NATIONAL MISSION ON HIMALAYAN STUDIES (NMHS)

FINAL TECHNICAL REPORT (FTR)

(2019 to 2022)

Title of the project

Morphological and biochemical characterization, mitigating climate change impact and quality honey production in buckwheat (*Fagopyrum* spp.) under organic condition: A community participation approach for doubling livelihood empowerment


Submitted by

DR. SHAON KUMAR DAS
Senior Scientist, (Agril. Chemistry/Soil Science)
ICAR Research Complex for NEH Region, Sikkim Centre, Gangtok
Tadong, Gangtok, Sikkim-737102

Template/Pro forma for Submission

NMHS-Himalayan Institutional Project Grant

NMHS-FINAL TECHNICAL REPORT (FTR)

Demand-Driven Action Research and Demonstrations

NMHS Grant Ref. No.:	**NMHS/2019-20/SG66/66**

Date of Submission:	1	2	0	6	2	0	2	3
	d	**d**	**m**	**m**	**y**	**y**	**y**	**y**

PROJECT TITLE (IN CAPITAL)

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION, MITIGATING CLIMATE CHANGE IMPACT AND QUALITY HONEY PRODUCTION IN BUCKWHEAT (*FAGOPYRUM* SPP.) UNDER ORGANIC CONDITION: A COMMUNITY PARTICIPATION APPROACH FOR DOUBLING LIVELIHOOD EMPOWERMENT

Project Duration: *from* (**24.02.2019**) *to* (**24.02.2022**).

***Submitted to*:**
Er. Kireet Kumar
Scientist 'G' and Nodal Officer, NMHS-PMU
National Mission on Himalayan Studies, GBP NIHE HQs
Ministry of Environment, Forest & Climate Change (MoEF & CC), New Delhi
E-mail: nmhspmu2016@gmail.com; kireet@gbpihed.nic.in; kodali.rk@gov.in

***Submitted by*:**
[*Dr. Shaon Kumar Das*]
[*ICAR RC for NEH Region, Sikkim Centre, Tadong, Gangtok, Sikkim-737102*]
[*Mobile No.: 9475680474*]
[*E-mail: shaon.iari@gmail.com*]

GENERAL INSTRUCTIONS:

1. The Final Technical Report (FTR) has to commence from the start date of the Project (as mentioned in the Sanction Order issued by NMHS-PMU) till completion of the project duration. Each detail has to comply with the NMHS Sanction Order.
2. The FTR should be neatly typed (in Arial with font size 11 with 1.5 spacing between the lines) with all details as per the enclosed format for direct reproduction by photo-offset printing. Colored Photographs (high resolution photographs), tables and graphs should be accommodated within the report or annexed with captions. Sketches and diagrammatic illustrations may also be given detailing about the step-by-step methodology adopted for technology development/ transfer and/ or dissemination. Any correction or rewriting should be avoided. Please provide all information under each head in serial order.
3. Any supporting materials like Training/ Capacity Building Manuals (with detailed contents about training programme, technical details and techniques involved) or any such display material related to project activities along with slides, charts, photographs should be brought at the venue of the Annual Monitoring & Evaluation (M&E) Workshop and submitted to the NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora 263643, Uttarakhand. In all Knowledge Products, the Grant/ Fund support of the NMHS should be duly acknowledged.
4. The FTR Format is in sync with many other essential requirements and norms desired by the Govt. of India time-to-time, so each section of the NMHS-FTR needs to be duly filled by the proponent and verified by the Head of the Lead Implementing Organization/ Institution/ University.
5. Five (5) hard-bound copies of the Project Final Technical Report (FTR) and a soft copy of the same should be submitted to the **Nodal Officer, NMHS-PMU, GBP NIHE HQs, Kosi-Katarmal, Almora, Uttarakhand**.

The FTR is to be submitted into following two (02) parts:

Part A – Project Summary Report

Part B –Detailed Project Report

In addition, the Financial and other necessary documents/certificates need to be submitted along with the Final Technical Report (FTR) as follows:

Annexure I	**Consolidated and Audited Utilization Certificate (UC) & Statement of Expenditure (SE)**, including the interest earned for the last Fiscal year and the duly filled GFR-19A (with year-wise break-up).
Annexure II	**Consolidated Interest Earned Certificate**
Annexure III	**Consolidated Assets Certificate** showing the cost of the equipment in Foreign/ Indian currency, Date of Purchase, etc. (with break-up as per the NMHS Sanction Order and year wise).
Annexure IV	**List of all the equipment, assets and peripherals** purchased through the NMHS grant with current status of use, including location of deployment.
Annexure V	**Transfer of Equipment** through Letter of Head of Institution/Department confirming the final status of equipment purchased under the Project.
Annexure VI	**Details, Declaration and Refund of any Unspent Balance transferred through Real-Time Gross System (RTGS)/ PFMS in favor of NMHS GIA General**

DSL: Date of Sanction Letter

2	4	0	2	**2**	**0**	**2**	**0**
d	d	m	m	y	y	y	y

DPC: Date of Project Completion

2	4	0	2	**2**	**0**	**2**	**2**
d	d	m	m	y	y	y	y

Part A: Project Summary Report

1. Project Description

i.	Project Grant Ref. No.:	NMHS/2019-20/SG66/66					
ii.	Project Category:	**Small Grant**	**SG**	Medium Grant		Large Grant	
iii.	Project Title:	Morphological and biochemical characterization, mitigating climate change impact and quality honey production in buckwheat (Fagopyrum spp.) under organic condition: A community participation approach for doubling livelihood empowerment.					
iv.	Project Sites (IHR States/ UTs covered) (*Location Maps attached*):	Sikkim					
v.	Scale of Project Operation:	Local		Regional	**R**	Pan-Himalayan	
vi.	Total Budget:	**3627328.00**					
vii.	Lead Agency:	ICAR RC for NEH Region, Sikkim Centre, Gangtok, Sikkim					
	Lead PI/ Proponent:	Dr. Shaon Kumar Das					
	Co-PI/ Proponent:	Dr. Chandan Kapoor					
viii.	Implementing Partners:	ICAR RC for NEH Region, Sikkim Centre, Gangtok, Sikkim					
	Key Persons (Contact Details, Ph. No., E-mail):	Dr. Shaon Kumar Das Email: shaon.iari@gmail.com Contact: 9475680464					

2. Project Outcomes

2.1. Abstract/ Summary (not more than 250-300 words)

Fifteen accessions of common buckwheat (*Fagopyrum esculentum*) and 21 of tartary buckwheat (*Fagopyrum tataricum*) were studied for 15 quantitative and 21 qualitative traits to understand the nature and magnitude of genetic variability using multivariate approach. Wide variations among buckwheat accession for quality attributes have shown ample potential to be exploited for further improvement of desirable quality attributes and antioxidant properties. 02 front line

demonstration of suitable germplasm of buckwheat in each district and total 08 demonstrations in four district of Sikkim was done. 15 best selected germplasm were characterized biochemically for different anticancer activity. The genotype varied significantly in their proximate composition with moisture content, crude protein, crude fat, ash and crude fibre on dry weight basis. 10 best germplasm were selected for maximum rhizosphere phosphorus enhancement activity in Sikkim. Results also revealed that, as a cover crop, buckwheat increased soil-P availability. Soil-P availability was not affected by buckwheat, but the concentration of rhizosphere tartrate^{2-} was significantly higher ($p < 0.005$) in low-P *vs.* P-fertilized plots which suggested that organic-anion root exudation may have a role in buckwheat-rhizosphere P dynamics. Impact of climate change on organic buckwheat production technology and mitigation study was carried in 04 districts (02 blocks per district, 04 villages per block and 02 beneficiaries per village. Total beneficiaries were 64 number from all the district and developed first HONEY VILLAGE (01 in each district and total 04 number) in Sikkim through quality honey production with honey bee as a pollinator component. Finally, 02 training per year for quality honey production technology (on/off campus training) was also conducted.

2.2. Objective-wise Major Achievements

Objective I: Georeference survey, collection and morphological characterization of unexplored buckwheat germplasm

Major achievement

Fifteen accessions of common buckwheat (*Fagopyrum esculentum*) and 21 of tartary buckwheat (*F. tataricum*) were studied for 15 quantitative and 21 qualitative traits to understand the nature and magnitude of genetic variability using multivariate approach. Significant variation for quantitative traits was recorded for seven traits in common buckwheat and for eleven traits in tartary buckwheat. In common buckwheat seed yield correlated positively with days to maturity while negatively associated with seed weight whereas in case of tartary type number of cymes/plant, seeds/cyme, days to flowering and days to maturity showed positive correlation with seed yield. First five and four principal components (PCs) respectively, in common and tartary type revealed majority of the variability in the accessions which further grouped the test accessions of both the species in to four clusters each. The accessions also showed considerable variation for qualitative traits.

Objective II: Biochemical characterization and identification in selected germplasm of buckwheat for anticancer activity

Major achievement

Buckwheat can be used as nutritious and energizing foods which contribute nutritional and food security. After the analysis of the different nutritional quality attributes of various buckwheat genotypes the result revealed that the accession along with the released variety were found to have superior desirable nutritional quality parameters in comparison with local variety Titey. Protein content was highest in Titey (11.2%) followed by Shimla B-1 (11.1%) and lowest in IC49671 & IC108510 (10.4%). Significant variation was estimated for tryptophan content for various genotypes ranges from 66.3 to 76.9 mg/g N. The crude fibre content showed the range of variation from 3.71 to 4.78 % in dry mature grains of tartary buckwheat. %. Maximum nitrogen free content was recorded in IC108518 (76.97%) followed by IC202268 (75.37%) and lowest was found in IC36805 (71.41%). Maximum Fe content was found in genotype IC108518 (3.50) followed by IC109549 (3.49) and lowest was recorded in genotype IC49671 (2.50 mg/100g). Similarly, total phenol content ranged from 378.41 to 652.71 mg GAE/100g with an average content of 518.68 mg GAE/100g. Therefore, development and utilization of such functional foods will not only improve the nutritional status of the population but also helps those suffering from degenerative diseases. Wide variations among genotypes for quality attributes have shown ample potential to be exploited for further improvement of desirable quality attributes and other properties.

Objective III: Evaluation for rhizosphere phosphorus enhancement activity from the selected germplasm of buckwheat

Major achievement

Phosphorus levels declined significantly (1.3-fold decrease) in all treatments between the third (at buckwheat harvest) and fourth (after mowing or tilling) sampling dates ($p < 0.05$). No significant differences in extractable soil P as a function of buckwheat treatment over time ($F_{2, 47} = 0.59$, $p = 0.57$), interaction between buckwheat treatments and time ($F_{6, 47} = 0.46$, $p = 0.77$), or post-buckwheat manipulation treatment ($F_{1, 23} = 3.14$, $p = 0.09$) were detected. At the time of buckwheat harvest, there were no significant differences in extractable soil P, buckwheat dry shoot, or dry root mass between control and added-P plots, suggesting that, if P availability was limiting for growth, compensatory P acquisition by buckwheat was present in unamended plots. Oxalic acid (protonated oxalate^{2-} at mobile phase pH, 3.8), if present in the samples, was not detected (with instrumental detection limit for oxalic acid of 0.5 l g mL^{-1}). However, tartaric acid ($C_6H_4O_5$, MW = 150 g mol^{-1}) was detected in rhizosphere-soil extractions by its retention time (6.7 min) and its mass spectrum (mass 149, negative ion mode).

