

DETAILED PROJECT PROGRESS REPORT

OF

**Collection, evaluation and conservation of native crops
germplasm from Uttarakhand hills and pre-breeding through
community participation**

Funded under

National Mission on Himalayan Studies (NMHS)-Medium Grant



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UTTARAKHAND

NMHS Progress Report
(Period from 01/04/2017 to 31/03/2018)

Project Information

Project ID: NMHS/2015-16/MG02/02	Sanction Date: 31-03-2016
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Project Title:	Collection, evaluation and conservation of native crops germplasm from Uttarakhand hills and pre-breeding through community participation
BTG:	Conservation and Sustainable Use of Biodiversity

PI and Affiliation (Institution):	Dr Anand Singh Jeena Professor, Genetics and Plant Breeding & Nodal Officer, Pantnagar centre for Plant Genetic Resources G.B. Pant Univ. of Agriculture & Technology, Pantnagar-263145 Distt Udham Singh Nagar UTTARAKHAND
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Project Partner Name	Affiliations	Role & Responsibilities
Partner 1 Pantnagar Centre for Plant Genetic Resources (Lead Institution)	G.B. Pant University of Agriculture and Technology, Pantnagar-263145 Distt Udham Singh Nagar UTTARAKHAND	<ol style="list-style-type: none"> 1. Survey, collection and purification of germplasm / land races of native crops of Uttarakhand. 2. Evaluation and characterization of strains for identification of specific distinguishable traits by morphological characters and biochemical and molecular techniques. 3. Ex-situ conservation of identified strains (germplasm) at Pantnagar Centre for Plant Genetic Resources for future use. 4. Prebreeding of strains having useful qualitative and quantitative characters. 5. Technological backstopping for trainings and awareness campaigns.
Partner 2 Field agency-NGO	Himalayan Gram Vikas Samiti, Gangolihat (Pithoragarh) (Field agency-NGO).	<ol style="list-style-type: none"> 1. Motivation of farmers and organization of trainings for creating awareness on germplasm collection <i>in situ</i> conservation and seed production. 2. Collaboration for survey and collection of land races of native crops from different districts of Uttarakhand. 3. In-situ conservation of identified strains (germplasm) by the community groups at the farmers' field.

Financial and Resource Information

Total Grant:	50,53,800/-	Grant Received Date:	08.06.2016 I st year grant 13.07.2017 II nd year grant
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Objectives of the Project:

- i) Survey, collection and purification of germplasm / land races of native crops (millets, pulses and vegetables) of Uttarakhand.
- ii) Evaluation and characterization of strains for identification of specific distinguishable traits by morphological characters and biochemical and molecular techniques
- iii) In-situ conservation of identified strains (germplasm) by the community groups at the farmers' field and ex-situ conservation at Pantnagar Centre for Plant Genetic Resources (PCPGR), respectively for future use.
- iv) Development of model mechanism for *in-situ* conservation linked with seed production (millets and pulses) and seedling production (vegetables) of identified unique germplasm for income generation.

Key Activity	Description on how it will be done, with whom etc
Result #1: Creating awareness among farming community for conservation of germplasm and registration of land races.	
Activity 1.1	Entry level activities with farming community and their SHGs to acquaint with project objective through NGO
Activity 1.2	Training of SHGs for <i>in-situ</i> conservation of germplasm and seed/ seedling production through NGO.
Activity 1.3	Encouragement of farmers' SHGs for bulk production of vegetables and millets utilizing germplasm / landraces possessing unique characteristics for income generation.
Result #2: Survey, collection and purification of germplasm / land races of native crops (millets and vegetables) of Uttarakhand	
Activity 2.1	Survey and exploration of plant germplasm in hilly district of Uttarakhand with community participation and collection.
Activity 2.2	Purification of germplasm at PCPGR experimental fields and glasshouse.
Activity 2.3	Seed multiplication of purified strains
Result #3: Evaluation and morphological characterization of strains for identification of specific distinguishable traits.	
Activity 3.1	Morphological characterization of germplasm lines as per the descriptor
Activity 3.2	Evaluation of germplasm for nutritive values.
Result #4: Biochemical and molecular characterization of Identified strains	
Activity 4.1	Identification of suitable biochemical and/or molecular markers for differentiation among the germplasm through literature search.
Activity 4.2	Designing primers for crop wise identified markers
Activity 4.3	Biochemical and /or molecular analysis of the germplasm
Result #5: In-situ and ex-situ conservation of identified strains (germplasm)	
Activity 5.1	Distribution of seed of purified and identified germplasm to the farming community for conservation vis-à-vis seed production.
Activity 5.2	Training of farmers for maintaining seed purity and quality of the germplasm during <i>in-situ</i> conservation. (Proposed investigations will also suggest suitable models for <i>in situ</i> conservation of valuable genetic wealth of Uttarakhand with involvement of local population and NGO's, so that quality characteristic of the species must be retained.)
Activity 5.3	Training of farmers for processing and packaging of seed and nursery production for income generation.
Activity 5.4	Ex-situ conservation of germplasm at PCPGR in mid-term storage facility with complete passport data of lines

