 4) Wood is durable and used for making agriculture implement like handles of spade, axes and pickaxe etc. The wood, weighing 801 kg/cu. m, is whitish with little reddish-brown heartwood. It is fine textured with distinct growth rings. It is hard, tough with good elasticity and strength properties. It becomes difficult to work by hand after seasoning. The timber is used for oar shafts, poles, frames, tool handles and other purposes where strength and elasticity are required. It is thought to be suitable for paper production and branches are used for making baskets.
- 5) Lopped one year old shoot when placed in running or shallow stream is used for the extraction of fibre which is used for cordage and clothing.
- 6) Fibre is used for making ropes for tying animals, nivar for cots, small baskets, bags and decorative materials etc.
- 7) Debarked branches/ twigs of *Grewia optiva* make it an excellent source of torchwood as it catches fire easily during rainy season also.
- 8) Poor ladies in rural areas use mucilage of the bark for washing their hair.
- 9) Boundary or barrier or support: The tree is often planted in hedges and field boundaries.

10) Intercropping: The tree is planted combined with climax grass.

### 6.6 Strengthening Networking in IHR

- We are in contact with 35 Gram panchayat under the project with the participation of Forest department and Animal Husbandry Departments. As this project has been taken with the involvement of State Forest Department, Additional PCCF Research and Training, Sundernagar. The Forest department has the direct linkage with the village farmers, stakeholders. The 15 Forest Divisions viz. Forest Division: Hamirpur, Forest Division: Dehra, Forest Division: Bilaspur, Forest Division: Renuka, Forest Division: Solan, Forest Division: Joginder Nagar, Forest Division: Una, Forest Division: Suket, Forest Division: Karsog, Forest Division: Nachan, Forest Division: Rajgarh, Forest Division: Nahan, Forest Division: Kunihar, Forest Division: Nalagarh, Forest Division: Mandi were contacted and involved to procure the superior planting material from this department and to coordinate activities at the field levels in low and mid zone of Himachal Pradesh.
- 2) We are making all possible collaborative efforts with the involvement of the animal husbandry, forest department. These are the departments that have a direct link with the village panchayat and farmers. Therefore, with the collaborative efforts of different departments, we have organized Mass media lectures, workshops for imparting practical training regarding the cultivation of superior selections.
- 3) Under the project, we have supplied quality planting material of superior nutritive selection of Clonal Seed Orchard to 15 Forest Divisions (Hamirpur, Dehra, Bilaspur, Renuka, Solan, Joginder Nagar, Una, Suket, Karsog, Nachan, Rajgarh, Nahan, Kunihar, Nalagarh, Mandi) during the year 2020 and 2021, for conservation and establishment of gene bank in each selected and identified forest division through SFD, for demonstration/ field testing trial/ block plantation. Conserved planting material in the gene bank can be further mass multiplied and supplied to other forest divisions, this will strengthen our network.
- 4) The quality planting material has also been supplied to the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc. these farmers can also supply superior planting material to another panchayat areas through cuttings and can strong their network with other farmers.
- 5) More additional selections can be made with the involvement of local people.
- 6) Practical training has been imparted to local people regarding the propagation of this species under nursery conditions. They can raise their nursery and sell them in the market which will increase the market network of hill farmers.
- 7) The methodology and approaches developed under the project can be used by other forest institutions in the Himalayan regions for screening superior nutritive strains in other regions of IHR. This will strengthen our network and the outcome will be enormous.

- 8) Other approaches can be used to strengthen our network in hilly regions:
  - a) Promoting education, awareness and outreach programmes and events,
  - b) Developing an online system for monitoring and dissemination of results of the studies
  - c) Creating a central capacity building/training unit or establishing Centre for Himalayan studies.
  - d) Supporting research programs on *Grewia optiva* in Himalayan Universities
  - e) Organizing seminar, conferences, symposia, colloquium, etc. regarding propagation and value-added products of *Grewia optiva*.
  - f) Promoting regional, national, international and conferences, workshops, etc. regarding the use of *Grewia optiva* in hilly regions.

### 7 EXIT STRATEGY AND SUSTAINABILITY

## 7.1 How effectively the project findings could be utilized for the sustainable development of IHR

The superior genotypes identified and screened under the project are much superior in fodder quality than the already practiced material. We recommend that old material which is existing on the cropland situation on the farmer's field should be gradually replaced with new screened genotypes in phased manner. To popularize the screened genotypes for the improved plants efforts are going on at all levels like state forest Department Himachal Pradesh, State Agriculture Department/Animal Husbandry and Panchayat level and progressive farmers. We are educating the importance of this valuable germplasm to end users (ultimate beneficiary) for farming of these genotypes developed under the project. This activity will continue for the benefits of end users in the interest of the farmers of the hilly state in India in general and Himachal Pradesh (in Particular).

### 7.2 Efficient ways to replicate the outcomes of the project in other parts of IHR

- Total nineteen superior nutritive selections have been screened from this study i.e., 10 superior trees have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Nine (09) best selections/ populations with crude protein more than 24 % have been identified and screened from thirty-five selected populations on the basis of morphological, fodder quality characteristics. These nineteen nutritive selections will be used to raise the demonstration block/Seed Orchard and from where production of genetically improved quality planting material will be raised and can be supplied to Forest divisions, farmers of other parts of IHR.
- > The Forest divisions and institutes of other hilly regions can conserve the quality planting material in Germplasm bank, and Seed Orchards and can establish their own

- Demonstration models. They can mass multiply quality planting material to produce true to type mothers replica.
- ➤ The methodology and approaches developed under the project can be used by forest institutions of Himalayan regions for screening of superior nutritive strains in other regions of IHR.
- Morphological DUS specific descriptors which have developed under the project can be utilized further for the identification of superior nutritive selection in other IHR regions.
- > The database generated can be used as baseline data for screening of superior nutritive selections.
- Mass media lectures, workshops, and field training can be organized to popularise this species, to impart practical training regarding the propagation of *Grewia optiva* through cutting, seed, and grafting, etc.
- Correlation and prediction model developed under the project can be used to set important variable for predicting the growth of different genotypes of *Grewia optiva*.
- For production of quality planting stock, nursery has been raised at Shilli Experimental Farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance/ at nursery stage level. This quality planting material can be supplied for cultivation in other areas of Hilly regions.

### 7.3 Identify other important areas not covered under this study needs further attention

❖ The twenty one new populations (five from Uttarakhand and sixteen from the cropland area of Himachal Pradesh) have been identified, added/selected to extend the research on *Grewia optiva*. The selected sites of Uttarakhand and Himachal Pradesh will be explored for variations and broader base of the germplasm of the species for further establishment of germplasm bank. This study will also help in comparative analysis among and between the selected populations of H.P and Uttarakhand to screen out the best nutritive selections and to find out valuable material for future breeding programmes. We can also develop superior hybrids through hybridization between superior selections of the both the states. We can make cross breedings. The details of important selected sites of Himachal Pradesh and Uttarakhand which have not been covered under this study but need further attention are:

Selected sites	District (State)
1. Harsar Khas	Kangra (H.P.)
2. Sujanpur Tihra	Hamirpur (H.P.)
3.Dramman	Kangra (H.P.)
4.Ghumarwin	Bilaspur (H.P.)