Objective IV: Impact of climate change on organic buckwheat production technology and mitigation strategies through community participation

Major achievement

Buckwheat is an important crop of the mountain regions at elevations above 1400 m amsl for grain and green leaves. In the higher Himalayas, up to 4500 m, this is the only crop which can be grown successfully. There are two species of buckwheat cultivated in the Himalayas hills (*F. esculentum* and *F. tataricum*). Due to their multiple uses, short growing periods, high nutritional and medicinal values, wide adaptability has become popular not only to researcher but also for farmers and consumers globally. It grows in a wider range from low to higher mountains of Sikkim. Maxent modelling was used to quantify the current suitable habitat and predicted future suitable area under different climate change scenarios, based on representative concentration pathways in two different time periods (2040 and 2060 AD) using climatic predictive variables and species localities. The most suitable habitat area of cultivation, area loss, and gained for buckwheat were determined. The model showed that about 59.2% area of state is climatically suitable for buckwheat within the elevation range of 502-4381 m. Habitat suitability of buckwheat would shrink by 6.6% and 7.3% under RCPs 4.5 and by 6.7% and 7.4% under 8.5 RCPs in the year 2040 and 2060, respectively. The loss and gain area analysis also indicated that suitable area would be lost more than gained in both 2040 and 2060. These findings are expected to support planning and policy framing for climatic resilience smart agriculture practice to meet the livelihood and food security problems in the mountains of Sikkim Himalaya. Due to medicinal value of buckwheat, its demands are increasing. The increasing demands of buckwheat would be fulfilled by increasing production under climatically suitable area under climate change scenarios.

Objective V: Quality honey production with honey bee as a pollinator component in different regions of Sikkim through demonstration and training through participation approach

Major achievement

Quality honey production technology was developed through buckwheat production which empowered the rural tribal farmer's. The transfer of quality honey production through buckwheat cultivation to the rural women and tribal communities helped to overcome the problem of unemployment to these strata of the society. They were skilled and sufficient to produce quality honey and also supplied to the other growers. The produce had easy access to the market. The selected villages were strengthened to emerged as new "HONEY VILLAGE" of Sikkim.

2.3. Outputs in terms of Quantifiable Deliverables*

S#	Quantifiable Deliverables*	Monitoring Indicators*	Quantified Output/ Outcome achieved	Deviations, if any, & Remarks thereof:
	Collection of >15 accessions of common buckwheat (Fagopyrum esculentum) and>21 of Tartary buckwheat (F. tataricum)	Number of Accession collected (Nos.)	Achieved 100%	Nil
	Developed demonstration model toconserve the germplasm of target species (8Nos in 4 Districts)	Demonstration Model developed (Nos.)	Achieved 100%	Nil
	Biochemical characterization includinganticancerous activity of elite germplas (15Nos.)	Biochemical Characterization (Nos.)	Achieved 100%	Nil
	Rhizosphere phosphorus enhancementactivity on elite germplasm (10 Nos.)	Rhizosphere phosphorus enhancement activity (Nos)	Achieved 100%	Nil

	Conduct production technology and mitigation study on 04 district (64 beneficiaries including 52 SC/ST)	HONEY VILLAGE developed (Nos.)	Achieved 100%	Nil
	Development of first HONEY VILLAGE(4 Nos.)	Number of Training/Awareness Programme Organized (Nos.)	Achieved 100%	Nil
	Conduct training Programme quality honey production technology (02 Nos.)	Number of beneficiaries village/ local people (Nos.)	Achieved 100%	Nil
	Develop the knowledge products: 01Policy, 01 manual document and 2-3 publications in well reputed journal	No. of Reports/Research articles/Policy documents prepared and published (Nos.)	Achieved 70%	Under review

*As stated in the Sanction Letter issued by the NMHS-PMU.

2.4. Strategic Steps with respect to Outcomes (in bullets)

S#	Particulars	Number/ Brief Details	Remarks/ Attachment
1.	New Methodology/ Technology developed, *if any*:	Quality organic honey production under buckwheat cultivation	Brief description attached in respective objective (Part-B)
2.	New Ground Models/ Process/ Strategy developed, *if any*:	Soil acidity management with buckwheat cultivation	Brief description attached in respective objective (Part-B)
3.	New Species identified, *if any*:	Various buckwheat germplasms have been identified	Brief description attached in respective objective (Part-B)
4.	New Database established, *if any:*	Biochemical properties of buckwheat database have established	Brief description attached in respective objective (Part-B)
5.	New Patent, *if any*:	Nil	Nil
	I. Filed (Indian/ International)		
	II. Technology Transfer, *if any*:		
6.	Others, *if any*	Nil	Nil

Note: Further details may be summarized in DPR Part-B, Section-5. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

3. New Data Generated over the Baseline Data

Data generated in this project are mentioned as Table in the DPR Part-B section.

Note: Further details may be summarized in DPR Part-B. Database files in the requisite formats (Excel) may be enclosed as annexure/ appendix separately to the soft copy of FTR.

4. Demonstrative Skill Development and Capacity Building/ Manpower Trained

Skill development and capacity building data generated in this project are mentioned as Table in the DPR Part-B section.

Note: Further details may be summarized in DPR Part-B. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

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

S#	Linkages /collaborations	Detail of activities (No. of Events Held)*	No. of Beneficiaries
1.	Sustainable Development Goals (SDGs)/ Climate Change/INDC targets addressed	08	64
2.	Any other:		

Note: Further details may be summarized in DPR Part-B, Section-6. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

6. Project Stakeholders/ Beneficiaries and Impacts

Beneficiaries list has been mentioned at DPR Part-B.

Note: Further details may be summarized in DPR Part-B, Section-6. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

7. Financial Summary (Cumulative)

Please attach the consolidated and audited Utilization Certificate (UC) and Year-wise Statement of Expenditure (SE) separately, *ref*. **Annexure I.**

Attached in the last section of this final report

8. Major Equipment/ Peripherals Procured under the Project** (*if any*)

S#	Name of Equipment	Quantity	Cost (INR)	Utilisation of the Equipment after project
1.	UV visible spectrophotometer (190-1100 nm, double beam, spectral bandpass 1.5 nm, wavelength accuracy 0.5 nm)	01	650000	Will be utilized for Institute research work
2.	Refrigerator (260 lt, double door, inverter four star)	01	30000	Will be utilized for Institute research work
3	Microwave (25 lt, frequency 2200 MHZ, turntable Diameter (mm): 300	01	20000	Will be utilized for Institute research work

4	Laptop set (Core i4 7th Gen, 4 GB/128 GB SSD/Windows 10 Home, 13-ah0042tu, lithium battery	01	55000	Will be utilized for Institute research work
5	Camera set (20 MP Digital SLR Camera + 18-55 is STM Lens + memory card + carry bag)	01	45000	Will be utilized for Institute research work

Details should be provided in details **(*ref.* Annexure III &IV).

9. Quantification of Overall Project Progress

S. No.	Parameters	Total (Numeric)	Remarks/ Attachments/ Soft copies of documents
1.	IHR States/ UTs covered:	*01*	*Attached in DPR Part-B*
2.	Project Sites/ Field Stations Developed:	*08*	*Attached in DPR Part-B*
3.	Scientific Manpower Developed (PhD/M.Sc./JRF/SRF/ RA):	*05*	*Attached in DPR Part-B*
4.	Livelihood Options promoted	*64*	*Attached in DPR Part-B*
5.	Technical/ Training Manuals prepared	*01*	*Attached in DPR Part-B*
6.	Processing Units established, if any	*Nil*	*Attached in DPR Part-B*
7.	No. of Species Collected, if any	*36*	*Attached in DPR Part-B*
8.	No. of New Species identified, if any	*10*	*Attached in DPR Part-B*
9.	New Database generated (Types):	*01*	*Attached in DPR Part-B*
	Others (if any)		

Note: Further details may be summarized in DPR Part-B. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

11. Knowledge Products and Publications:

S#	Publication/ Knowledge Products	*Number*		*Total Impact Factor*	*Remarks/* Enclosures
		National	International		
1.	Journal – Research Articles/ Special Issue:		01	2.6	
2.	Book – Chapter(s)/ Monograph/ Contributed:		01		
3.	Technical Reports:	02			
4.	Training Manual (Skill Development/ Capacity Building):	01			
5.	Papers presented in Conferences/Seminars:	03			
6.	Policy Drafts/Papers:	0			Writing
7.	Others, if any: Extension folder	0			Writing

Note: Please append the list of KPs/ publications (with impact factor, DOI, and further details) with due Acknowledgement to NMHS. Supporting materials may be enclosed as annexure/ appendix separately to the FTR.

12. Recommendation on Utility of Project Findings, Replicability and Exit Strategy

Particulars	Recommendations
Utility of the Project Findings:	The project funding was utilized for the for doubling livelihood empowerment
Replicability of Project/ Way Forward:	Such type of project activity can be replicated in other himalayan states
Exit Strategy:	The selected local progressive farmers were benefitted through implementing this project

(SHAON KUMAR DAS)
Name & Signature of Principal Investigator

साउन कुमार दास
Shaon Kumar Das
वैज्ञानिक (कृषि रसायन)
Scientist (Agricultural Chemistry)
भा.कृ.अनु.प. राष्ट्रीय जैविक कृषि अनुसंधान संस्थान
ICAR-National Organic Farming Research Institute
Tadong, Gangtok-737102, Sikkim

(**PROJECT PROPONENT/ COORDINATOR**)

(Signed and Stamped)

Signature
Name:
Head of Institute
Director (ICAR-NEH)

Director
ICAR Research Complex for NEH Region
Umroi Road, Umiam, Meghalaya-793103

24/5/23

(**HEAD OF THE INSTITUTION**)

(Signed and Stamped)

Place: ICAR RC for NEH Region, Sikkim Centre, Tadong, Gangtok, Sikkim.
Date: 24/05/2023

PART B: DETAILED PROJECT REPORT

The Detailed report should include an Executive Summary and it should have separate chapters on (i) **Introduction**, (ii) **Methodologies/Strategy/Approach**, (iii) **Key Findings and Results**, (iv) **Overall Achievements**, (v) **Project's Impacts in IHR** (vi) **Exit Strategy** and Sustainability, (vii) **References**, and (viii) **Acknowledgements** (acknowledging the financial grant from the NMHS, MoEF&CC, GoI).

Other necessary details/ Supporting Documents/ Dissemination Materials (*New Products/ Manuals/ Standard Operating Procedures (SOPs)/ Technology developed/Transferred, etc, if any*) may be attached as Appendix(ces).

INTRODUCTION

Buckwheat is one of the best crops at higher altitude. It belongs to the family Polygonaceae which are distinct from the monocot cereals (family, Poaceae) and belong to the category of dicot pseudo cereals. Among the minor cereals buckwheat is an important crop in feeding the mankind and animals to meet the ever increasing demand of rapidly expanding population of Sikkim. It is an important crop of the mountain regions at elevations above 1400 m amsl for grain and green leaves. In the higher Himalayas, up to 4500 m, this is the only crop which can be grown successfully. The economic importance of buckwheat rests mainly with the high nutritive value of their grains and the presence of a high content of the drug rutin and quercetin. It is evident from the many researches that the groat is a good protein supplement. Buckwheat grains have been found to contain a high carbohydrate content chiefly starch. Buckwheat protein quality is high due to high concentration of most essential amino acids especially lysine, threonine, tryptophan and the sulfur containing amino acids, however, due to a high content of crude fibre and tannin the true digestibility is below 80 per cent. It has also been reported by many workers that low prolamins content in buckwheat corresponds to favourable amino acid composition compared with other cereals. Buckwheat may be a valuable supplement to cereal grains as its high lysine content compensates for the limiting lysine content in diets consisting predominantly of cereals. Buckwheat foliage is an important source of the glycoside rutin (quercitin-3-rutlnoside). Rutin is used in medicine in the treatment of increased capillary fragility with associated hypertension, leading to haemorrhage, purpurea and bleeding from kidney. It has been found to have antioxidant, anticarcinogenic, antimutagenic, and antifungal properties. It contributes to controlling blood sugar which has been shown to lower risk of diabetes, cancer and heart disease. The main reasons for the low productivity are grain shattering and irregular time of maturity due to indeterminate growth habit of the crop. Due to its gradual formation and maturity, harvesting is done periodically and finally the crop is cut and threshed when the rest of the seeds are fully matured. Besides, buckwheat tends very heavy branching capacity and weak stem, which makes them susceptible to lodging. Being an underutilized crop, biochemical and morphological work in this crop is scanty mainly due to its complex reproductive mechanisms. It is widely grown in the Northeastern states including Sikkim, Arunachal Pradesh, Manipur, Nagaland, Meghalaya (West Khasi Hills). But majority area of this crop is in Sikkim. The average yield of this pseudocereal is 972.27 kg/ha with an area of 3.57 thousand hectares and production of 3.47 thousand tonnes in Sikkim (2015-16) against 2.01 thousand tonnes (2003-04). Niti Aayog has designated four high value crops of which buckwheat is one of them in Sikkim. No research has been done for morphological and biochemical analysis of buckwheat till now. Besides climate change impact study on this crop in mountain ecosystem is also scanty. Buckwheat has higher tolerance to soil acidity than any other cereal/grain crops. It has the ability to acidify its rhizosphere which is necessary for its different metabolic processes. The acidification mechanism may be enzyme and organic acid exudation, root-associated mycorrhizae, root acid, organic ligands, nutrient scavenging activity *etc*. Based on these, we hypothesize that buckwheat root rhizosphere has the ability to mobilize the rhizosphere P. If this happen then obviously the intensity of mobilizing root rhizosphere phosphorus by the different buckwheat germplasm will vary. Finally, quality honey production through buckwheat cultivation and its promotion through community participation approach for livelihood security study will add a new vision in this study. Hailed as a fabulous substitute for maple syrup, buckwheat honey is often drizzled over toasts, crepes, pancakes or waffles. Buckwheat honey, nature's superfood, is considered as one of the most medicinal honeys around. Darker honeys are often recommended for children who are coughing as they contain more bioactive compounds. Many studies have shown that buckwheat honey is more effective than over-the-counter cough syrup for treating a cough.