Detailed Project Progress Report

Abstract

The project was started with an objective to explore and preserve the genetics wealth of Uttarakhand with respect to millets, pulses, vegetables and spices. With NGO partner (HGVS, Gangolihat) three meetings with SHG's were conducted in villages Kotera, Kanalichinna and Khatigaon in Pithoragarh district to create awareness on importance of *in-situ* Germplasm conservation. Survey and exploration visits were conducted during the year and a total of 302 accessions of different crops like finger millet, barnyard millet, horse gram, pulses, mustard and vegetables were collected from Champawat, Pithoragarh, Chamoli and Rudrprayag districts. A collection of 89 accessions of Barnyard millet, 92 accessions of finger millet and 32 accessions of mustard were evaluated in field trial at PCPGR to assess the diversity and variability in the collections. Biochemical analysis for micronutrient content in 89 accessions was carried out and 92 accessions of finger millet were also evaluated for their response to PEG induced drought stress in laboratory conditions. Similarly other collections of various cereals, pulses and vegetables will be evaluated in coming season. Two trainings on maintaining quality during seed production, processing and packaging of produced seed were also conducted at Bhainti murari (Lohaghat) in Champawat, Rithad (Devnai) in Bageshwar and one training on *in-situ* conservation of germplasm was conducted at Narainbagar in Chamoli districts. Farmers were given knowledge about *in-situ* conservation of germplasm and seed production of farmers' varieties. Potential areas where germplasm repository could be established were also identified. Qualitative evaluation and molecular profiling of the germplasm is going on.

Objective : Survey, collection and purification of germplasm / land races of native crops of Uttarakhand.

Result #1: Creating awareness among farming community for conservation of germplasm and registration of land races.

Activity 1.1	Entry level activities with farming community and their SHGs to acquaint with project objective through NGO
Activity 1.2	Training of SHGs for <i>in-situ</i> conservation of germplasm and seed/ seedling production through NGO.

The different categories of farmers inhabit in the project area ranging from poor farmers with no knowledge to farmers turned entrepreneurs. Principal investigator has visited different areas and made surveys, it has been observed that farmers are still cultivating some of the very good strains of millets like finger millet, barnyard millet etc, pulses like Kulthi (Gahat), ricebean, black soybean (bhatt) etc, and vegetables. But they are unaware of the importance of germplasm and landraces. Therefore, for creation of awareness among the farming community and transferring necessary skill to them, Kisan goshies and farmers' training had been organized in different villages. The detail of participation in training and kisan goshies is presented in Table 1. Trainings aimed at *in situ* conservation, maintaining seed purity and quality of

Table 1. Details of the participants in various trainings and meetings					
S. No	Village	Date	Participants		
			M	F	Total
Farmers Trainings:					
1	Gangolihat	09/06/17	11	19	28
2	Lohaghat	29/01/18	10	13	23
3	Devnai	10/02/18	17	01	18
Farmers Meetings:					
1	Kotera	05/06/17	6	27	33
2	Kanalichinna	30/01/18	14	16	30
3	Khatigaon	09/02/18	18	1	19

the germplasm and processing and packaging of seed and nursery production for income generation. The trainings were organized by the NGO partner and technological backstopping was provided by PI. Farmers were motivated for conservation of genetic resources and awareness meetings were also conducted regarding germplasm conservation and its utilization.