Selected sites	District (State)
5.Daggar	Uttarakhand
6.Kalanjari Devi	Hamirpur (H.P.)
7.Sihunta	Chamba (H.P.)
8.Dodar(Sarkaghat)	Mandi (H.P.)
9.Kuthar	Solan (H.P.)
10. Kutuldi	Chamba block, Tehri Garhwal, U.K.
11.Awah devi	Hamirpur (H.P.)
12.Dharot	Solan(H.P.)
13.Neri	Shimla (H.P.)
14. Basni	Sirmaur (H.P.)
15. Jagdhar	Chamba block, Tehri Garhwal, U.K.
16. Shogi	Shimla (H.P.)
17.Sangrah	Sirmaur(H.P.)
18.Ghanahati	Shimla(H.P.)
19. Garkhal	Solan(H.P.)
20. Ranichauri	Chamba block, Tehri Garhwal, U.K.

### 7.4 Major recommendations for sustaining the outcome of the projects in future

- The 19 Nutritive selections screened under the project should be used to raise the demonstration block/Seed Orchard for production of quality planting material. The establishment of seed orchards will help in the multiplication of superior genotypes in bulk and transferring of genetically improved material to end-users. We recommend that old material which is existing on the cropland situation on the farmer's field should be gradually replaced with new screened genotypes in phased manner. This will be a best approach for sustainable development of social, economic and environment resources of IHR.
- ❖ The morphological DUS specific descriptors developed for elected genotypes under the project can be used for the categorization of superior genotypes in other parts of IHR.
- ❖ The superior seedling raised under the nursery conditions as well as the material that will be produced by mass multiplications from the nineteen nutritive selections can be planted in the other parts of IHR. This can be an efficient way to replicate the outcomes of the project in left over parts of IHR.
- ❖ The twenty new populations (four from Uttarakhand and sixteen from the cropland area of Himachal Pradesh) which have been added/selected to extend the research on *Grewia* optiva should be explored for variations and broader base of the germplasm of the species for further establishment of germplasm bank. This study will also help in comparative analysis among and between the selected populations of H.P and Uttarakhand to screen out the best nutritive selections and to find out valuable material for future breeding programme.

- ❖ The number of Mass media lectures, workshops, and training should be organized to popularise this species in hilly areas and to distribute quality planting material among farmers.
- Quality planting of superior nutritive selection of Clonal Seed Orchard which have been supplied to 15 Forest Divisions for conservation and establishment of gene bank in each selected and identified forest division through SFD, for Demonstration/ Field Testing trial/ block plantations etc. Beside this, quality plants that have been supplied for the farmers/stakeholders, identified through selected Forest Division to end users, farmers, women groups, self-help group etc. should be multiplied and supplied in the remaining unexplored parts of the IHR.
- ❖ Gene banks and its maintenance: Gene bank are actively well-maintained at various locations and utilized for germplasm. This proactive approach guarantees a continuous supply of genetic resources for diverse applications. Keeping up the excellent work in maximizing the benefits of these vital genetic repositories. The importance of maintaining and regularly using field gene banks is that gene banks are invaluable assets to our project, providing a rich source of germplasm for mass multiplication. Their long-term conservation is pivotal for ensuring the sustained success and impact of our project. I am committed to overseeing this aspect and maximizing the benefits of these genetic resources in line with our project's objectives. This perspective ensures that these valuable resources continue to benefit us for years to come.
- ❖ The project's outcomes are paramount for conserving *Grewia optiva* germplasm in the Himalayan region. We safeguard genetic diversity, crucial for species survival, amidst mounting environmental threats. *Grewia optiva*, a linchpin of biodiversity, sustains ecosystems and serves as a resource for local communities. Our focus on producing top-quality plants aids sustainable harvesting and eases pressure on wild populations. Additionally, this project advances scientific knowledge of the species and its habitat. Ultimately, our efforts ensure the long-term preservation of *Grewia optiva* and its multifaceted benefits to both nature and society.
- It's essential to recognize that while we have achieved success within the defined project parameters, we should also remain open to opportunities for broader sustainability beyond the project's boundaries. We will continue to assess and adapt our strategies to ensure longterm impact and success. It emphasizes a gradual, step-by-step approach instead of a sudden high-speed shift, ensuring the project's intended goals are met. This measured approach promotes stability and a more strategic achievement of targets.

### REFERENCES/ BIBLIOGRAPHY

The data in the concerned project was collected, analysed and presented in this report. The research papers published from the present three years data which are as under. It is a record of bonofide research work carried out under the adhoc research project entitled "Biological Screening, Conservation and Establishment of Gene Bank of *Grewia optiva* Drummond (Beul)"

### **Research Papers (Published)**

<u>HP Sankhyan</u>, Jyoti Dhiman, Neerja Rana and Krishan Chand (2022). Genetic Variability among different populations of *Grewia optiva* Drummond in low and mid hill zones of Himachal Pradesh. *Indian Journal of Agroforestry* 24 (2): 30-38.

<u>H.P. Sankhyan\*</u>, Jyoti Dhiman, Neerja Rana, Krishan Chand and Prachi (2022) Physiochemical characteristics of the Soil and their Correlation with Leaf Fodder Quality Parameters of *Grewia optiva* Drummond of the Himachal Pradesh. *Biological Forum - An International Journal* 14(2a) 1-8

<u>H.P. Sankhyan</u>, Jyoti Dhiman, Krishan Chand, Prachi and Karishma (2021) Physico-Chemical Properties of Soil under *Grewia optiva* (Beul) Populations of Himachal Pradesh. *Indian Journal of Forestry.* 44 (1)193-200

<u>H.P. Sankhyan,</u> Jyoti Dhiman, Krishan Chand, Prachi, Karishma and Bhupender Negi (2021) Soil Nutrient Analysis Underneath (*Grewia optiva*) Population. *International Journal of Bioresource and Stress Management.* 12(6):645-654.

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<u>Dr. HP Sankhyan</u>, Dr. Sanjeev Thakur, Sunil Kumar, Karishma and Prachi (2021). Analysis of qualitative characteristics among and between populations of *Grewia optiva* Drummond (Beul). *International Journal of Fauna and Biological Studies*. 8(1): 76-82

<u>HP Sankhyan.</u>, Sanjeev Thakur, Sunil Kumar, Karishma and Prachi (2020). Variation in morphological descriptors among different populations of *Grewia optiva* Drummond (Beul) in Himachal Pradesh. *Journal of Pharmacognosy and Phytochemistry*. 9(5):249-254

Let's focus on enhancing the rigor and depth of our work to ensure that our future research papers meet the highest standards. Quality is a top priority, and by refining our research methods, data analysis, and reviewing processes, we can achieve better outcomes. Your dedication to continuous improvement is valuable, and together, we'll work on producing high-quality research that makes a significant impact. To enhance research quality as it takes time to publish a paper in high NAAS rating above 6, we are focusing on rigorous methodology, comprehensive literature reviews, robust peer review, and fostering interdisciplinary collaborations. As we will duly acknowledge the fund support of NMHS and further collaboration so that this will ensure our research as well-founded, innovative and impactful for the coming future. Moreover good quality research papers are being worked out as and when there will be published in good NAAS rated reputed journals, duly acknowledging funding support of NMHS, will also be communicated and intimated to NMHS after publication

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# APPENDIX-I To APPENDIX-VII

### **APPENDICES**

### **Appendix 1 – Details of Technical Activities**

- Established Demonstration Model -cum- Gene Bank of *Grewia optiva* (Beul) at Naganji Farm with total 165 genotypes belonging to 55 families, (planted in 3 replication) at a spacing of 2mx2m.
- Establishment 'On Field Demonstration Model' -cum- Gene Bank of Grewia optiva (Beul) at Khaltoo Farm with total 60 genotypes belonging to 20 families, (planted in 3 replications) at spacing of 2x2m
- The additional germplasm has been selected/ introduced from Chamba block of Tehri Garhwal, Uttarakhand and other cropland sources of Himachal Pradesh to find out another morpho variants and superior nutritive strains of *Grewia optiva*.