Methodology

Objective 1: *Georeference survey, collection and morphological characterization of unexplored buckwheat germplasm*

For morphological characterization, the morphological descriptors for this crop published by Bioversity International (IPGRI 1994) will be used. The descriptors for buckwheat reported in this work will follow the international standardized documentation system for the characterization and study of the genetic resources as promoted by Bioversity. Accessions will be selected to represent a diversity of morphological types and origins. On the basis of the genebanks' description of these accessions, seed characteristics, and farmers' grouping of cultivars, accessions will be assigned. A total of 50 descriptors, which correspond to different plant characteristics and parts, including leaves, flowers and inflorescences, infructescences, and seeds, and overall architecture will be established. Twenty-four plants per accession will be arranged in a randomized complete block design, with three blocks and eight replications (one plant = one replication) per block. Fifteen randomly selected representative plants per accession (five per block) will be evaluated. The number of samples evaluate per accession, with an equivalent number of measurements taken from each plant, will be: 15 for whole-plant traits and inflorescence traits (except inflorescence rachis internode length), 30 for leaf and inflorescence rachis internode length, 75 for flower (except petal length and width) and fruit traits, and for seeds per fruit, 150 for seed size, and 375 for petal length and width. Samples for a given descriptor will be evaluated on the same day, when appropriate. For each descriptor, we will calculated the following parameters: mean, maximum value, minimum value, range, maximum value/minimum value ratio, standard deviation (SD), coefficient of variation (CV) and broad-sense heritability (H^2). Data will be also subjected to analysis of variance (ANOVA) tests and, where significant ($P<0.05$) differences will be observed among accessions, means will be separated by the Student-Newman-Keuls multiple range test. Pearson linear coefficients of correlation (r) will be calculated from non-parametric regression analyses between pairs of descriptors for the traits. The traits will be subjected to frequency distribution. Z scores will be used to standardize the data and then use for principal component analysis (PCA). The extracted principal components (PCs) will be further used in the Ward's hierarchical clustering to assess the morphological/phenotypic diversity in buckwheat from northeast India. The experiment layout will be a Randomized Block Design (RBD) with three replicates per accession. All the analyses will be performed in SPSS.

Objective 2: *Biochemical characterization and identification in selected germplasm of buckwheat for anticancer activity*

Determination of total antioxidant capacity

The total antioxidant capacity will be determined by the TEAC method by Re et al. 1999, which is based on the capacity of antioxidants to capture the radical 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$).

DPPH assay

DPPH radical scavenging activity will be measured at 515 nm using a spectrophotometer. Results will be expressed in mM of ascorbic acid equivalent (AAE)/g dry weight.

ABTS assay

ABTS assay will be carried out at 734 nm using the spectrophotometer. Results will be expressed in mM of ascorbic acid equivalent (AAE)/g dry weight.

FRAP assay

FRAP assay will be conducted using the method of Wong et al. 2006 with some modifications. The increase in absorbance will be measured using spectrophotometer at 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the extracts will be calculated as mM GAE/g dry weight from the GAE-FRAP standard curve.

Superoxide anions scavenging activity

The superoxide anions scavenging activity will be measured by spectrophotometer at 560 nm against methanol as the control.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity will be determined by using 2-deoxyribose oxidation assay. Its absorbance will be measured with a spectrophotometer at 515 nm.

Nitric oxide activity

Nitric oxide will be generated from sodium nitroprusside and will be measured by the Greiss reaction. Finally, 2 ml of water will be added and absorbance will be taken at 546 nm.

Organic acid and sugar composition

Soluble solids content (SSC; %) will be measured with a hand held refractometer. Titratable acidity (TA) will be determined by titrating diluted juice with 0.1 mol/L NaOH to the phenolphthalein-end point and will be expressed as g/100 g of anhydrous citric acid. The SSC:TA ratio will be calculated from the SSC and TA values. Glucose, fructose and sucrose contents will be determine using a commercial kit. Analyses of malic and citric acids will be also performed with commercial enzymatic kits. Uronic acid contents will be determined spectrophotometrically using the m-hydroxybiphenyl method, using galacturonic acid as standard.

Total phenol estimation

Total phenolic compounds of fruit extracts will be determined by Folin-Ciocalteu method and absorbance will be measured at 765 nm.

Phenolic acids and flavonoids analysis

The polyphenolic composition will be determined by using HPLC system with a reversed-phase C_{18} column. The polyphenolic composition will be determined according to method as described by Uddin et al. (2014) with slight modification. The detection and quantification of gallic acid and caffeic acid will be done at 254 nm, chlorogenic acid, epigallocatechin gallate, vanillic acid, p-coumaric acid and naringin will be done at 280 nm, trans-ferullic acid and ferullic acid at 329 nm while quercetin and kaempferol will be detected at 370 nm.

Estimation of vitamin A (β-carotene)

Beta-carotene extraction will be performed using high performance liquid chromatography (HPLC) analysis.

Chemical compositions of gluten-free flours

Proximate composition analyses will be carried out in triplicate according to AOAC (2000) for dry matter (DM; method 930.15), ash (method 942.05), crude protein (method 976.05) and crude lipid (method 954.02 without acid hydrolysis) contents. Furthermore, the total starch and the total dietary fiber will be carried out using specific assay kits and following manufacturer’s procedure.

Determination of total phenolics and total anthocyanins

The total phenolic content (TPC) of flours will be determined colorimetrically according to the Folin-Ciocalteu assay (Rocchetti, Chiodelli, et al., 2017). Absorbance will be recorded at 765 nm after 40 min at 20°C. Total anthocyanin content will be evaluated exploiting the pH differential method (Wrolstad, Durst & Lee, 2005).

Determination of total flavonoid contents

Total flavonoid content of the extracts will be investigated using the aluminum chloride colorimetric method (Chang, Yang, Wen, & Chern, 2002).

Rutin and quercetin concentration analysis

Rutin and quercetin concentrations of the extracts will be measured using high-performance liquid chromatography. The analysis will be performed on a C_{18} column with degassed mobile phase, a mixture of 1% phosphoric acid solution and methanol.

Determination of amino acid composition

Amino acids of the buckwheat flours will be extracted using 8% trichloroacetic acid according to the method of Saikusa, Horino, and Mori (1994). HPLC measurement will be performed. The peaks will be detected at 338 and 262 nm.

Determination of diabetic enzyme inhibition of the phenolic extracts

Alpha-amylase inhibition activity will be identified according to the method of Thilagam, Parimaladevi, Kumarappan, and Mandal (2013) with minor modification.

Determination of fatty acid composition

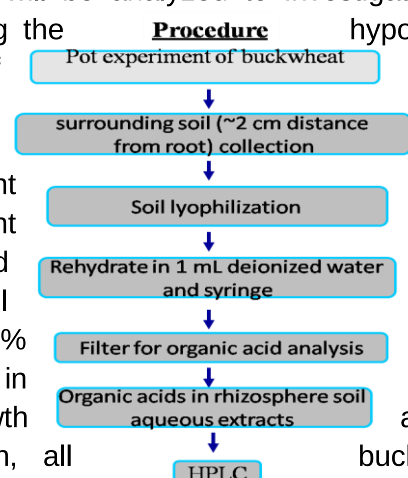
Free fatty acid composition of three buckwheat varieties will be determined using a gas-liquid chromatography (GLC) as reported by Hung, Maeda, Tsumori, and Morita (2007).

Statistical analysis

The value for each sample will be calculated as the mean ± SD. Each assay will be performed with three replications. Analysis of variance and significant difference among means will be tested by ANOVA with completely randomized design (CRD) of experiment. Pearson's correlation coefficients (r) will be calculated for the traits. Z scores will be used to standardize the data and then use for principal component analysis (PCA). The extracted principal components (PCs) will be further used in the Ward's hierarchical clustering to assess the grouping of landraces. All the analyses will be performed in SPSS. Clustering and PCA will reveal distinct groups of landraces exhibiting different ranges of anticancer agents which may be used for quality breeding.

Objective 3: *Evaluation for rhizosphere phosphorus enhancement activity from the selected germplasm of buckwheat*

In this study, the effect of buckwheat incorporation on bulk soil extractable P in control and added-P in pots will be determined to assess the role of buckwheat in increasing P availability for subsequent crops. Further, organic acid anions in buckwheat rhizosphere soil will be analyzed to investigate possible buckwheat soil-P acquisition mechanisms, specifically testing the hypothesis that buckwheat increases its access to soil P by exudation of organic acid anions. Different types of collected germplasm will be sown in the pot. Before sowing of seeds of buckwheat, soil will be analyzed for available phosphorus, pH, Al, K, Ca, Mg, N. Plant will be grown with uniform addition of manures and organic plant protectant chemicals. Two treatments (buckwheat with and without P fertilizer) each for individual selected germplasm will assigned to three replicate in each pot. Superphosphate (0-3-0, with ~25% of total P as orthophosphate), will be applied to added-P treatments in each pot under study. To determine differences in buckwheat growth among the treatments and observe possible organic-anion exudation, all buckwheat plants and surrounding soil (~2 cm distance from root) will be collected 21 days after germination. Root and shoot tissue will be separated. The roots of plants will be rinsed thoroughly, and ~1 g of rhizosphere soil (1 mm of soil surrounding roots) will retained for organic acid analysis following Fan et al. (1997). The collected rhizosphere soil will lyophilize immediately to prevent microbial degradation, rehydrate in 1 mL deionized water, and syringe- filter for organic acid analysis. Roots and



shoots will oven-dry (24 h at 50°C) and weighed. Analysis of organic acids in rhizosphere soil aqueous extracts will be completed by using a liquid-chromatography Changes in P availability over time and as a function of buckwheat treatments will analyze using Repeated Measures ANOVA, with pairwise analysis sampling dates using Tukey's Multiple Comparison Test.