Result #2: Survey, collection and purification of germplasm / land races of native crops (millets and vegetables) of Uttarakhand

Activity 2.1	Survey and exploration of plant germplasm in hilly district of Uttarakhand with community participation and collection.
Activity 2.2	Purification of germplasm at PCPGR experimental fields and glasshouse.
Activity 2.3	Seed multiplication of purified strains

Through the exploration visits villages of Champawat, Pithoragarh and Chamoli are covered for conservation of the genetic resources of millets like Finger Millet, Barnyard Millet etc Pulses like Horse gram (Gahat), Rice-Bean, Black Soybean (bhatt) and vegetables. Survey and exploration resulted in collection of 302 accessions of the crop germplasm from Champawat, Pithoragarh, Rudraprayag and Chamoli district. The detailed of accessions collected during period under report are presented in Table 2. As the farmers’ indigenous strains of different crops collected from different geographical niches are generally a mixture, which has to be purified for their characters through grow out test at PCPGR experimental fields in the respective crop seasons. Accordingly, if possible sowing of the accessions collected the period under report have been done according to season and rest were kept for coming season. It was serve the purpose of purification as well as seed multiplication for the evaluation trials.

Objective : Evaluation and characterization of strains for identification of specific distinguishable traits by morphological characters and biochemical and molecular techniques

Result #3: Evaluation and morphological characterization of strains for identification of specific distinguishable traits.

Activity 3.1	Morphological characterization of germplasm lines as per the descriptor
Activity 3.2	Evaluation of germplasm for nutritive values.

During the year 2017-18, germplasm evaluation was carried out under the Project and Three different experiments were conducted on three crops such as Finger millet (92), Barnyard millet (89) and Rapeseed (32) during Kharif and Rabi 2017-18 respectively at PCPGR fields. The field experiments with 92 accessions of finger millet was conducted to evaluate for morphological traits for the first year, however, the experiment on Barnyard millet (89 accessions) was confirmity trial for morphological as well as Nutritive value triats. The trial on rapeseed was the preliminary evaluation trial. The results of all the three experiments conducted are presented below.

Experiment 1. Finger Millet:

Finger Millet accessions were planted in augmented block design with the objective of evaluation and characterisation. The experiment consisted of 92 test entries and five checks in four blocks. Each entry was represented by a single row of 3 m spaced at 0.3 m. Fourteen quantitative characters viz., Days to fifty percent flowering, Plant height, No. of tillers, Leaf length, Leaf breadth, Peduncle length, Finger length, Finger width, No. of fingers, Spike length, Straw weight, Spike

weight, Test weight, and Grain yield were recorded and results have been summarized in Table 3. The wide range of variation was recorded for traits like Plant height and Spike weight i.e. 70.0-165.6 and 75.0-550.0 along with high mean 128.14 cm and 241.30 g respectively. Four qualitative characters *viz.*, Grain colour, Grain shape Seed surface and Pericarp persistence were also recorded and results have been summarized in Table 4. None of the entry recorded white grain colour, whereas 56 entries recorded light brown, 22 copper brown and 14 were dark brown. Most of the entries were having Round Grain shape (44), Rough Seed surface (55) and Non persistent Pericarp (70).

Table 3. Mean and Range for different quantitative characters in Finger millet.

S. No	Characters	Mean	Range
1	Days to 50% Flowering	88.57	67.0-108.0
2	Plant height (cm)	128.14	70.0 -165.6
3	No. of tillers	3.14	1.3-8.6
4	Leaf length (cm)	35.49	23.6-48.6
5	Leaf breadth (cm)	1.05	0.6-1.4
6	Peduncle length (cm)	22.96	11.8-34.3
7	Finger length	9.20	4.4-16.1
8	Finger width	1.20	0.7-8.8
9	Number of Fingers	6.21	3.3-10.6
10	Spike length	10.69	5.7-19.6
11	Straw weight (kg/plot)	1.17	0.5-3.0
12	Spike weight (g)	241.30	75.0-550.0
13	Test weight (g)	76.94	12.0-230.0
14	Grain yield (kg/plot)	3.36	2.4-4.6

Table 4. Distribution of Finger millet accessions for different qualitative characters.