### Data and Brief outcome from Evaluation of populations of *Grewia optiva* Drummond project

Dist.	Population	Population	LDM(%)	CP(%)	CF(%)	TA(%)	EE(%)	NFE(%)
		Name						
		77 .1 . 1	10.16	24.42	10.54	10.76	4.05	20.50
S	1	Kothi kanwal	42.46	24.43	19.54	12.76	4.35	39.59
SOLAN	2	Unchagaon	46.25	24.96	21.05	12.02	4.12	40.23
À	3	Nerikalan	31.59	25.12	20.19	13.02	4.38	41.98
	4	Gaddo	43.79	21.98	19.98	12.13	5.03	39.98
	5	Devera	33.25	20.12	21.5	13.68	4.34	40.14
	Mean		39.47	23.32	20.45	12.72	4.44	40.38
SI	6	Machair	50.91	25.02	19.66	12.94	4.62	42.54
RN	7	Jajjar	47.35	24.56	20.32	12.68	5.12	41.98
SIRMAUR	8	Neharbag	41.94	20.43	21.51	11.87	4.69	42.05
JR	9	Badon	34.85	21.22	20.49	11.64	5.33	43.31
	10	Dharkyari	40.48	20.19	21.03	12.06	4.35	40.67
	Mean		43.11	22.28	20.60	12.23	4.82	42.11
U	11	Kant	29.56	19.23	20.05	12.42	4.28	42.7
UNA	12	Navami	32.25	19.11	20.45	12.03	4.98	39.89
	13	Kharunibangana	35.98	20.17	20.51	11.93	5.21	42.85
	14	Thanakalan	37.19	19.89	20.33	12.54	4.86	40.88
	15	Lamlehri	36.99	20.33	20.76	12.31	4.53	41.14
	Mean		34.39	19.74	20.42	12.24	4.77	41.49
K	16	Katoi	40.165	20.15	21.21	12.11	4.21	43.25
AN	17	Balugloa	40.05	22.34	21.43	19.87	4.38	42.34
KANGRA	18	Purana kangra	33.86	23.43	20.54	12.64	5.22	41.33
$\triangleright$	19	Dohan	37.59	22.65	21.15	13.81	5.11	42.24
	20	Balla	49.01	25.05	19.87	12.08	4.98	40.98
	Mean	L	40.13	22.72	20.84	14.10	4.78	42.02
H	21	Janhen	42.23	23.92	21.02	11.55	5.18	42.39
AM	22	Jhinkari	46.16	24.98	20.43	11.76	5.24	39.68
HAMIRPUR	23	Harbalneri	33.65	23.69	20.52	12.12	5.21	42.17
UR	24	Anu khurd	40.46	20.94	19.56	12.04	5.27	40.21
	25 Bhaleth		42.76	25.11	20.03	12.23	5.22	42.43
	Mean		41.05	23.72	20.31	11.94	5.22	41.37
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Dist.	Population	Population	LDM(%)	CP(%)	CF(%)	TA(%)	EE(%)	NFE(%)
		Name						
Z	26	Patta	35.52	19.34	21.22	12.61	5.36	42.17
MANDI	27	Gangal	34.96	19.73	20.27	11.98	4.98	40.19
DI	28	Bagla	35.90	20.22	19.12	11.87	5.44	42.23
	29	Balt	32.47	20.52	20.12	12.24	5.43	40.56
	30	Bharnoi	31.89	19.68	20.32	12.64	4.98	43.22
	Mean		34.15	19.89	20.21	12.26	5.23	41.67
В	31	Ghumarwin	36.67	23.93	19.21	11.43	4.78	41.45
ILA	32	Barthi	42.12	25.15	19.97	13.12	5.33	44.21
BILASPUR	33	Kuthera	39.28	20.98	21.34	12.97	5.67	39.87
UR	34	Jukhala	32.36	23.42	20.17	11.98	4.98	39.67
	35	Nehari	37.65	22.72	20.12	12.45	5.61	40.34
	Mean		37.62	23.24	20.16	12.39	5.27	41.10
	SEM		0.843*	0.464*	0.414*	0.264*	0.104*	0.913*

- The study on leaf fodder quality parameters (proximate principles) *viz.*, Leaf fresh weight (g), Leaf dry weight (g), Leaf dry matter (%), Ether extract (%), Crude protein (%), Crude fibre (%), Total ash (%), Nitrogen free extract (%) of thirty-five selected populations has been completed.
- The fodder quality parameters (leaf fresh weight, leaf dry weight, leaf dry matter, ether extract, crude protein, crude fibre, total ash, nitrogen free extract) have been recorded for all the selected thirty-five populations and their statistical analysis has been completed. The Nine (09) nutritive selections/populations *viz.*, Kothi Kanwal (Solan), Unchagaon (Solan), Nerikalan (Solan), Machair (Sirmour), Jajjer (Sirmour), Balla (Kangra), Jhinjkari (Hamirpur) and Barthi population of Bilaspur district have been identified and screened with crude protein percentage more than 24 percent from thirty-five selected and identified populations of *Grewia optiva* Drummond.
- A nursery has been raised at Shilli farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the selected germplasm have been developed at population level as well as progeny performance / at nursery stage level.







❖ Morphological descriptors of 35 populations of seven districts of Himachal Pradesh have been developed, and 210 superior plants have been identified and screened biologically. Morphological data of 35 populations, which have been recorded and completed on cropland situation, have

been analysed statistically. Data base generated on 210 genotypes as Example/ Reference Varieties under the project from 35 populations, besides data generated from 23 families in Clonal Seed Orchard.

❖ Ten (10) best families and superior trees namely; CH-3, MA-3,SI-15,SO-3,HA-2,CH-1,SO-10,HA-4,SI-16 and SI-14 have been identified and screened from Clonal Seed Orchard on the basis of morphological, molecular characterization and progeny performance in field experimental/ trial studies. The Morphological descriptors have been prepared for these 10 best screened families.