Objective 4: *Impact of climate change on organic buckwheat production technology and mitigation strategies through community participation.*

Activity 1: Assess the yield and quality of seeds and other growth parameters of the buckwheat growing at different altitudes and in different regions of the Sikkim

Methodologies:

- Georeference survey and site selection at different location of each district of Sikkim
- Yield estimation of buckwheat (seed, haulm, harvest index)
- Yield attributing character assessment (plant height, branches/plant, spikes/plant, seed/spikes, 10000 seed weight, growth rate, above ground and below ground biomass)
- Yield and yield attributing characters monitoring
- Input-output relationship

Focus area of action: Yield & yield attributes of buckwheat

Performance indicator: Information on the climate change impact on buckwheat production

Activity 2: Assess ambient concentration of GHG's and soil health (nutrients and microbiology at different sites in the Sikkim State

Methodologies:

- Assessment of concentration of GHG's from the selected site
- Soil sample collection from the selected site
- Processing of soil samples
- Soil analysis
- Soil nutrient monitoring

Focus area of action: GHG's and soil health

Performance indicator: Information on the GHG's and soil health parameters

Activity 3: Delineate buckwheat growing areas with the help of RS and GIS technology.

Methodologies: RS and GIS based map as per the procedure

Focus area of action: RS and GIS

Performance indicator: map on buckwheat growing area

Activity 4: Adaptation strategies like screening of germplasm and adoption of climate resilient technology for mitigation

Methodologies:

- Collection of germplasm from different altitude and zones varying in temperature
- Screening of germplasm under plant growth chamber (temperate tolerate and cold tolerate)
- Conservation agriculture practices (mulching, zero tillage, minimum tillage, residue incorporation *etc.*) for mitigation of climate change impact

Focus area of action: screening and adaptation

Performance indicator: Information of climate resilient buckwheat germplasm

Objective 5: *Quality honey production with honey bee as a pollinator component in different regions of Sikkim through demonstration and training through participation approach*

Activity 1: Selection of villages and farmers to carry demonstration and training activities on quality honey production technology

Methodologies:

- Survey will be conducted to identify the villages with atleast 30% population of tribal communities and unemployed women from four districts of Sikkim (east, west, north and south)
- Two blocks/district, four villages/block and one-two SHGs/village of each districts of Sikkim will be selected
- The selected farmers will be assisted to form SHGs
- This activity will be carried out with the collaboration of KVKs of the respective district

Focus area of action: Identification of beneficiaries

Performance indicator:

- Information on the population structure of the village and their interest in honey bee rearing for quality honey production
- Identification, group and subgroup categorization of the beneficiaries into group of 20-25 farmers

Activity 2: Organization of 3-5 days on-campus and off campus training on cultivation of honey production in batches for 25 selected farmers

Methodologies:

- Preparation of training calendar so that the training programmes can be organized in different slots of year
- The hands on training will be conducted at ICAR-NOFRI, Gangtok where the farmers will be trained in detail on honey production through honey bee rearing and other necessary details if necessary
- The trained beneficiaries will be facilitated to take up quality honey production on a small scale at their own places.

Focus area of action: Induction of scientific knowledge and experience on quality honey production

Performance indicators:

- Total number of beneficiaries (number of women, number of tribal farmers)
- Total input distributed to the beneficiaries
- The performance of honey production capability of farmers on small scale

Activity 3: Development of a demonstration honey bee units at the ICAR-NOFRI for the honey growers

Methodologies:

- A small honey production unit (bee hives) will be built at ICAR-NOFRI, Gangtok for the purpose of demonstration to the farmers as well as production of quality honey

Focus area of action: Display of technology to the farmers

Activity 4: Establishment of honey bee units for quality honey at selected villages of different regions of Sikkim

Methodologies:

- Among the selected beneficiaries, all of will be strengthened on honey production through honey bee
- A centralized bee hives units will be maintained and honey bee will be distributed among the beneficiaries

Focus area of action: Development of infrastructure for sustaining the project

Performance indicator:

- Development of centralized honey bee supply unit
- Development of honey production unit through bee
- Production of quality honey from each selected villages

Objective I: Georeference survey, collection and morphological characterization of unexplored buckwheat germplasm

Abstract

Fifteen accessions of common buckwheat (*Fagopyrum esculentum*) and 21 of tartary buckwheat (*F. tataricum*) were studied for 15 quantitative and 21 qualitative traits to understand the nature and magnitude of genetic variability using multivariate approach. Significant variation for quantitative traits was recorded for seven traits in common buckwheat and for eleven traits in tartary buckwheat. In common buckwheat seed yield correlated positively with days to maturity while negatively associated with seed weight whereas in case of tartary type number of cymes/plant, seeds/cyme, days to flowering and days to maturity showed positive correlation with seed yield. First five and four principal components (PCs) respectively, in common and tartary type revealed majority of the variability in the accessions which further grouped the test accessions of both the species in to four clusters each. The accessions also showed considerable variation for qualitative traits.

Morphological Characterization

The analysis of variance revealed significant differences for plant height, number of internodes, number of leaves, 1000 seed weight, days to flowering, days to maturity and seed yield in common buckwheat while tartary type differed significantly for plant height, number of branches, leaf blade length and width, 1000 seed weight, shoot diameter, petiole length, days to 50% flowering, days to maturity and seed yield. Significant differences in the buckwheat germplasm for both common and tartary buckwheat have been reported in several studies worldwide. The extent of variability was high in tartary type accessions, which implies wider scope of employing selection based on more number of desirable traits. Summary statistics of both the species has been shown in Table 1. Heritability estimates in case of common buckwheat were high for plant height, number of internodes, number of leaves, days to flowering, days to maturity, seed weight, seed diameter and petiole length while in tataricum type plant height, number of branches, number of leaves, leaf blade width, days to flowering, days to maturity, seed weight, seed diameter, petiole length showed high heritability. Number of cymes per plant and seeds per cyme showed low heritability in both the species, as these traits are highly influenced by the environment. Characters showing high heritability in both the species respond to selection as these are least influenced by the environment, thereby improvement in these traits can be made by employing selection on individual plants selected in both the species for respective traits. Flowering started on 22nd day after sowing (50% flowering) in common buckwheat entries EC323730, EC125937 and EC58322 while entry EC125935 and EC58332 were earliest to attain maturity (75 days). Entry EC21874 grew tallest (90.40cm) whereas EC286396 recorded highest 1000 seed weight (33.08g). Entry PRB1 was the highest yielder (2388 kg/ha). In tartary type flowering started on 29th day after sowing in IC49669, IC108518 and Himpriya. Himpriya was earliest to attain maturity in 79 days. Maximum height attained by entry Shimla B1 (105.70 cm) whereas IC26600 recorded maximum 1000 seed weight (45.14 g). Entry IC109728 was the highest yielder (2247 kg/ha). PRB1 and VL7 were used as checks for common buckwheat whereas Himpriya and Shimla B1 for tataricum type. In case of tataricum type, accessions IC109728, IC26600, IC109729, Titey, IC15393, IC109433 and IC49671 were superior to both the checks for seed yield, however in case of common buckwheat none of the entries surpassed the checks for seed yield. Entry PRB1 and IC109728 can be recommended as high yielding varieties for buckwheat cultivation areas of Sikkim after multilocational trials. The frequency of twenty one qualitative characters in thirty six buckwheat accessions has been shown in Table 2 which show a variable range of characters. Majority of the entries were indeterminate type with semi erect type branch shoot habit. Red stem colour predominates in twenty eight accessions. Grey, brown, black and mottled seed colour exist in different frequencies (16:8:1:11). Leaf blade shape for all the accessions were of saggitate type. Except two entries, inflorescence was of non-branched type. Triangular seed shape recorded for 15 accessions while ovate type for rest of the entries. Except four buckwheat accessions, pink margin colour of the leaf was present in all the material. Lodging resistance was recorded for majority of the accessions as a desirable character in buckwheat particularly in common type for reaping high seed yield as this species has low seed set due to genetic and climatic factors. Buckwheat is an underutilized crop where the varietal development work is mainly hindered by the incompatible nature of common buckwheat and availability of very few literature on its genetic improvement. However, better crop production through agronomic practices have been reported for yield improvement. In short run, selection strategies may prove to be beneficial in both the species but realizing its potential as a climate resilient crop, improvement in yield particularly in common buckwheat is needed which is only possible through breeding strategies that overcome reproductive barriers for enhancing seed yield. Alternate breeding methodology for common buckwheat (*F. esculentum* Moench) involving the use of self-incompatibility (Sh) gene derived from *F. homotropicum* Ohnishi have been reported. The agricultural system has been witnessing effects of climate change thereby it is prerogative to explore potential crops like buckwheat. Conserving the genetic resources along with cataloguing and documentation after evaluation at multi locations will figure out the best adaptable lines in different locations. The in-situ and ex-situ conservation strategies needs to be strengthened and specific adaptations from diversity rich areas and their conservation on-farm is important especially in marginal and low-input areas of Himalayan region.

Table 1: Summary statistics of quantitative traits in common and tartary buckwheat

Character	Species type	Mean	Std Dev	Std Error	Variance	CV	Range	Heritability	Genetic Advance
Plant height (cm)	*F. esculentum*	80.13	6.89	0.25	47.49	8.61	67.00-93.40	0.714	8.991
	F. tataricum	86.90	12.15	1.87	147.66	13.98	65.20-105.70	0.742	16.537
No. of internodes	*F. esculentum*	7.25	0.99	0.18	0.99	13.73	5.20-9.60	0.820	1.571
	F. tataricum	13.08	1.09	0.16	1.19	8.36	9.60-15.00	0.486	0.893
No. of branches	*F. esculentum*	3.26	0.61	0.11	0.38	18.93	2.20-4.40	0.488	0.503
	F. tataricum	4.22	0.95	0.14	0.92	22.72	2.40-6.40	0.873	1.636
No. of leaves	*F. esculentum*	22.28	4.02	0.73	16.17	18.04	13.60-32.00	0.643	4.378
	F. tataricum	41.21	9.36	1.44	87.67	22.76	23.00-63.40	0.782	13.821
Leaf blade length (cm)	*F. esculentum*	6.37	0.45	0.08	0.20	7.16	5.54-7.24	0.019	0.013
	F. tataricum	5.33	0.49	0.07	0.24	9.19	4.54-6.52	0.590	0.502
Leaf blade width (cm)	*F. esculentum*	5.28	0.36	0.06	0.13	6.84	4.68-6.08	-0.612	-0.283
	F. tataricum	5.11	0.71	0.10	0.50	13.90	4.10-7.16	0.741	0.959
Length of cyme (cm)	*F. esculentum*	5.17	0.58	0.10	0.34	11.37	3.52-6.08	0.405	0.396
	F. tataricum	4.95	0.95	0.14	0.90	19.25	2.94-7.16	0.514	0.816
No. of cymes/plant	*F. esculentum*	25.15	5.62	1.02	31.59	22.34	19.00-46.60	-0.214	-1.607
	F. tataricum	32.02	6.58	1.01	43.34	20.55	19.80-49.20	0.298	2.985
Seeds/cyme	*F. esculentum*	12.35	2.47	0.45	6.12	20.03	6.40-18.40	0.321	1.283
	F. tataricum	15.37	3.02	0.46	9.16	19.68	10.60-25.80	0.028	0.118
Days to 50% flowering	*F. esculentum*	23.40	0.89	0.16	0.80	3.82	22.00-25.00	0.878	1.536
	F. tataricum	38.26	5.39	0.83	29.12	14.10	29.00-52.00	0.991	11.088
Days to maturity	*F. esculentum*	78.46	2.09	0.38	4.39	2.67	75.00-83.00	0.961	4.089
	F. tataricum	88.95	6.06	0.93	36.76	6.81	79.00-108.00	0.982	12.263
Seed yield (kg/ha)	*F. esculentum*	1521.11	391.16	71.41	153010.64	25.71	1005.56-2388.00	0.680	81.454
	F. tataricum	1543.52	504.28	77.81	254299.78	32.67	383.33-2247.00	0.532	83.011
1000 seed weight (gm)	*F. esculentum*	28.06	2.07	0.37	4.31	7.40	24.50-33.08	0.995	4.323
	F. tataricum	26.87	5.61	0.86	31.49	20.88	19.78-45.14	0.999	11.681
Stem diameter (mm)	*F. esculentum*	4.90	0.38	0.07	0.14	7.86	4.04-5.57	0.954	0.752
	F. tataricum	4.43	0.52	0.08	0.27	11.73	3.50-5.50	0.993	1.075
Petiole length	*F. esculentum*	4.23	1.24	0.22	1.55	29.47	2.12-7.60	0.998	2.606