S. No.	Character	Class	Accessions
1	Grain color	White	0
		Light Brown	56
		Copper Brown	22
		Dark Brown	14
2	Grain shape	Round	44
		Reniform	27
		Ovoid	21
3	Seed surface	Smooth	37
		Rough	55
4	Pericarp Persistence	Non Persistent	70
		Persistent	22

Experiment 2. Barnyard Millet:

Barnyard millet accessions (89) were evaluated for morphological attributes and nutritive value. The experiment was laid out in Randomised block design with 89 entries and two checks *viz.* VL 181 and PRJ 1 in two replications. Each entry was represented by a single row plot of 3 m x 0.3 m size. Six quantitative characters *viz.*, Basal tiller No, Flag Leaf Blade Length, Peduncle Length, Panicle length, Plant height and Grain yield were recorded and results of analysis was presented in Table 5. Highly significant differences among the accessions were observed for all six yield and yield attributing characters owing to their varied geographical distribution. Most of the characters showed a varied range of variation among the accessions. Widest range was observed for Grain yield (167.6-475.95 g/plot) with a mean of 284.36g/plot. Coefficient of variation was observed higher at phenotypic level than genotypic level for traits like Basal tiller number and Grain yield. High

variability along with moderate heritability and genetic advance offers greater scope of selection for improvement of peduncle length and panicle length. Plant height recorded lower heritability along with lower genetic advance over mean.

Similarly, 11 qualitative characters *viz.*, Plant growth habit, Days to fifty percent flowering, Plant pigmentation, Inflorescence shape, Culm branching, lodging, Grain colour and Grain shape were recorded and results have been summarized in Table 6. All the 89 entries were having erect growth habit and lower raceme branching was absent. Most of the entries recorded oval grain shape (79), absence of lodging (59), slender raceme (64) and branched Culm (83). The accessions were equally distributed for presence (46) and absence (43) of plant pigmentation.

Table 5. Mean, range, Genetic (GCV), Phenotypic (PCV) coefficient of variability, Heritability (h^2_{bs}) and Genetic advance as percent of mean (GAM) for different quantitative characters in Barnyard millet.

S. No.	Character	GM	Range	GCV	PCV	GAM	h^2_{bs}
1.	Basal tillers (No.)	1.73	1.10-2.90	13.70	27.61	14.02	0.25
2.	Flag leaf blade length (cm)	32.62	25.91-42.10	7.93	13.97	3.05	0.32
3.	Peduncle length (cm)	16.72	13.33-21.60	7.71	13.80	8.87	0.31
4.	Panicle length (cm)	23.86	15.72-30.88	8.38	14.18	10.20	0.35
5.	Plant Height(cm)	105.97	72.90-133.3	2.61	10.09	2.68	0.06
6.	Grain Yield (g/plot)	284.36	167.6-475.95	14.00	27.76	14.55	0.25

Table 6. Distribution of Barnyard millet accessions for different qualitative characters.

S. No.	Character	Class	Accessions
1	Plant growth habit	Erect	89
		Decumbent	-
		Prostrate	-
2	Days to 50 % flowering	Early	27
		Medium	41
		Late	21
3	Plant pigmentation	Absent	43
		Present	46
4	Inflorescence shape	Cylindrical	33
		Pyramidical	42
		Globose-elliptic	14
5	Culm branching	Absent	6
		Present	83
6	lodging	Absent	59
		Present	30
7	Grain colour	Straw white	10
		Light grey	39
		Grey	40
8	Grain shape	Concave	10
		Oval	79
9	Lower raceme Shape	Straight	55
		Curved	34
10	Lower raceme thickness	Slender	64
		Thick	25
11	Lower raceme branching	Absent	89
		Present	-

Nutritive value in terms of micronutrient content was also evaluated for all these barnyard millet entries. Micronutrients such as Fe (mg/kg), Zn (mg/kg), Cu (mg/kg) and Mn (mg/kg) were estimated through Atomic Absorption Spectrophotometry (AAS) and results are presented in Table 7. PCPGR 7904 (6.638mg/kg) and GP-2011-129 (7.150 mg/kg) were found to record significantly higher Cu (mg/kg) compared to best check PRJ 1 (3.70 mg/kg) and PCPGR 7886 (82.3 mg/kg) recorded significantly higher Fe (mg/kg) compared to best of the check PRJ 1 (47.5 mg/kg). For Mn (mg/kg), 57 lines were found to be significantly better than VL 181 (9.688 mg/kg) but none of the line was found to be significantly better than PRJ 1 (19.16 mg/kg). The lines PCPGR 7904, GP-2011-129 could be a good source of copper in diet. Similarly, the line PCPGR 7886 can be utilized as good source of Iron in diet.