**Best 10 Families for Fodder** 

CODE		DISTRICT	LOCATION
1.	CH-3	Chamba	Balu
2.	MA-3	Mandi	Bambla
3.	SI-15	Sirmour	Madhobag
4.	SO-3	Solan	Dharja
5.	HA-2	Hamirpur	Patta Balakhar
6.	CH-1	Chamba	Chanad
7.	So-10	Solan	Jaunaji
8.	HA-4	Hamirpur	Kanal
9.	SI-16	Sirmour	Nainatikkar
10.	SI-14	Sirmour	Saraha Chakli

















- ❖ For production of quality planting stock, nursery has been raised at Shilly farm of the department from the seeds collected from superior families and of superior populations under different ecological niches in different districts of Himachal Pradesh and DUS specific descriptors of the progeny performance / at nursery stage level.
- ❖ This species is difficult to raise through cutting but raising planting material through cuttings has been standardized to develop with hormonal treatment (IBA 500 mg/l) auxin concentration) for true to type (mothers' replica) material under this project.





❖ Till date, the total production of quality planting material in nursery is about 55,000 plants/ saplings, out of which 34,200 plants have been distributed for research purpose and to farmers, stakeholders, self help groups through fifteen Forest Divisions of Himachal Pradesh. At present about more than 20,000 plants/ saplings are still available with the Department which have been screened out under the project.











The additional germplasm was selected/ introduced from Chamba block of Tehri Garhwal, Uttarakhand and other cropland sources of Himachal Pradesh to find out another morpho variants and superior nutritive strains of *Grewia optiva*.

A survey has been undertaken during the month of March — April working on this species *Grewia optiva* in four altitudinal zones viz; 400 to 800 m, 801-1200 m, 1201-1600 m and 1601-2000 m above mean sea level (a m s l), of Himachal Pradesh (HP) and Uttarakhand (UK), keeping in view the rich genetic diversity of *Grewia optiva* populations. Total twenty populations were identified, marked and selected (five in each altitudinal zones; 4 from HP and 1 from UK). Therefore, sixteen populations from eight districts of HP viz., Chamba, Kangra, Hamirpur, Bilaspur, Mandi, Solan, Sirmaur and Shimla and four populations from Daggar and Chamba block of Tehri Garhwal Uttarakhand (UK) has been taken to make comparative analysis of superior nutritive selection of UK with the state of HP which represents the major share of population under study. Total 80 morphologically best genotypes of 20-30 cm diameter class (four in each population) were marked accordingly for the study of variation in morphological, fodder characteristics between and among the selected populations and screening of the best nutritive strains. The plant height (m), basal diameter (cm), internodal length, no. of primary branches were recorded simultaneously for each selected genotype.

Details of population(additional germplasm) has been added from Himachal Pradesh and Uttarakhand.

<b>Population Name</b>	Altitudinal range	District				
-	(above mean sea level)					
	I. Altitudinal range 400m-800 m amsl					
1.Harsar Khas	482m	Kangra (H.P.)				
2.Sujanpur Tihra	515m	Hamirpur (H.P.)				
3. Dramman	740m	Kangra (H.P.)				
4. Ghumarwin	700m	Bilaspur (H.P.)				
5.Daggar	770m	Uttarakhand (U.K.)				
	II. Altitudinal range 800m	-1200 m amsl				
6. Kalanjari Devi	854 m	Hamirpur (H.P.)				
7. Sihunta	900 m	Chamba (H.P.)				
8. Dodar(Sarkaghat)	982m	Mandi (H.P.)				
9. Kuthar	1127m	Solan (H.P.)				
10. Kutuldi	1136m	Chamba block, Tehri Garhwal, U.K.				
	III. Altitudinal range 1200m-16	600 m amsl				
11. Awah devi	1219m	Hamirpur (H.P.)				
12. Dharot	1500m	Solan(H.P.)				
13. Neri	1520m	Shimla (H.P.)				
14. Basni	1552m	Sirmaur (H.P.)				
15. Jagdhar	1585m	Chamba block, Tehri Garhwal, U.K.				
	IV. Altitudinal range 1600m-20	000 m amsl				
16. Shogi	1712m	Shimla (H.P.)				
17. Sangrah	1830m	Sirmaur(H.P.)				
18. Ghanahati	1900m	Shimla(H.P.)				
19. Garkhal	1800m	Solan(H.P.)				
20. Ranichauri	1730m	Chamba block, Tehri Garhwal, U.K.				







Seeds were collected during the months of November and December from twenty selected populations of HP and UK. The data for fruit characteristics viz., fruit length, fruit breadth, and thickness was recorded simultaneously. The fruit length was recorded between the range of 7.28-13.18mm, the fruit breadth ranged between 6.23-16.08mm and thickness ranged between 6.75mm-13.06mm. Before storage, the fruit pulp was removed by giving slight hot water treatment The seed characteristics viz., seed length (5.66-7.05 mm) and seed breadth (3.88-6.20mm) were also recorded with the help of digital vernier caliper.













The seeds were sown during the month of April-May in the seed beds at Shilli nursery Solan (H.P.). The seeds of each 80 genotype were sown in three replications and in each replication 10 seedlings were taken for recording observations on nursery related parameters. The nursery performance of seedlings was evaluated for nine months. The germination percentage observed was maximum in genotypes from Solan and Chamba (90-95%) followed by Hamirpur and Bilaspur (75-90%) whereas it was minimum in genotypes collected from Uttarakhand district (50-70%). Similar trend was followed by other germination parameter such as germination value and germination index. 100 per cent survival was observed after 1 month among the germinated seeds.

Morphological data on parameters *viz.*, seedling height (cm), collar diameter (mm), petiole length (cm), internodal length (cm), branch angle (°) and leaf area (cm²) was observed and noted on monthly basis.











Nursery seedlings are evaluated for morphological parameters (mentioned above) on monthly intervals. The genotypes will be assessed after one-year monthly data from June 2021 to July 2022. The results from the latest data collected shows that genotypes from Solan, Sirmaur, Hamirpur, Bilaspur, Kangra and Chamba are performing better in overall growth whereas, genotypes from Shimla, Mandi and Uttarakhand have least values for growth parameters. Collar diameter ranged from 0.2-0.9 mm, minimum range was observed for genotypes from Mandi and Uttarakhand. Internodal length ranged from 1 - 4cm the maximum range observed in genotypes collected from Chamba district and minimum for genotypes collected from Ranichauri (UK). Number of branches observed are ranging between 3-14. Branch angle ranged from 10-55° for all the genotypes.

In the months of November- December 2021, leaf samples were collected to record leaf parameters from twenty selected population. Leaf area was recorded in the range from 45.99 to 102.21 cm². The Minimum leaf area was recorded in genotypes collected from Ranichauri (Uttarakhand), Jagdhar (Uttarakhand), Mandi and the maximum was recorded in samples collected from Daggar (Uttarakhand), Chamba and Solan. Leaf length was recorded in range between 7.12 to 17.78cm; the maximum was recorded in samples from Daggar (UK), Kutuldi (UK) and Chamba (HP) while the minimum in samples from Mandi (HP) and Ranichauri (UK). Leaf width observed ranges between 3.2to9cm, the minimum was recorded in samples from Daggar (UK), Jagdhar (UK) and maximum in samples from Kangra (HP) and Kutuldi (UK). The fresh weight of 100 leaves recorded in the range of 57.89-129.45 g, the maximum was recorded for genotypes from Kutuldi (UK) and Chamba and the minimum was recorded for genotypes from Mandi district. The dry

weight of 100 leaves recorded in range of 35.42-64.66g, maximum reported for Jagdhar (UK) and Bilsapur genotypes and minimum for genotypes from Mandi and Ranichauri (UK).