(cm)	*F. tataricum*	3.68	1.05	0.16	1.11	28.73	2.10-6.50	0.997	2.194

Table 2: Frequency of qualitative characters for common and tartary buckwheat accessions

Descriptors	Features	Frequency ratio
Cotyledon/seedling leaf colour	3 green, 5 pink, 7 red	36:0:0
Growth and branch shoot habit	3 semi-erect, 5 semi-erect longer, 7erect longer, 9 erect longer	36:0:0:0
Degree of determination	1 indeterminate, 5 intermediate, 9 determinate	33:0:3
Plant branching	1 very weak, 3 weak, 5 intermediate, 7 strong, 9 very strong	0:23:12:1:0
Stem colour	3 green, 5 pink, 7 red	0:8:28
Lodging suspect	1 very resistant, 5 intermediate, 9 very susceptible	31:5:0
Leaf colour	3 green, 5 pink, 7 red	36:0:0
Leaf margin colour	3 green, 5 pink, 7 red	4:32:0
Leaf vein colour	3 green, 5 pink, 7 red	27:9:0
Petiole colour	3 green, 5 pink, 7 red	17:10:9
Leaf blade shape	1 ovate, 2 hastate, 3 sagittate, 4 cordate,	0:0:36:0
Compactness of inflorescence	3 cyme loose, 5 cyme semi compact, 7 cyme compact	20:13:3
Branched inflorescence	0 Yes, 1 No	2:34
Colour of inflorescence stalk	3 green, 5 pink, 7 red	2:34:0
Flower colour	1 white, 3 greenish yellow, 7 pink, 9 red	7:21:8:0
Flower abortion	3 low, 5 intermediate, 7 high	22:11:3
Seed colour	3 grey, 5 brown, 7 black, 9 mottled	16:8:1:11
Seed shape	1 triangular, 2 ovate, 3 conoidal	15:21:0
Seed surface	1 smooth, 2 irregular/wrinkled, 3 other	15:21:0
Seed quality	3 poor, 5 intermediate, 7 good	0:7:29
Threshability	3 difficult, 5 intermediate, 7 easy	9:9:18

Objective II: Biochemical characterization and identification in selected germplasm of buckwheat for anticancer activity

Abstract

Buckwheat can be used as nutritious and energizing foods which contribute nutritional and food security. After the analysis of the different nutritional quality attributes of various buckwheat genotypes the result revealed that the accession along with the released variety were found to have superior desirable nutritional quality parameters in comparison with local variety Titey. Protein content was highest in Titey (11.2%) followed by Shimla B-1 (11.1%) and lowest in IC49671 & IC108510 (10.4%). Significant variation was estimated for tryptophan content for various genotypes ranges from 66.3 to 76.9 mg/g N. The crude fibre content showed the range

of variation from 3.71 to 4.78 % in dry mature grains of tartary buckwheat. %. Maximum nitrogen free content was recorded in IC108518 (76.97%) followed by IC202268 (75.37%) and lowest was found in IC36805 (71.41%). Maximum Fe content was found in genotype IC108518 (3.50) followed by IC109549 (3.49) and lowest was recorded in genotype IC49671 (2.50 mg/100g). Similarly, total phenol content ranged from 378.41 to 652.71 mg GAE/100g with an average content of 518.68 mg GAE/100g. Therefore, development and utilization of such functional foods will not only improve the nutritional status of the population but also helps those suffering from degenerative diseases. Wide variations among genotypes for quality attributes have shown ample potential to be exploited for further improvement of desirable quality attributes and other properties.

Variation in biochemical constituents of tartary buckwheat

The data on variation in biochemical constituents of various genotypes of tartary buckwheat (*Fagopyrum tataricum*) are presented in Table 1. The grain samples of selected sixteen tartary buckwheat genotypes were evaluated using standard biochemical methods. The moisture content roughly indicates the degree of maturity and accumulation of different nutrients in food crops. It is an important criterion contributing towards acceptability of the crop harvest. The moisture percentage of food grains is an important consideration for domestic consumption as well as large scale storage. Among the different tartary buckwheat (*F. tataricum*) accessions it was noticed that IC24301 and VL-7 contributed highest moisture content (11.2%) and lowest in IC108518 (10.4%). Similarly, protein content was highest in Titey (11.2%) followed by Shimla B-1 (11.1%) and lowest in IC49671 & IC108510 (10.4%). The fat content was maximum in Titey, IC49669, IC108510, Shimla B-1 (2.6%) and lowest in IC49671 and IC202268 (1.8%). Similarly, ash content was highest in VL-7 (2.6%) and lowest in IC49669, IC49671, IC108510 & Himpriya (1.8%). Crude fibre was maximum in IC49671 and IC109728 (7.8%), and minimum in IC108518 and IC49669 (7.1%). Carbohydrate content of tartary buckwheat genotypes ranged significantly from 61.2 to 66.9 per cent. The maximum value for carbohydrate content was observed in accession IC108518 and IC108510 (66.9%), and the minimum in IC202268 (61.2%). Raghuvanshi et.al., (2021) reported 66.01 to 72.89% carbohydrate in buckwheat flour from India which was in similar conformity with the present study. Tryptophan is an essential amino acid involved in various metabolic processes in the human body. Significant variation was estimated for tryptophan content for various genotypes ranges from 66.3 to 76.9 mg/g N. The maximum value for the tryptophan content was found in IC243184 (76.9 mg/g N) followed by PRB-1 (76.7) and minimum in IC108518 (66.3). The values obtained in the present investigation with regard to released varieties viz., VL-7, Shimla B-1, PRB-1 recorded the highest value for moisture content, protein, fat, ash and tryptophan content and lowest value was recorded in Himpriya for ash content. David and Persis, (2000) reported the lower moisture content of buckwheat flour justifies the suitability for long term storage without deterioration. Sato *et al*., (2001) estimated the protein content in the tartary buckwheat flour ranges from 10.32 to 13.84 per cent. The chemical composition of grain, bran and flour of tartary buckwheat was analysed by Bonafaccia et al., (2023) and found 2.81 % ash content in grains, 4.97 % in bran and 1.8 % in the flour. Wide variations among genotypes for quality attributes have shown ample potential to be exploited for further improvement of desirable quality attributes and antioxidant properties. The genotypes superior in individual quality trait i.e., protein, essential amino acids methionine, tryptophan, dietary fibre in grains were identified.

Nutritional quality of various genotypes of tartary buckwheat

Significant difference was observed for their proximate composition of buckwheat germplasm, *viz.*, moisture content, crude protein, crude fat, crude fibre, ash content and nitrogen free extract, starch, amylose and resistant starch. The data on variation in nutritional quality of various genotypes of tartary buckwheat (*Fagopyrum tataricum*) are presented in Table 2. Value in respect to moisture content in tartary buckwheat genotypes ranges from 7.52 to 9.11%. Shimla B-1 (9.11 %) recorded the highest moisture content followed by IC49671 (9.03 %) and lowest in PRB-1 (7.52%). Similarly crude fat content was highest in Himpriya (3.62%) and lowest was recorded in Shimla B-1 (1.97%). The crude protein content varied from 7.23 to 9.53 %. Maximum crude protein content was recorded in accession IC36805 (9.53%) and lowest in PRB-1 (7.23%). Ash content was highest in IC49669 (2.93%) followed by IC109728 (2.88%) and lowest was recorded in IC108510 (1.83%). The crude fibre content showed the range of variation from 3.71 to 4.78 % in dry mature grains of tartary buckwheat. Highest crude fibre content was found in accession IC109549 (4.78%) and lowest was recorded in Himpriya (3.71%). The buckwheat germplasm under study were found poor in their protein and fibre content then the values reported earlier. Raghuvanshi et al., (2021) reported crude fat, crude protein, total ash and crude fibre in buckwheat flourin the range of 0.75 to 2.33%, 10.43 to 11.23%, 1.82 to 3.10% and 3.53 to 4.80%, respectively. Zheng *et al.* (1998) dehulled buckwheat groats were found to contain 75% starch, 13.9% protein, and 2.3% lipid. The ash content of buckwheat flour (2.32%) observed in this study is comparable with that reported by Mota et. al., (2019). Average ash and crude fibre were comparatively higher in the buckwheat accessions. Crude fibre and ash were found higher than the values obtained in this study. Such variations in the Proximate composition in different germplasm may be attributed to their genetic variation, their stage of maturity, climate and soil conditions from where the samples were collected as well as methods of determination. Nitrogen-free extract (NFE) consist of carbohydrates, sugar, starches, and a major portion of materials classed as hemicelluloses in food and feeds. NFE is calculated by difference. When the sum of crude protein, fat, moisture, ash and fibre is subtracted from 100, the difference is NFE. As NFE is calculated by difference, all the errors associated with proximate analysis are additive in the estimate of nitrogen- free extract. Nitrogen free content of tartary buckwheat genotypes ranged significantly from 71.41 to 76.97%. Maximum nitrogen free content was recorded in IC108518 (76.97%) followed by IC202268 (75.37%) and lowest was found in IC36805 (71.41%). Hussain *et.al*. (2017) Reported NFE contents of common buckwheat flour as 68.27%. Average NFE in buckwheat genotype in the present study was 74.08% (Table 2). Starch is the major component of buckwheat endosperm and is composed of 25% amylose and 75% amylopectin. Evaluation of genotypes for starch content revealed significant variation from 63.18 to 72.61%. Accession IC36805 (72.61%) recorded the highest starch content followed by IC108510 (72.48%). Soral-Smietana et.al., (2022) reported relatively high amylose content (42-52%) in buckwheat samples. Maximum amylose content was recorded in IC36805 (24.00%). Resistant starch (RS) is a form of starch that is not digested in the small intestine but have unique health benefits that include glycemic control, control of fasting plasma triglyceride and cholesterol levels and absorption of minerals. Similarly resistant starch was recorded highest in accession IC202268 (20.53%) and lowest was recorded in IC26600 (15.20%). Skrabanja et.al., (2021) reported 7-37% of RS in buckwheat seeds. The RS content of sixteen buckwheat genotypes was in the range of 15.20-20.53% with an average content of 17.64% (Table 2). The genotypes differed significantly in RS content in buckwheat genotypes might be attributed to the varietal differences along with other inherent factors such as granular structure, amylose contains amylose retrogradation, amylopectin chain length distribution and amylose-lipid complexes.