Table 7. Mean and Range for different Micronutrients (Zn, Fe, Cu and Mn) in Barnyard millet

Micronutrients	Mean	Range	SEM	CD @ 5%
Zn (mg/kg)	30.88	17.7-41.0	5.00	14.04
Cu (mg/kg)	3.77	1.2-7.1	0.98	2.76
Fe (mg/kg)	39.41	23.6-82.3	9.25	25.99
Mn (mg/kg)	16.60	9.06-22.9	2.25	6.32

Experiment 3. Mustard:

Mustard accessions were evaluated in Randomised block design with 32 entries and three checks Kranti, PPS-1 and PT 303 in three replications. Each entry was represented by a single row plot of size of 3m x 0.5m. Eight quantitative characters viz; Plant height, Shoot length, Siliqua length, Beak length, No of Siliqua, No of seeds/ Siliqua, Siliqua density and Grain yield were recorded and results are presented in Table 8. Highly significant differences among the accessions were observed for all eight yield and yield attributing characters owing to their varied geographical locations. Most of the characters showed a varied range of variation among the accessions. Widest range was observed for plant height (78-164 cm) with a mean of 128.46 cm. The grain yield was found around a mean of 38.32 g per plot with a range of variation from 5.4-71.0 g per plot. Coefficient of variation was observed higher at phenotypic level than genotypic level for traits like Shoot length, Beak length, No of seeds/ Siliqua, Siliqua density and Grain yield High variability along with high heritability and genetic advance offers greater scope of selection for improvement of Siliqua density and Grain yield. Four qualitative characters viz., Siliqua texture, Siliqua Angle, Seed Colour and Seed Size were recorded and summarized in Table 9. All thirty-two entries recorded small seed size having less than 5 g/ 100- seeds. Twenty entries exhibited yellow seed colour and Semi-appressed Siliqua angle, while most of the entries (18) were having undulated Siliqua texture.

Table 8. Mean, range, genetic (GCV), phenotypic (PCV) coefficient of variability, heritability (h^2_{bs}) and genetic advance as percent of mean (GAM) for different quantitative characters in Mustard.

S.No.	Character	GM	Range	GCV	PCV	GA	GAM	h^2_{bs}
1.	Height(cm)	128.46	78-164	16.58	16.63	43.73	34.04	0.91
2.	Shoot Length (cm)	49.05	20.6-80.0	23.66	23.85	23.71	48.34	0.89
3.	Siliqua length(cm)	5.15	2.58-6.72	16.11	17.85	1.54	29.94	0.81
4.	Beak length(cm)	1.38	0.44-2.07	25.39	29.50	0.62	45.01	0.74
5.	No of siliqua	30.26	15-45	17.91	19.58	10.21	33.73	0.84
6.	No of seeds/ siliqua	17.18	7-39	27.96	30.89	8.96	52.15	0.82
7.	Siliqua Density	0.67	0.28-1.6	40.62	42.12	0.54	80.70	0.93
8.	Grain Yield (g/plot)	38.32	5.4-71.0	44.93	45.24	35.22	91.91	0.78

Table 9. Distribution of Mustard accessions for different qualitative characters.

S. No.	Character	Class	Accessions
1	Siliqua texture	Constricted	12
		Smooth	2
		Undulated	18
2	Siliqua angle	Appressed	20
		Semi- appressed	5
		Open	7
3	Seed colour	Yellow	20
		Reddish brown	2
		Brown	3
		Dark brown	4
4	Seed size	Black	3
		Small	32
		Medium	-
		Bold	-

Result #4: Biochemical and molecular characterization of Identified strains

Activity 4.1	Identification of suitable biochemical and/or molecular markers for differentiation among the germplasm through literature search.
Activity 4.2	Designing primers for crop wise identified markers
Activity 4.3	Biochemical and /or molecular analysis of the germplasm