Table: Physicochemical characteristics of soil samples collected underneath thirty-five different populations of *Grewia optiva* Drummond.

Dist.	Population	Population	pН	EC	Organic	N	P	K	Soil Texture	Bulk
		Name		(dSm <sup>-1</sup> )	Carbon	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )		Density
					$(g kg^{-1})$					
	1	Kothi kanwal	7.32	0.25	7.40	295.83	14.93	240.05	Sandy Loam	1.14
SOLAN	2	Unchagaon	7.54	0.19	10.70	343.92	30.61	282.67	Clay loam	1.13
$\mathbb{Z}$	3	Nerikalan	7.66	0.17	7.30	327.19	33.60	489.81	Clay loam	0.99
2	4	Gaddo	6.70	0.33	6.60	278.06	23.89	360.88	Sandy Loam	1.17
	5	Devera	7.02	0.30	3.50	270.79	17.92	212.80	Clay loam	1.16
	Mean		7.24	0.24	7.1	303.15.	24.19	337.24		1.11
S	6	Machair	6.76	0.36	14.65	378.45	57.12	439.28	Silt Clay	1.70
R									Loam	
SIRMAUR	7	Jajjar	7.09	0.48	16.85	357.43	33.06	376.81	Silt Loam	1.24
F	8	Neharbag	7.52	0.54	19.05	379.51	49.40	512.46	Loam	1.39
		Badon	6.98	0.47	12.6	343.79	63.96	378.60	Sandy Clay	1.08
	9								Loam	
		Dharkyari	6.91	0.26	15.75	368.22	35.68	285.96	Sandy Clay	1.15
	10								Loam	
	Mean		7.05	0.42	15.78	365.48	47.84	398.62		1.31

Dist.	Population	Population	pН	EC	Organic	N	P	K	Soil Texture	Bulk
		Name		(dSm <sup>-1</sup> )	Carbon	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )	(kg ha <sup>-1</sup> )		Density
					$(g kg^{-1})$					
UNA	11	Kant	6.60	0.14	10.02	280.36	25.60	285.38	Gravelly Loam	1.21
	12	Navami	7.80	0.17	11.20	290.60	29.33	349.30	Sandy Clay Loam	1.04
	13	Kharunibangana	6.89	0.14	16.09	360.60	33.78	290.49	Loam	1.08
	14	Thanakalan	7.20	0.19	14.16	320.80	56.89	349.88	Sandy Clay Loam	1.18
	15	Lamlehri	7.22	0.22	10.08	280.18	46.70	477.21	Gravelly Loam	1.16
	Mean	I	7.14	0.11	12.31	307.60	38.47	350.45		1.13
KANGRA	16	Katoi	6.65	0.15	9.90	282.24	24.64	283.36	Sandy, Clay, Loam	1.21
Q	17	Balugloa	7.65	0.16	11.25	291.64	29.12	348.32	Loam	1.08
$\approx$	18	Purana kangra	6.71	0.10	10.05	363.77	33.60	284.48	Loam	1.15
	19	Dohan	6.01	0.11	14.10	319.87	53.76	349.44	Gravelly Loam	1.13
	20	Balla	7.21	0.19	7.05	272.83	44.80	577.92	Sandy Loam	1.22
	Mean		6.84	0.14	10.47	306.07	37.18	368.70		1.15
HAMIRPUR	21	Janhen	7.30	0.47	17.21	370.50	43.60	240.43	Sandy Clay Loam	1.46
)R	22	Jhinkari	7.11	0.60	20.82	380.31	40.42	260.30	Sandy Loam	1.13
PUF	23	Harbalneri	7.10	1.15	27.25	372.13	35.46	272.10	Sandy Loam	1.11
~	24	Anu khurd	7.20	0.82	25.06	344.91	37.90	212.66	Gravelly Loam	1.19
	25	Bhaleth	7.13	1.32	26.08	380.33	34.70	161.30	Clay loam	1.14
	Mean		7.16	0.85	23.28	369.63	38.35	229.35		1.14
7	26	Patta	5.70	0.18	2.70	231.00	37.50	316.00	Clay Loam	1.15
MAN	27	Gangal	5.40	0.21	1.01	132.00	35.50	480.00	Loam	1.17
DI	28	Bagla	6.30	0.12	1.48	115.00	18.80	182.00	Sandy Loam	1.38
	29	Balt	5.10	0.15	1.10	134.00	35.80	258.00	Sandy clay Loam	1.01
	30	Bharnoi	6.30	0.18	2.63	136.00	18.80	414.00	Sandy clay Loam	1.30
	Mean		5.76	0.16	1.78	149.5	29.28	330.00		1.20
ВІ	31	Ghumarwin	7.38	0.33	22.10	362.70	42.00	246.70	Clay Loam	1.33
LA	32	Barthi	7.68	0.25	27.50	419.20	45.62	328.80	Loam	1.20
BILASPUR	33	Kuthera	5.92	0.12	15.20	378.32	45.20	213.40	Sandy Loam	1.18
R	34	Jukhala	7.26	0.81	17.21	364.40	37.20	297.80	Clay Loam	1.08
	35	Nehari	7.40	0.46	27.12	386.30	38.10	279.84	Sandy Loam	1.22
	Mean		7.12	0.39	21.82	382.18	41.62	267.30		1.18
	SEM	level of significance	0.09	0.08	0.76	11.84	2.31	7.72		0.02

[Significant at 1% level of significance, where; EC- electrical conductivity, OC-organic carbon, BD-bulk density, N-nitrogen, P-phosphorus, K-potassium]

The analysis on physicochemical properties viz. pH, OC, EC, bulk density and in macronutrients (N, P, K) of 35 soil samples collected underneath *Grewia optiva* population have been completed. There was a significant difference observed in pH, OC, EC, bulk density and in macronutrients (N,

P, K) within selected population of each district. The lowest pH of 5.1 was reported beneath Balt population of Mandi district, whereas the highest beneath Nawami population of Una district. In this study pH range of moderately acidic to moderately alkaline reported best for the growth of this species. The highest EC 1.32 (dSm<sup>-1</sup>) was reported beneath Bhaleth population of Hamirpur district, whereas minimum 0.10 (dSm<sup>-1</sup>) beneath old Kangra population. EC values affects uptake of nutrients by the tree. The highest organic carbon recorded in soil of Barthin population of Bilaspur district, whereas lowest found in Gangal Population of Mandi district. The highest Nitrogen recorded in soil of Barthin Population of Bilaspur district, whereas lowest found underneath Bagla Population of Mandi district. The highest available phosphorus recorded in soil of Badon population of Sirmour district, whereas the lowest for Bharnoi population of Mandi district. The highest available Potassium was recorded for Balla population of Kangra district, whereas lowest Bharnoi population of Mandi district. Soils of different textures were founded underneath populations of different districts viz; 50% of soil texture observed as sandy loamy and sandy clay loamy followed by clay loamy (20%), gravelly loamy (10%), loamy (10%) and remaining silty clay loamy (5%) and silty loam (5%). The highest BD was recorded w.r.t. the Machair population of Sirmour district whereas the lowest in the soil of Nerikalan population of Mandi district