Total soluble protein and its fractions of F. esculentum genotypes

The total soluble protein and its fractions of F*. esculentum* genotypes (% dry weight) were presented in Table 3. From the above data revealed that highest total soluble protein was found in accession IC188701 (7.40%) followed by Shimla B (7.35%) and lowest was recorded in EC18864 (4.58%). Soluble protein level reflects the proteins available in diet. The total soluble protein content ranged from 4.58% to 7.40% with an average of 6.29% for all the sixteen buckwheat genotypes tested. Marshal and Pomeranz (1982) reported protein content in whole buckwheat and buckwheat groates as 13.80 and 16.40%, respectively which was quite higher than the reported value. Such variation might be due to the differences in the varieties, environment and nutrient status of the soil. Value in respect to albumin content in tartary buckwheat genotypes ranges from 17.05 to 21.55 %. Maximum albumin content was recorded in accession EC18864 (21.55 %) and lowest was found in EC218784 (17.0%) with an average of 18.48%. Globulin was found as the major protein fraction in *F. esculentum* followed by glutelin, albumin and prolamin. Significant variation was estimated for globulin content for various genotypes ranges from 50.47 to 55.30%. Maximum globulin was found in EC218784 (55.30%) followed by EC125937 (54.94%) and variety Shimla B (55.30%) recorded the lowest globulin content. Marshal and Pomeranz (2021) studied that in buckwheat storage globulins account for about 70% of total proteins in the seeds and albumin fraction range from 18 to 32% of total protein depending on cultivar are in agreement with the present study. Maximum prolamin content was recorded in variety Himpriya (3.95%) followed by accession EC18864 (3.87%) with an average of 3.27%. Significant variation was estimated for glutelin content for various genotypes ranges from 25.94 to 22.20 %. Similarly, variety Shimla B (25.94%) recorded the maximum glutelin content followed by IC188701 (25.59%) and lowest was found in EC125935 (22.20 %). Javornik et. al., (2022) reported buckwheat protein consists of 18.2% albumin, 43.3% globulin, 0.8% prolamin, 22.7% glutelin, and 5.0% other nitrogen residue are in similar conformity with the present studied and found higher albumin, globulin, prolamin and glutelin content than the reported value. Maximum extraction efficiency was estimated in genotype EC216631 (90.26%) followed by EC18864 (90.17%) and lowest was recorded in IC188701 (85.54%) with an average extraction efficiency of 88.14%.

Mineral and phytochemical contents of *F. tataricum* genotypes

In the present investigation, five minerals, Ca, P, Fe, Na, and K were estimated and results are presented in Table 4. Maximum Ca content was found in variety VL-7 (215.33) followed by accession IC26600 (210.32) and lowest was recorded in IC109549 (144.00) with an average of 177.57 mg/100g. Awasthi and Thakur (2010), however, reported lower Ca values, but much higher P values for the released varieties Himprya, PRB-1, Shimla-1 and VL-7. Similarly, P content was found maximum in IC109549 (282.00) followed by Titey (281.00) and lowest was found in variety VL-7 242.61 mg/100g dry weight. Ikeda et.al., (2019) reported phosphorous content in buckwheat flour ranges from 265 to 510mg/100g with an average of 359 mg. Results reported by Mota et.al., (2019) also observed similar pattern in mineral contents of buckwheat genotypes. Significant variation was estimated for Fe content for various genotypes ranges from 2.50 to 3.50 mg/100g. Maximum Fe content was found in genotype IC108518 (3.50) followed by IC109549 (3.49) and lowest was recorded in genotype IC49671 (2.50 mg/100g). Similarly, K content was found maximum in IC26600 (298.27) followed by PRB-1 (284.60) and lowest was found in variety IC24301 237.00 mg/100g with an average of 257.68 mg/100g dry weight.

Significant variation was estimated for Na content for various genotypes of *F. tataricum*. Maximum Na content was found in genotype Himpriya (4.24) followed by IC108518 (4.07) and lowest was recorded in variety Shimla B-1 (1.56) with an average of 2.55 mg/100g. Buckwheat has varied total phenolic content depending on the method of extraction, and calibration standard used, i.e. gallic acid or ferulic acid. Maximum total phenol content was found in Himpriya (652.71) followed by IC108518 (640.33) and lowest was recorded in genotype IC49671 (378.41). Similarly, total phenol content ranged from 378.41 to 652.71 mg GAE/100g with an average content of 518.68 mg GAE/100g (Table 4). Buckwheat also has high levels of flavonoids. In the present study, total flavonoids content ranged between 33.80 to 60.11 mg QE/100g with an average content of 46.57 mg/100. Maximum flavonoids content was found in accession IC108518 (60.11) followed by IC202268 (54.42) and lowest was recorded in genotype IC49671 (33.80). The flavonoids contents in present study were found to be slightly higher than the value reported by Jiang et.al., (2019). Such variations might be mainly because of the methods, solvent and calibration standards used for estimating flavonoid contents and the genetic makeup of the materials. It observed that variation in mineral contents in buckwheat genotypes might be attributed to the varietal differences, stage of maturity, condition of growth, fertilization and the nutrient status of the soil. Buckwheat seeds are good sources of many phenolic compounds, particularly phenolic acids and flavonoids.

Conclusion

The buckwheat grains were found to have satisfactory nutritional profile of the sixteen buckwheat germplasm used in the study. Buckwheat protein contains well-balanced amino acids with high biological value compared to other cereal proteins. Buckwheat has also abundant source of dietary minerals like calcium, zinc, copper, and manganese. Its leaves are rich source of β-carotene and calcium and can be effectively used for micronutrient deficiencies of the population. Buckwheat flour is a good source for diabetic patients due to low glycemic index.

Table 1. Variation in biochemical constituents of tartary buckwheat

S.l. No.	*F. tataricum*	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	Carbohydrate (%)	Methionine (mg/g N)	Tryptophan (mg/g N)
1.	Titey	10.9	11.2	2.6	1.9	7.2	63.4	90.5	75.1
2.	IC109549	10.5	10.8	2.1	2.5	7.6	63.7	88.5	74.6
3.	IC108518	10.4	10.6	2.4	2.1	7.1	66.9	77.8	66.3
4.	IC49669	10.7	10.7	2.6	1.8	7.1	65.4	85.6	71.6
5.	IC24301	11.2	11.0	2.3	2.3	7.5	61.4	84.9	73.1
6.	IC26600	10.9	10.9	2.2	2.4	7.6	64.5	79.5	67.1
7.	IC49671	10.6	10.4	1.8	1.8	7.8	62.5	90.6	70.9
8.	IC36805	10.8	10.8	1.9	2.5	7.2	63.4	88.4	72.9
9.	IC108510	11.1	10.4	2.6	1.8	7.7	66.9	79.3	68.8
10	IC243184	11.0	10.6	2.1	2.1	7.2	66.1	80.6	76.9
11	IC202268	11.1	10.5	1.8	2.2	7.3	61.2	86.5	70.5
12	IC109728	10.6	10.7	1.9	2.4	7.8	64.5	81.7	69.4
13	Himpriya	10.9	10.5	2.4	1.8	7.4	63.5	86.7	68.4
14	PRB-1	10.5	11.0	2.3	2.3	7.6	64.8	88.4	76.7
15	Shimla B-1	11.1	11.1	2.6	1.9	7.5	65.6	89.6	66.7
16	VL-7	11.2	10.7	2.1	2.6	7.2	62.3	83.4	69.4
	Mean	10.8	10.7	2.2	2.2	7.4	64.1	85.1	71.2
	SE (±m)	0.08	0.07	0.06	0.05	0.06	0.11	0.28	0.03
	CD (5%)	0.20	0.21	0.17	0.15	0.17	0.32	0.88	0.86

Table 2: Proximate composition, total carbohydrate, starch, amylose and resistant starch content of *F. tataricum* genotypes (% dry weight)

S.l. No.	Germplasm	Moisture	Crude Fat	Crude protein	Ash	Crude fibre	Nitrogen free	Starch	Amylose	Resistant starch
1.	Titey	8.26	2.26	9.38	2.15	4.49	73.44	68.27	22.51	18.70
2.	IC109549	8.05	3.36	8.39	1.87	4.78	73.56	63.18	22.96	18.69
3.	IC108518	7.87	1.98	7.33	1.93	3.90	76.97	70.25	23.57	15.65
4.	IC49669	8.65	3.13	9.11	2.93	3.88	72.29	69.28	23.49	16.40
5.	IC24301	8.27	2.37	8.99	2.75	3.78	73.82	69.44	23.20	15.59
6.	IC26600	7.90	2.89	9.29	1.93	4.60	73.38	68.40	23.04	15.20
7.	IC49671	9.03	1.99	8.38	2.00	4.70	73.89	71.93	23.90	18.32
8.	IC36805	8.50	3.27	9.53	2.67	4.61	71.41	72.61	24.00	15.71
9.	IC108510	7.55	2.33	8.58	1.83	4.56	75.12	72.48	23.02	20.03
10.	IC243184	8.00	2.41	7.98	2.57	4.19	74.84	68.42	23.09	16.99
11.	IC202268	7.88	2.12	8.30	2.23	4.09	75.37	65.62	22.45	20.53
12.	IC109728	8.10	3.40	7.85	2.88	4.30	73.46	68.90	23.73	16.72
13.	Himpriya	8.72	3.62	7.30	1.88	3.71	74.77	69.35	23.26	19.89
14.	PRB-1	7.52	3.41	7.23	2.39	4.74	74.70	70.34	23.60	19.07
15.	Shimla B-1	9.11	1.97	8.09	2.73	3.91	74.17	68.90	22.95	19.27
16.	VL-7	7.59	2.99	8.79	2.32	4.16	74.13	70.55	22.61	15.40
17.	Mean	8.18	2.72	8.40	2.32	4.28	74.08	69.22	23.21	17.64
18.	$CD_{0.05}$	0.171	0.173	0.156	0.193	0.0325	0.428	0.242	0.0936	0.545
19.	S.Ed	0.084	0.085	0.077	0.095	0.016	0.210	0.119	0.046	0.268

Table 3: Total soluble protein and its fractions of F. *esculentum* genotypes (% dry weight)

Germplasm	Total protein	Albumin	Globulin	Prolamin	Glutein	Total	Extraction efficieny (%)
PRB1	7.10	17.96	54.53	3.56	23.95	6.18	87.04
VL7	6.80	18.26	54.78	3.07	23.89	5.86	86.18
IC202226	4.80	17.88	54.12	3.53	24.47	4.25	88.54
EC218784	5.40	17.05	55.30	3.53	24.12	4.81	89.07
EC218742	7.30	17.86	54.81	3.73	23.60	6.44	88.18
EC286936	6.16	18.86	53.66	2.38	25.09	5.46	88.64
EC272442	5.62	17.91	53.92	3.42	24.75	4.97	88.43
IC188701	7.40	17.38	54.82	2.21	25.59	6.33	85.54
EC125937	7.15	17.83	54.94	3.50	23.73	6.28	87.83
EC125935	6.46	20.07	54.35	3.37	22.20	5.63	87.15
EC58332	6.16	17.73	54.30	2.56	25.41	5.47	88.80
EC323730	6.42	17.69	54.64	3.50	24.17	5.71	88.94
EC18864	4.58	21.55	51.33	3.87	23.24	4.13	90.17
EC216631	5.34	19.09	54.56	3.73	22.61	4.82	90.26
Himpriya	6.56	17.32	50.77	3.95	27.96	5.83	88.87
Shimla B	7.35	21.23	50.47	2.36	25.94	6.36	88.53
Mean	6.29	18.48	53.83	3.27	24.42	5.53	88.14
$CD_{0.05}$	02709	0.055	0.178	0.0203	0.067	-	-
S.Ed(±)	0.133	0.027	0.087	0.010	0.033	-	-

Table 4: Mineral and phytochemical contents of *F. tataricum* genotypes (mg/100g dry weight)

Germplasm	Mineral content					Phytochemical content	
	Ca	**P**	**Fe**	**K**	**Na**	**Total phenol content (mg GAE/100g)**	**Total flavonoid content (mg QE/100g)**
Titey	148.33	281.00	3.31	248.00	2.22	540.19	48.84
IC109549	144.00	282.00	3.49	247.67	2.56	523.15	44.13
IC108518	169.00	243.00	3.50	251.67	4.07	640.33	60.11
IC49669,	156.00	263.00	3.29	248.33	1.97	442.61	40.22
IC24301	152.64	265.60	2.80	237.00	1.89	582.10	43.51
IC26600	210.32	274.00	3.14	298.27	3.52	461.33	43.20
IC49671	181.67	271.00	2.50	256.67	2.06	378.41	33.80
IC36805	179.30	265.00	2.84	250.11	2.00	538.19	51.92
IC108510	176.00	261.60	2.93	245.00	1.88	425.77	40.28
IC243184	187.60	260.30	3.43	246.50	1.93	533.00	51.97
IC202268	205.60	262.70	3.28	242.00	1.76	577.81	54.42
IC109728	192.31	259.30	3.30	254.00	2.23	523.08	51.90
Himpriya	174.00	266.00	3.15	267.33	4.24	652.71	49.62
PRB-1	184.00	255.00	2.83	284.60	3.53	546.67	44.91
Shimla B-1	165.00	272.60	3.37	280.33	1.56	554.33	51.30
VL-7	215.33	242.61	3.03	265.33	3.36	379.20	36.06
Mean	177.57	264.07	3.14	257.68	2.55	518.68	46.57
$CD_{0.05}$	7.487	14.543	0.145	6.652	0.305	27.721	4.271
S.Ed(±)	3.676	7.140	0.071	3.266	0.150	13.609	2.097