As per the objective of the project the purified indigenous strains will be characterized by biochemical and molecular markers, but we have done preliminary studies for drought tolerance among the finger millet genotypes as we found uniformity in the accessions growing in the field experiment. With the objective of evaluating 92 Finger Millet accessions for their response to abiotic stress, drought stress was induced by using Poly ethylene glycol (PEG) in laboratory conditions. All the entries were subjected to three different treatments such as Control (No PEG), 15 % PEG concentration and 21 % PEG concentration in two replications. The traits like germination percentage, Root length and Shoot length were recorded after 8 days of treatment. No entry was germinated at 21% PEG concentration, hence results of 15% concentration were compared with control. The entries showing lesser reduction in germination, root and shoot length were rated as tolerant to drought (**Gupta et al., 2014**). The results for mean performance of drought tolerant finger millet entries are presented in Table 10. The results of preliminary evaluation will be further tested under field trials and marker based screening will also be carried out.

Table 10. Mean Performance of Drought Tolerant Entries of Finger Millet for Germination, Root Length and Shoot Length Under PEG Induced Drought Stress.

S. No	Entry	Germination (%)		Root length (cm)		Shoot length (cm)	
		Control	15% PEG	Control	15% PEG	Control	15% PEG
1	116	80.0	70.0	3.07	2.70	5.8	3.95
2	120	80.0	70.0	3.30	2.42	3.8	3.9
3	126	70.0	70.0	3.23	2.30	4.62	2.43
4	130	60.0	60.0	3.57	2.50	4.18	1.53
5	131	65.0	55.0	2.95	1.17	4.53	1.35
6	141	65.0	80.0	3.08	2.60	3.44	2.85
7	187	90.0	70.0	3.50	3.17	3.7	3.71
8	272	70.0	90.0	3.38	2.70	4.62	3.05
9	393	65.0	80.0	2.5	1.43	4.3	3.14
10	396	90.0	80.0	2.1	1.97	8.02	3.13
11	461	85.0	70.0	3.10	2.73	3.4	2.37
12	652	60.0	70.0	2.70	2.23	3.53	2.9

Objective : In-situ conservation of identified strains (germplasm) by the community groups at the farmers' field and ex-situ conservation at Pantnagar Centre for Plant Genetic Resources (PCPGR), respectively for future use.

Result #5: In-situ and ex-situ conservation of identified strains (germplasm)

Activity 5.1	Distribution of seed of purified and identified germplasm to the farming community for conservation vis-à-vis seed production.
Activity 5.2	Training of farmers for maintaining seed purity and quality of the germplasm during <i>in-situ</i> conservation. (Proposed investigations will also suggest suitable models for <i>in situ</i> conservation of valuable genetic wealth of Uttarakhand with involvement of local population and NGO's, so that quality characteristic of the species must be retained.)
Activity 5.3	Training of farmers for processing and packaging of seed and nursery production for income generation.
Activity 5.4	Ex-situ conservation of germplasm at PCPGR in mid-term storage facility with complete passport data of lines

As per the plan the purified strain was provided to the farmers for cultivation and *in-situ* conservation, but the material was not handle properly by the farmers due to unawareness. Hence, awareness programmes of the current year was more focused on “How to handle the germplasm during multiplication and storage” rather than “importance of germplasm and its conservation” as discussed during first year’s awareness goathies. Hence the farmers encouraged to conserve the germplasm in their farm itself and five farmers in different locations were selected to act as germplasm savior.

S.No.	Name of the Farmer	Location	AES
1	Mr Ratan Singh	Hupali (Pithoragarh)	Valley
2	Mr Pan Singh	Birthe (Pithoragarh)	Mid-hills
3	Mr Jagdish Prasad	Chakh (Pithoragarh)	Mid-hills
4	Mr Raghuvar Murari	Bhainti-murari (Champawat)	Mid-hills
5	Mrs Vimala Nagarkoti	Dhapolasera (Bageshwar)	Mid-hills

Objective : Development of model mechanism for *in-situ* conservation linked with seed production (millets and pulses) and seedling production (vegetables) of identified unique germplasm for income generation.

Discussion is going on with the farmers’ SHGs and an incentive based model system will be developed for registration of their varieties in order to share benefits of conservation of valuable landraces. Unique germplasm identified will ultimately be submitted to NBPGR for registration. Seed and seedling production will also be linked with *in-situ* conservation to add on income of farming community. The germplasm lines and farmers varieties will be utilized as prebreeding lines which will be given to the breeders for integration in their breeding programs for development of superior varieties. This will directly assure benefits to the farming community in the long run.