A survey was under taken during the month of March – April in four altitudinal zones *viz*, 400 to 800 m, 801-1200 m, 1201-1600 m and 1601-2000 m above mean sea level (a m s l), of Himachal Pradesh (HP) and Uttarakhand (UK), keeping in view the rich genetic diversity of *Grewia optiva* populations. Total twenty populations were identified, marked and selected (five each in altitudinal zones; 4 from HP and 1 from UK). Therefore, sixteen populations from eight districts of HP *viz.*, Chamba, Kangra, Hamirpur, Bilaspur, Mandi, Solan, Sirmaur and Shimla and four populations from Daggar and Chamba block of Tehri Garhwal Uttarakhand (UK) were taken to make comparative analysis of superior nutritive selection of UK with the state of HP which represents the major share of population under study. In total, 80 morphologically best genotypes of 20-30 cm diameter class (four in each population)were marked accordingly for the study of variation in morphological, fodder characteristics between and among the selected populations and screening of the best nutritive strains. The plant height (m), basal diameter (cm), internodal length, no. of primary branches were recorded simultaneously for each selected genotype.

Details of selected populations of Himachal Pradesh and Uttarakhand.

Site name	Altitudinal range (above mean sea level)	District			
I. Altitudinal range 400m-800 m amsl					
Harsar Khas	482m	Kangra (H.P.)			
SujanpurTihra	515m	Hamirpur (H.P.)			
Dramman	740m	Kangra (H.P.)			
Ghumarwin	700m	Bilaspur (H.P.)			
Daggar	770m	Uttarakhand (U.K.)			
		!			

Site name	Altitudinal range (above mean sea level)	District			
II. Altitudinal range 800m-1200 m amsl					
Kalanjari Devi	854 m	Hamirpur (H.P.)			
Sihunta	900 m	Chamba (H.P.)			
Dodar(Sarkaghat)	982m	Mandi (H.P.)			
Kuthar	1127m	Solan (H.P.)			
Kutuldi	1136m	Chamba block, Tehri Garhwal, U.K.			
III. Alt	itudinal range 1200m-1600 m am	nsl			
Awah devi	1219m	Hamirpur (H.P.)			
Dharot	1500m	Solan(H.P.)			
Neri	1520m	Shimla (H.P.)			
Basni	1552m	Sirmaur (H.P.)			
Jagdhar	1585m	Chamba block, Tehri Garhwal, U.K.			
IV. Alti	tudinal range 1600m-2000 m an	nsl			
Shogi	1712m	Shimla (H.P.)			
Sangrah	1830m	Sirmaur(H.P.)			
Ghanahati	1900m	Shimla(H.P.)			
Garkhal	1800m	Solan(H.P.)			
Ranichauri	1730m	Chamba block, Tehri Garhwal, U.K.			









Seeds were collected during the month of November and December from twenty selected populations of HP and UK. The data for fruit characteristics viz., fruit length, fruit breadth, and thickness were recorded simultaneously. The fruit length between the range of 7.28-13.18mm, fruit breadth ranged between 6.23-16.08mm and thickness ranged 6.75mm-13.06mm. Before storage fruit pulp was removed by giving slight hot water treatment The seed characteristics viz.,

seed length (5.66-7.05 mm) and seed breadth (3.88-6.20mm)were also recorded with the help of digital vernier caliper.

The seeds were sown during the month of April-May 2021, in the seed beds at Shilly nursery Solan (H.P.). The seed of each 80 populations were sown in three replications and in each replication 10 seedlings were taken for recording observations on nursery related parameters. The nursery performance of seedlings was evaluated for nine months. Germination percentage observed was maximum in genotypes from Solan and Chamba (90-95 %) followed by Hamirpur and Bilaspur (75-90 %) whereas it was minimum in genotypes collected from Uttarakhand district (50-70%). Same trend was followed by other germination parameter such as germination value and germination index. 100 per cent survival was observed after 1 month.

Morphological data on parameters *viz.*, seedling height (cm), collar diameter (mm), petiole length (cm), internodal length (cm), branch angle (°) and leaf area (cm²) were being observed and noted on monthly basis.

Nursery seedlings are being evaluated for morphological parameters (mentioned above) on monthly intervals. Till date, data pertaining to 9 months has been noted. The genotypes will be assessed based on one-year monthly data from June 2021 to July 2022. Results from the latest data shows that genotypes from Solan, Sirmaur, Hamirpur, Bilaspur, Kangraand Chamba performed best in overall growth whereas the genotypes from Shimla, Mandi and Uttarakhand has least values for growth parameters. Collar diameter ranged from 0.2-0.9 mm, minimum range was observed for genotypes from Mandi and Uttarakhand. Internodal length ranged from 1 - 4cm the maximum range observed in genotypes collected from Chamba district and minimum for genotypes collected from Ranichauri (UK). Number of branches observed in range between 3-14. Branch angle ranged from 10-55° for all the genotypes.

During the months of November- December 2021, leaf samples were collected to record leaf parameters from twenty selected population. Leaf area was recorded in the range between 45.99-102.21 cm². The minimum leaf area recorded in genotypes collected from Ranichauri (Uttarakhand), Jagdhar (Uttarakhand), Mandi and maximum recorded in samples collected from Daggar (Uttarakhand), Chamba and Solan. Leaf length recorded in range between 7.12-17.78 cm, the maximum was recorded in the samples from Daggar (UK), Kutuldi (UK) and Chamba and minimum in samples from Mandi and Ranichauri (UK). Leaf width is ranged between 3.2-9cm, the minimum was recorded in samples from Daggar (UK), Jagdhar (UK) and the maximum in samples from Kangra and Kutuldi (UK). The fresh weight of 100 leaves was recorded in range of 57.89-129.45 g, the maximum was recorded for genotypes from Kutuldi (UK) and Chamba and minimum recorded for genotypes from Mandi district. The dry weight of 100 leaves recorded in the range of 35.42-64.66g, maximum reported for Jagdhar (UK) and Bilsapur genotypes and minimum for genotypes from Mandi and Ranichauri (UK).