Objective III: Evaluation for rhizosphere phosphorus enhancement activity from the selected germplasm of buckwheat

Indian soils are deficient in phosphorus (nearly 49 per cent). In soil system P is very immobile and thus in acid soil fixation is a great problem. Soils of Sikkim are acidic in nature. Sustainable management of limiting/fixed soil P in acid soil can be enhanced by buckwheat cultivation. In extracting soil phosphorus of low availability from the acid soil, it is an efficient crop and this is possibly through the mechanisms such as enzyme and organic acid exudation, root-associated mycorrhizae, root acid, organic ligands, nutrient scavenging activity *etc*. These different mechanisms promote P release and/or compete for P adsorption sites. On the second and third sampling dates, average Bray soil P levels (l g [g dry soil]$^{-1}$) across all treatments did not differ from each other or the initial levels ($p > 0.05$). Phosphorus levels declined significantly (1.3-fold decrease) in all treatments between the third (at buckwheat harvest) and fourth (after mowing or tilling) sampling dates ($p < 0.05$). No significant differences in extractable soil P as a function of buckwheat treatment over time ($F_{2, 47} = 0.59$, $p = 0.57$), interaction between buckwheat treatments and time ($F_{6, 47} = 0.46$, $p = 0.77$), or post-buckwheat manipulation treatment ($F_{1, 23} = 3.14$, $p = 0.09$) were detected. At the time of buckwheat harvest, there were no significant differences in extractable soil P, buckwheat dry shoot, or dry root mass between control and added-P plots, suggesting that, if P availability was limiting for growth, compensatory P acquisition by buckwheat was present in unamended plots. Oxalic acid (protonated oxalate^{2-} at mobile phase pH, 3.8), if present in the samples, was not detected (with instrumental detection limit for oxalic acid of 0.5 l g mL^{-1}). However, tartaric acid ($C_6H_4O_5$, MW = 150 g mol^{-1}) was detected in rhizosphere-soil extractions by its retention time (6.7 min) and its mass spectrum (mass 149, negative ion mode). Tartaric acid was 15 times greater in rhizosphere samples from control plots ([0.30 ± 0.07] l g [mL soil solution]$^{-1}$) than added-P plots ([0.02 ± 0.05] l g mL^{-1}), a significant difference (Table 1). The absence of oxalic acid (protonated oxalate^{2-}) in buckwheat rhizosphere soils could be attributed to sufficient P availability. However, the presence of tartaric acid in rhizosphere soil adds additional information to the understanding of buckwheat–rhizosphere soil interactions. Significant differences in rhizosphere tartrate^{2-} concentration between low-P and fertilized plots suggest that this acid anion may be produced by buckwheat as a response to low soil-P availability. Lack of difference in buckwheat biomass between the treatments suggests that this may be a compensatory mechanism to enable greater P uptake. This study reveals a possible new mechanism for the enhanced P-uptake capabilities of buckwheat. The identification of tartrate^{2-} exudation as a potential P-uptake mechanism in buckwheat may guide development of improved buckwheat varieties and cover crop-management strategies for more sustainable soil-P management in agro-ecosystems.

Table 1. Mean (± SD) soil-P availability, dry buckwheat plant biomass, and rhizosphere tartaric acid concentration at the time of buckwheat harvest (21 d after germination). N = 4 for each treatment with exception of rhizosphere tartaric acid concentration (N = 3). Within the same variable, means with different letters differ significantly (p < 0.05); dm = dry matter.

Variable	**Added P**	**No Added P**	**Control**	**Statistic$_{(d.f)}$**	***P* value**
Soil-P availability / l g (g dry soil)$^{-1}$	3.90(±0.59)^{a}	4.59 (± 0.66)^{a}	4.31 (± 0.39)^{a}	$F_{2,11}$ = 1.65	0.245
Buckwheat shoot biomass / g dm plant^{-1}	3.90(±0.59)$_{a}$	0.33 (± 0.06)^{a}	-	$F_{1,7}$ = 1.39	0.285
Buckwheat root biomass / g dm plant^{-1}	0.26(±0.08)^{a}	0.26 (± 0.07)^{a}	-	$F_{1,7}$ = 0.009	0.927
Rhizosphere tartaric acid concentration / µg g^{-1}	0.24(±0.07)^{a}	0.56 (± 0.03)^{b}	-	$F_{1,6}$ = 53.05	< 0.005

Quantifiable outputs

As a cover crop, buckwheat increased soil-P availability. Buckwheat was grown in low-P and P-fertilized field plots, and organic anions were measured in rhizosphere soil. Soil-P availability was not affected by buckwheat, but the concentration of rhizosphere tartrate^{2-} was significantly higher (p < 0.005) in low-P *vs.* P-fertilized plots. This suggests that organic-anion root exudation may have a role in buckwheat-rhizosphere P dynamics. The local germplasm was very much efficient to increase the soil-P as compared with another germplasms and hence, local Mithey and Titey should be promoted in mid hill of Sikkim to increase the phosphorus use.

Objective IV: Impact of climate change on organic buckwheat production technology and mitigation strategies through community participation

Abstract

Buckwheat is an important crop of the mountain regions at elevations above 1400 m amsl for grain and green leaves. In the higher Himalayas, up to 4500 m, this is the only crop which can be grown successfully. There are two species of buckwheat cultivated in the Himalayas hills (*F. esculentum* and *F. tataricum*). Due to their multiple uses, short growing periods, high nutritional and medicinal values, wide adaptability has become popular not only to researcher but also for farmers and consumers globally. It grows in a wider range from low to higher mountains of Sikkim. Maxent modelling was used to quantify the current suitable habitat and predicted future suitable area under different climate change scenarios, based on representative concentration pathways in two different time periods (2040 and 2060 AD) using climatic predictive variables and species localities. The most suitable habitat area of cultivation, area loss, and gained for

buckwheat were determined. The model showed that about 59.2% area of state is climatically suitable for buckwheat within the elevation range of 502-4381 m. Habitat suitability of buckwheat would shrink by 6.6% and 7.3% under RCPs 4.5 and by 6.7% and 7.4% under 8.5 RCPs in the year 2040 and 2060, respectively. The loss and gain area analysis also indicated that suitable area would be lost more than gained in both 2040 and 2060. These findings are expected to support planning and policy framing for climatic resilience smart agriculture practice to meet the livelihood and food security problems in the mountains of Sikkim Himalaya. Due to medicinal value of buckwheat, its demands are increasing. The increasing demands of buckwheat would be fulfilled by increasing production under climatically suitable area under climate change scenarios.

Distribution of suitable habitat

Under current climatic conditions, the overall distribution of most suitable habitat of buckwheat was predicted within 502-4381 m elevations; however, major suitable occurred in the mid hill region of the Sikkim state excluding lowland and upper elevation zone. At present scenario total suitable habitat for buckwheat cultivation was 59.2.6% of Sikkim including cultivated land, shrub, bare and grass land of the state. Physiographically, the predicted potential suitable habitat suggested that the most suitable areas for buckwheat were in the West Sikkim. Similarly, mid hills and lower parts of mountain regions of central and western part of the state showed better suitability than eastern and northern parts of the state.

Future distribution prediction

The elevation range of predicted suitable habitat for buckwheat from the model indicated that habitat suitability would slightly shrink in uppermost elevation and expand towards the lower most elevation zone in future scenarios with different RCPs for 2040 and 2060. The number of pixels within different elevation of Sikkim was counted under all RCPs to predict climatically most suitable area. Elevation wise predicted habitat suitability having probabilities with more than 50% indicated that 1200-2400 m elevation has most suitable habitat for buckwheat followed by 2400–3600 m with greatest numbers of concentration of pixels (potential suitable area or predicted area).

Changes on suitable area in future scenarios

Potential suitable habitat of buckwheat under changing climatic scenarios in future is discussed here. Across all scenarios in two different periods, the predicted suitable area, changing scenarios of stable area and quantification of loss, gain and net loss or gain of area in each individual case is presented. This model clearly identified that suitable area for buckwheat would shrink in future with respect to current habitat under all RCPs for in 2040 and 2060. Under all

RCPs the net shrinkage would range from 1.9 to 4.3% by 2040 and 1.2 to 3.4% by 2060. Much shrinkage would occur in eastern part; however, the area of gain and loss showed mixed pattern of increasing and decreasing under different RCPs for the periods between 2040 and 2060. This result indicates that as the radioactive forcing increased the suitable habitat for buckwheat neither increases nor decreases proportionally, but showed mixed pattern of gaining and losing from different parts of the state.

Objective V: Quality honey production with honey bee as a pollinator component in different regions of Sikkim through demonstration and training through participation approach

Beekeeping is an agro-based occupation that provides income and employment generation for rural and tribal families. It plays a vital role in the present context of the commercialization of agriculture and liberalization of the economy. It covers the entire scope of honeybee resources, bee products, beekeeping practices, pollination services, and their interface with business systems and ecological integrity. Selection of villages and farmers to carry demonstration and training activities on quality honey production technology with honey bee a pollinator component was done. Organization of 3-5 days on-campus and off campus training on production of honey production in batches for 20-25 selected farmers was also done. Development and demonstration of honey bee units (beehives) at the ICAR-Sikkim Centre with honey bee a pollinator component for the honey growers was created. Establishment of honey bee units (beehives) for quality honey with honey bee a pollinator component at selected villages of different regions of Sikkim was done. The beneficiaries were identified and selected with the help of the Krishi Vigyan Kendra (KVKs) in each district of Sikkim. The plan was covered two blocks/district, four villages/block and one-two SHGs/village of each districts of Sikkim. We have conducted different scientific training program in each and every village and hands-on-training was also given to the interested honey bee farmers. Need based training along with infrastructure under buckwheat production system for quality honey production were developed under Sikkim condition. Besides, field demonstration for quality honey production with honey bee as a pollinator component in different villages for livelihood security was also done.

Number of district:	04
Number of block per district:	02
Number of village per block:	04
Number of beneficiaries per village:	02

Total number of beneficiaries will be covered:	04 × 02 × 04 × 02 = 64*

**Total beneficiaries were 64 and among which ST = 40, SC = 12 and others = 08 (general, OBC etc). This represented that out of total beneficiaries ≥80% farmers was from SC/ST community.*

Finally, quality honey production technology was developed through buckwheat production which empowered the rural tribal farmer's. The transfer of quality honey production through buckwheat cultivation to the rural women and tribal communities helped to overcome the problem of unemployment to these strata of the society. They were skilled and sufficient to produce quality honey and also supplied to the other growers. The produce had easy access to the market. The selected villages were strengthened to emerged as new “HONEY VILLAGE” of Sikkim.

Economics of Honey Production

The beekeeping started with two boxes/hives, extractors, bee veil, gloves, decapping knife, hive tools, etc. initially 5 farmers were keep only one honey extractor.

The current price is as under.

S. No.	Items	Price
1.	Bee Hive/Box	2500 × 10 =25,000
2.	Honey extractor	6500
3.	Bee veil	300 × 5 =1500
4.	Bee gloves	100 × 5 = 500
5.	Sale of honey	Rs. 1000/kg × 150 kg = 1,50,000
6.	Production of honey	Avg. 15 kg × 10 = 150 kg

The bee farmer can start with 5-10 colonies which can sell 5-10 colonies each after proper division with retention of 5-10 colonies with them. Also, the farmers can produce 2-3 colonies each year from a single colony with modern scientific management.