## Appendix 2 – Copies of Publications duly Acknowledging the Grant/ Fund Support of NMHS Please See Enclosure –XVII

### **Research Papers (Published)**

- 1. <u>H.P. Sankhyan\*</u>, Jyoti Dhiman, Neerja Rana, Krishan Chand and Prachi (2022) Physiochemical characteristics of the Soil and their Correlation with Leaf Fodder Quality Parameters of *Grewia optiva* Drummond of the Himachal Pradesh. *Biological Forum An International Journal* 14(2a) 1-8
- H.P. Sankhyan, Jyoti Dhiman, Krishan Chand, Prachi and Karishma (2021) Physico-Chemical Properties of Soil under *Grewia optiva* (Beul) Populations of Himachal Pradesh. *Indian Journal of Forestry*. 44 (1)193-200
- 3. <u>H.P. Sankhyan</u>, Jyoti Dhiman, Krishan Chand, Prachi, Karishma and Bhupender Negi (2021) Soil Nutrient Analysis Underneath (*Grewia optiva*) Population. *International Journal of Bio-resource and Stress Management.* 12(6):645-654.
- 4. <u>Hari Paul Sankhyan</u>, Jyoti Dhiman, Prachi and Shanti Swarup Sharma (2021). Seed sources variation in growth traits of *Grewia optiva* (Beul). *Indian Journal of Forestry*. 44 (4), 148-154
- 5. <u>HP Sankhyan</u>, Sanjeev Thakur, Sunil Kumar, Karishma, Prachi and Krishan Chand (2021). Morphological characteristics among different populations for screening of beul trees (*Grewia optiva*) Drummond. *International Journal of Chemical Studies*. 8(4):1845-1848.
- 6. <u>Dr. HP Sankhyan</u>, Dr. Sanjeev Thakur, Sunil Kumar, Karishma and Prachi (2021). Analysis of qualitative characteristics among and between populations of *Grewia optiva* Drummond (Beul). *International Journal of Fauna and Biological Studies*. 8(1): 76-82
- 7. <u>HP Sankhyan.</u>, Sanjeev Thakur, Sunil Kumar, Karishma and Prachi (2020). Variation in morphological descriptors among different populations of *Grewia optiva* Drummond (Beul) in Himachal Pradesh. *Journal of Pharmacognosy and Phytochemistry*. 9(5):249-254

### **Research Papers Accepted for Publication**

- HP Sankhyan, Jyoti Dhiman, Neerja Rana and Krishan Chand (2022). Genetic Variability among different populations of *Grewia optiva* Drummond in low and mid hill zones of Himachal Pradesh. *Indian Journal of Agroforestry*
- 2. <u>HP Sankhyan,</u> Jyoti Dhiman, Krishan Chand, Shikha Thakur, Prachi, Karishma (2022). Fodder quality evaluation of selected populations of *Grewia optiva* Drummond in Himachal Pradesh. *Chemical Engineering*

### **Published Technical Bulletin in Hindi**

- HP Sankhyan, Jyoti Dhiman, Prachi, Karishma, Bhupinder Negi and Sunil Kumar (2021), Beul ki Vaigyanik Vriksharopan Takneek, Utpadan Evam Mahatav, Tree Improvement and Genetic Resources, Dr YS Parmar University of Horticulture and Forestry, Nauni-Solan (HP) 173 230; ISBN978-81-927930-0-11: P 20
- Hari Paul Sankhyan, Jyoti Dhiman and Shikha Thakur (2022) Beul (*Grewia optiva*) ke Vividh Upyog, Anusandhan evam Samvardhan, Tree Improvement and Genetic Resources, Dr YS Parmar University of Horticulture and Forestry, Nauni-Solan (HP) 173 230 ISBN 978-81-927930-0-12: P 21

### **Research Papers Submitted for Publication**

- 1. HP Sankhyan, Jyoti Dhiman and Krishan Chand (2022). Evaluation of half-sib progenies of *Grewia optiva* Drummond under nursery conditions. *Indian Journal of Genetics and Plant Breeding*
- 2. HP Sankhyan, Jyoti Dhiman and Shikha Thakur (2022). Evaluation of genotypes of *Grewia optiva* Drummond under different tree spacing and estimation of their genetic parameters. *Journal of Mountain Sciences*.

# Research Publication from previous years work on the species by the Principal Investigator of the Project

<u>Sankhyan H.P.</u>, Bhagta Shikha and Thakur Sanjeev. 2019. Evaluation of the half sib progenies to identify prepotency of the mother clones of *Grewia optiva* Drummond. *Journal of Pharmacognosy and Phytochemsitry*. 8(4): 1265-1270

Bhagta Shikha, <u>Sankhyan H.P.</u>, Thakur, Sanjeev Bishist Rohit and Gupta R.K (2019). Evaluation of Seedling Seed Orchard of *Grewia optiva* Drummond for Morphometric and Fodder Quality Parameters. *Journal of Non-Timber of Forest Products* . 26(2) 1-5.

Bhat Sheeraz Saleem, Dand Suheel Ahmad, <u>Sankhyan HP</u>, Mir Nazim Hamid (2019)- *Grewia optiva* Drummod: An autobiography. In Van Sangyan (ISSN 2395 – 468X) Vol. 6. No. 10. Pages: 21-26 (Published by Tropical Forest Research Institute, Jabalpur, MP, India)

Bhagta Shikha, <u>Sankhyan H.P.</u>, Sharma Dushyant and Ashine Tesfaye (2019). Correlation and path coefficient analysis in *Grewia optiva* Drummond. *International Journal of Chemical Studies*. 7 (3):746-749.

Bhagta Shikha, <u>Sankhyan H.P.</u>, Sharma JP and Kumari Reena. (2019). Assessment of Variability in Half Sib Progenies of *Grewia optiva* Drummond for Various Qualitative and quantitative Traits in North Western Himalayas. *International Journal Current Microbiology Applied Sciences*. 8 (4):1661-1669.

## Appendix 3 – List of Trainings/ Workshops/ Seminars with details of trained resources and dissemination material and Proceedings

- Five local level workshops organized during the year 2019-2020 with the staff and representatives of local bodies/local people Enclosure-XIX
- Three practical field tailored training/ camps organized for particularly for women. Enclosure-XX
- One practical field tailored training/ camps organized for Pardhans of different Panchayats,
   Up-pardhans, Nursery Growers and Stack Holders including women, Forest Guards of HFRI.
   Enclosure-XXI
- One more Practical field training organised for the small farmers/ nursery growers of District Solan and Sirmaur for the production of quality planting material of *Grewia optiva* (Drummond) Beul during the Year 2022.

## Appendix 4 – List of New Products (utilizing the local produce like NTFPs, wild edibles, bamboo, etc.)

### Species Identified: 01 (LKTS) Grewia laevigata

Locally called as beuli. It is a lesser known tree species found in district Solan in grass land situation. Efforts are being made to multiply/ propagate this species but 95% seeds of the species were found not viable.

Few plants of *Grewia laevigata* have been found occuring in Kangra district.

This species is difficult to raise through cutting but raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/lit) auxin concentration) for true to type (mothers' replica) material under this project.



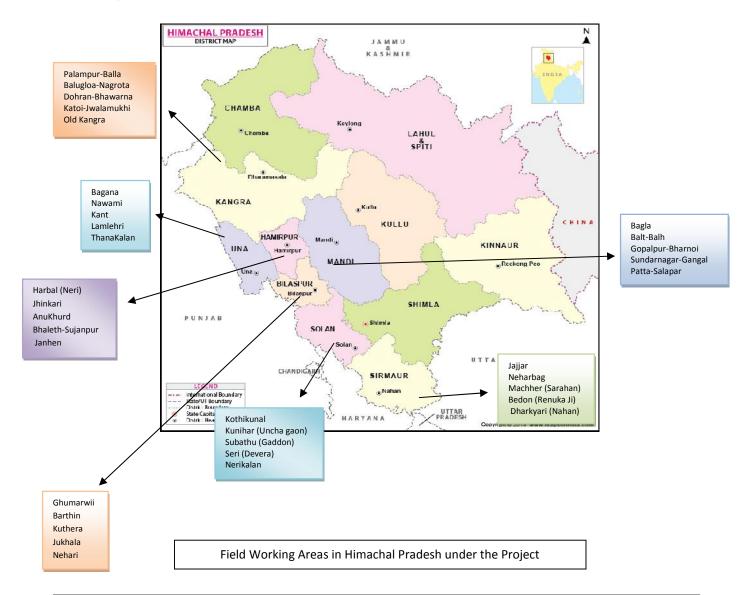




### Appendix 5 – Copies of the Manual of Standard Operating Procedures (SOPs) developed

The study on *Grewia optiva* Drummond (Beul) has been undertaken in low and mid hill zones of H.P., keeping in view the rich genetic diversity of *Grewia optiva* populations for recording morphological observations on plants and within populations. A total of thirty-five populations were identified, marked and selected (five in each district) in seven districts of Himachal Pradesh viz. Solan, Bilaspur, Mandi, Una, Hamirpur, Kangra and Sirmaur and 210 morphologically best genotypes (six in each population) were marked with the aim to ascertain the response of the different parameters towards the screening of the adopted best genotypes and forage productivity.

### Site Map



Districts and their locations of project activities

### Proximate composition (as recommended by AOAC(1995))

S. No	Characters	Methodology adopted as per methods recommended/ suggested by as under			
1.	Leaf fresh weight(g)	Recommended by AOAC(1995)			
2	Leaf dry wieght(g)	Recommended by AOAC(1995)			
3.	Leaf dry matter content(%)	Recommended by AOAC(1995)			
4.	Ether extract(%)	As per procedure outlined by AOAC(1995)and Sankaram(1966)			
5.	Crude fiber(%)	As described by AOAC(1995)and Sankaram(1966)			
6.	Crude protein (%)	Estimated by Microkjedahl method (Sankaram, 1966)			
7.	Total ash(%)	Procedure given by AOAC(1995) and Sankaram(1966)			
8.	Nitrogen free extract(%)	As per method recommended by AOAC(1995) and Sankaram(1966)			

### Mineral nutrients composition (according to methods recommended by AOAC(1995))

S. No.	Characters	Methodology adopted					
1.	Nitrogen(%)	Estimated by Microkjeldahl, AOAC(1995)					
2.	Phosphorus(%)	Recommended by yellow vanadomolybdate (Gupta, 2000)					
3.	Potassium (%)	Recommended by AOAC(1995) / and by dissolving 1.9069g KCL					
4.	Calcium(%)	Estimated by atomic absorption spectrometer(Gupta,2000)					
5.	Magnesium(%)	Recommended by atomic absorption spectrometer(Gupta,2000)					

### **Determination of Soil Characteristics**

Characteristics	Methods	References
pH	Eltop pH meter	AOAC, 1975
Electrical Conductivity (EC)	Conductivity meter method	AOAC, 1975
Organic Carbon (%)	Walkey and Blacks rapid method	Piper, 1966
Available Nitrogen (N)	Alkaline Permanganate method	Subbiah and Asiaja, 1956
Available Phosphorous (P)	Olsen method	Olsen <i>et al</i> ,1954
Available Potassium (K)	Flame photometer method	Merwin and Peach , 1951
Soil texture	Using qualitative method such as texture by feel and quantitative methods such as hydrometer method	Albert Atterberg, 1905
Bulk density	Apparent specific gravity of soil	Singh <i>et. al</i> , 1986

Characteristics	Methods	References
Statistical Analysis ANOVA	Single tree family block design	Panse and Sukhatme, 1967 and Chandel,1984
		Jain, J.P. , 1982
Critical difference (CD)	CD=S.E x t <sub>0.05</sub>	
Correlation coefficient	As per standard statistical method	Karl Pearson correlation coefficient
Variances	Vp=Vg+Me(Phenotypic variance)	
Coefficients of variability	As per standard statistical method	Burton and De Vane, 1953
Heritability	As per standard statistical method	Burton and De Vane, 1953 and Johnson <i>et.</i> <i>al</i> , 1955
Genetic advance	As per standard statistical method	Allard, 1960
Genetic gain	As per standard statistical method	Johnson et. al , 1955
Seed germination traits and important charaterstics of nursery raised seedlings	As per international rules of seed testing,	AOAC, 1995
	TZ test (Bonner 1974)	AOAC,1980
	Czabator method, Djavanshir and Pourbeik method	ISTA, 1966

## <u>Germination and survival percentage and seedling growth studies were obtained using following methods</u>

Characteristics	Methods	References
<ul> <li>Number of days to initial germination</li> <li>Number of days to complete germination</li> <li>Per Cent germination</li> <li>Germination Value(GV)</li> <li>Per cent survival (after 9 months)</li> </ul>	As per international rules of seed testing, TZ test (Bonner 1974) Czabator method, Djavanshir and Pourbeik method	AOAC, 1995 AOAC,1980 ISTA, 1966

### **Seedling Growth Characteristics**

Characteristics	Methods	References
<ul> <li>Seedling Height(cm)</li> <li>Collar diameter(cm)</li> <li>Number of Nodes</li> <li>Internodal length(cm)</li> <li>Number of branches per plant</li> <li>Branch length(cm)</li> <li>Numbers of leaves per plant</li> <li>Leaf shape</li> <li>Leaf length(cm)</li> <li>Leaf breadth(cm)</li> <li>Leaf area(cm²)</li> </ul>	As per international rules of seed testing, TZ test (Bonner 1974) Czabator method, Djavanshir and Pourbeik method	AOAC, 1995 AOAC,1980 ISTA, 1966 Bhat , SS, 2010

Biomass study			
- Leaf biomass per plant(g) at leaf harvesting stage - Root length(cm) - Shoot length(cm)  Poet fresh weight(g)	ethods a per international rules of red testing, TZ test (Bonner 1974) abator method, Djavanshir ad Pourbeik method	References AOAC, 1995 AOAC,1980 ISTA, 1966	

### Appendix 6 – Details of Technology Developed/ Patents filled

Locally called as beuli. It is a lesser known tree species found in district Solan in grass land situation. Efforts are being made to multiply/ propagate this species but 95% seeds of the species were found not viable.

Few plants of *Grewia laevigata* have been found occuring in Kangra district.

- ❖ This species is difficult to raise through cutting but raising planting material through cuttings have been standardized to develop with hormonal treatment (IBA 500 mg/lit) auxin concentration) for true to type (mothers' replica) material under this project.
- ❖ Nursery raising techniques of *Grewia optiva* have been standardized and procedure for seed collection from superior trees and certification has been standardized

### Appendix 7 – Any other (specify)

- Only 19 example varieties/ reference varieties developed and being maintained at Farm of the University, Nauni-Solan
- Clonal Seed Orchard of *Grewia optiva* evaluated and Model plantation of the species at different spacing established under the project.
- Quality planting material produced from the screened genotypes having crude protein more than 24 per cent.

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