Description of work undertaken

Sixty four (64) farmers has been provided with one honey bee box, uncapping knife, protective garments, gloves, net veil, bee net, and brush each. The farmers were from East district (24), West district (12), South district (16) and North district (12). After proper training (08 number) and awareness programme (04) we have distributed the honey bee production inputs. The farmers were installed the honey bee box set and from 2021 they are producing quality honey. The cost of one kg of pure organic honey was Rs. 1600/- and each farmer is earning average

Rs. 75000/- per year by selling pure honey. Thereafter, it was possible to develop 04 number of first HONEY VILLAGE (one from each district) in Sikkim.

Quantifiable outputs

We have already identified the place for development of first HONEY VILLAGE (4 Nos.) and already distributed honey bee box with proper training and awareness. Already many beneficiaries has producing quality honey. we have established the HONEY village already. In the third year honey were produced by the farmers during the buckwheat cultivation. For this, we have conducted 08 number of training programme on quality honey production technology and 04 number of awareness programme. The honey bee beneficiary farmers list are mentioned below and it is to inform that farmers are still producing quality organic honey after support from NMHS fund and they are very happy now.

List of skill developed manpower trained: (64)

S.N.	***Name of Farmer's***	***Village Name***	***Geographic Location and Altitude***		
			Altitude	***Latitude***	***Longitude***
1.	Purna Kumari Tamang	Timpyem East Sikkim	1304 m	27°18'39''N	88°33'42''E
2.	Kausila Bajgai	Timpyem East Sikkim	1334 m	27°18'43''N	88°33'47''E
3.	Sakhila Chettri	Timpyem East Sikkim	1330 m	27°18'47''N	88°33'49''E
4.	Domo Tamang	Timpyem East Sikkim	1305 m	27°18'54''N	88°25'42''E
5.	Mon Maya Chettri	Timpyem East Sikkim	1301 m	27°18'54''N	88°25'44''E
6.	Mon Maya Tamang	Timpyem East Sikkim	1286 m	27°10'52''N	88°28'20"E
7.	Bimla Tamang	Timpyem East Sikkim	1274 m	27°10'35''N	88°28'20"E
8.	Rabina Tamang	Timpyem East Sikkim	1275 m	27°10'02''N	88°29'26"E
9.	Lhumu Tamang	Timpyem East Sikkim	910 m	27°10'34"N	88°27'05"E
10.	Kumari Subba	Timpyem East Sikkim	911 m	27°31'20''N	88°31'08''E
11.	Ganga Maya Tamang	Timpyem East Sikkim	911 m	27°31'43''N	88°30'50''E
12.	Durga Devi Chettri	Timpyem East Sikkim	1338 m	27°18'46''N	88°33'78''E
13.	Sita Rai	Timpyem East Sikkim	1315 m	27°18'43''N	88°33'40''E
14.	Laxmi Tamang	Timpyem East Sikkim	1281 m	27°18'44''N	88°33'44''E
15.	Kumar Tamang	Nandok East Sikkim	815 m	27°18'46''N	88°33'48''E
16.	P.K. Tamang	Nandok East Sikkim	1293 m	27°18'48''N	88°33'50''E
17.	Pempa Doma Tamang	Nandok East Sikkim	1307 m	27°18'41''N	88°33'39''E
18.	Shyam Kumar Tamang	Nandok East Sikkim	1313 m	27°18'42''N	88°33'40''E
19.	Gyalpo Tamang	Nandok East Sikkim	975 m	27°18'44''N	88°33'41''E
20.	Ganga Sharma	Nandok East Sikkim	910 m	27°18'45''N	88°33'41''E

21.	Risikha Rai	Yangang S. Sikkim	864 m	27°18’43’’N	88°33’48’’E
22.	Sulaman Tamang	Yangang S. Sikkim	929 m	27°18’48’’N	88°33’51’’E
23.	Lilly Rai	Yangang S. Sikkim	879 m	27°15’46’’N	88°34’33’’E
24.	Amrita Lepcha	Yangang S. Sikkim	940 m	27°15’44’’N	88°34’46’’E
25.	Norki Bhutia	Yangang S. Sikkim	977 m	27°15’44’’N	88°34’46’’E
26.	Reeta Chettri	Yangang S. Sikkim	1097 m	27°15’36’’N	88°34’52’’E
27.	Phulmati Lepcha	Namthang S. Sikkim	1141 m	27°15’30’’N	88°34’58’’E
28.	Sazey Lepcha	Namthang S. Sikkim	937 m	27°15’30’’N	88°34’53’’E
29.	Tshering Doma Bhutia	Namthang S. Sikkim	738 m	27°18’54’’N	88°25’50’’E
30.	Chenga Bhutia	Namthang S. Sikkim	878 m	27°18’57’’N	88°25’38’’E
31.	Sabita Tamang	Passingdang N. Sikkim	880 m	27°18’18’’N	88°25’36’’E
32.	Norkith Lepcha	Passingdang N. Sikkim	1232 m	27°18’45’’N	88°26’04’’E
33.	Kiden Bhutia	Passingdang N. Sikkim	1372 m	27°31’45’’N	88°30’48’’E
34.	Sonam Doma Bhutia	Passingdang N. Sikkim	1307 m	27°31’41’’N	88°30’43’’E
35.	Sati Maya Manger	Passingdang N. Sikkim	1469 m	27°31’36’’N	88°30’43’’E
36.	Monika Manger	Dzongu N. Sikkim	1143 m	27°33’18’’N	88°27’21’’E
37.	Padma Maya Manger	Dzongu N. Sikkim	847 m	27°33’21’’N	88°27’16’’E
38.	Karma Doma Bhutia	Dzongu N. Sikkim	1154 m	27°10’33’’N	88°13’06’’E
39.	Tashi Bhutia	Dzongu N. Sikkim	1165 m	27°15’26’’N	88°09’02’’E
40.	Keshab Dahal	Dzongu N. Sikkim	1110 m	27°15’32’’N	88°08’57’’E
41.	Bishnu Maya Karki	Soreng W. Sikkim	1550 m	27°15’35’’N	88°08’54’’E
42.	Chumke Bhutia	Soreng W. Sikkim	1464 m	27°15’33’’N	88°08’55’’E
43.	Phurba Doma Bhutia	Soreng W. Sikkim	1432 m	27°15’33’’N	88°08’57’’E
44.	Tashi Doma Bhutia	Soreng W. Sikkim	1449 m	27°10’34’’N	88°13’08’’E

45.	Sonam Ongmu Bhutia	Soreng W. Sikkim	1457 m	27°33’21’’N	88°27’18’’E
46.	Nim Lhamu Bhutia	Dentam W. Sikkim	1225 m	27°33’23’’N	88°27’13’’E
47.	Karma Sonam Bhutia	Dentam W. Sikkim	1246 m	27°33’17’’N	88°27’25’’E
48.	Doma Sherpa	Dentam W. Sikkim	1220 m	27°10’37’’N	88°12’57’’E
49.	Layke Doma Bhutia	Dentam W. Sikkim	1175 m	27°10’32’’N	88°13’01’’E
50.	Sonam Bhutia	Dentam W. Sikkim	1203 m	27°10’34’’N	88°13’02’’E
51.	Jiwan Rai	Soreng W. Sikkim	1036 m	27°08'19"N	88°26'31"E
52.	Nar Bahadur Kami	Dentam W. Sikkim	907 m	27°08'35"N	88°26'30"E
53.	Kharka Bahadur Rai	Soreng W. Sikkim	995 m	27°08'07"N	88°26'33"E
54.	Man Bahadur Sarki	Dentam W. Sikkim	1027 m	27°08'19"N	88°26'34"E
55.	Yanki Lepcha	Soreng W. Sikkim	993 m	27°08'15"N	88°26'27"E
56.	Santosh Kumar Chettri	Dentam W. Sikkim	1047 m	27°08'21"N	88°26'25"E
57.	Sujana Biswakarma	Soreng W. Sikkim	1345 m	27°08'54"N	88°26'03"E
58.	Aleeshiba Rai	Dentam W. Sikkim	1304 m	27°08'46"N	88°26'08"E
59.	Simon Rai	Soreng W. Sikkim	1287 m	27°08'42"N	88°26'01"E
60.	Daniel Rai	Dentam W. Sikkim	1250 m	27°08'55"N	88°25'43"E
61.	Bikha Maya Rai	Soreng W. Sikkim	1208 m	27°08'38"N	88°25'58"E
62.	Nak Tshering Lepcha	Dentam W. Sikkim	1127 m	27°08'22"N	88°26'12"E
63.	Amous Gurung	Soreng W. Sikkim	992 m	27°08'24"N	88°26'36"E
64.	Chukit Lepcha	Dentam W. Sikkim	910 m	27°08'24"N	88°26'08"E

**Each farmer has been provided one honey bee box, uncapping knife, protective garments, gloves, net veil, bee net, and brush.*

Skill Development and Capacity Building/ activities

	
Honey bee box and kit distribution	Honey production training
	
Honey extraction by beneficiary farmer	Beneficiary farmer with honey bee box
	
Honey bee box distribution	Honey bee box distribution and training
	
Honey bee box made by beneficiary farmer	Beneficiary farmer

	
Honey bee box installation at farmers filed for quality honey production	Honey bee box installation at farmers filed for quality honey production
	
Honey bee box installation at farmers filed for quality honey production	Honey bee box installation at farmers filed for quality honey production
	
Biochemical analysis of buckwheat	Biochemical analysis of buckwheat
	
Field survey study for impact of climate	Field survey study for impact of climate change on

change on buckwheat	buckwheat
	
Training and awareness programme	Training and awareness programme
	
Farmers carrying their honey bee box	Buckwheat germplasm conservation
	
Honey extraction by beneficiary farmer	Beneficiary farmer with honey bee box
	
Honey bee box distribution and training	Honey bee box distribution and training

	
Honey bee box made by beneficiary farmer	Beneficiary farmer
	
	
Honey bee box installation at farmers filed for quality honey production	Honey bee box installation at farmers filed for quality honey production
	

FORM GFR 12 – A
[(See Rule 238 (1)]

UTILIZATION CERTIFICATE FOR THE YEAR 2022-2023 in respect of Recurring and Non- Recurring Grants

1. Name of the Project/Fellowships: **Morphological and biochemical characterization, mitigating climate change impact and quality honey production in buckwheat (*Fagopyrum* spp.) under organic condition: A community participation approach for doubling livelihood empowerment**
2. Whether recurring grants: Both recurring and non-recurring
3. Name of the Grantee Org.: ICAR RC for NEH Region, Sikkim Centre, Tadong, Gangtok, Sikkim-737102
4. Grants position at the beginning of the financial year: 2022-2023
   (i) Cash in Hand/Bank: **857048.00**
   (ii) Unadjusted advances: 0.00
   (iii) Total: **857048.00**
5. Details of grants received, expenditure incurred and closing balances:

Unspent Balances of Grants received years	Interest Earned thereon	Interest deposited back to the Govt.	Grant received during the year			Total Available funds (1+2+3+4)	Expenditure (excluding commitments)	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction	Date (ii)	Amount (iii)			
00.00	**00.00**	**00.00**	**Ref. No.: GBPNI/NMHS/2019-20/SG/317/52/106/74/60 Dated 15.06.2022**	**15/06/2022 with PFMS Payment Advice no. C062219780331**	**857048.00**	**857048.00**	**857048.00**	**00.00**

Component wise utilization of grants:

Grant-in-aid– General	Grant-in-aid– Salary	Grant-in-aid–creation of capital assets	Total
523448.00	**333600.00**	**00.00**	**857048.00**

Details of grants position as on **31.03.2023**

(i) Cash in Hand/Bank: **- 00.00**

(ii) Unadjusted Advances: 0.00

(iii) **Total: 00.00**

Cont...........2

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

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

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

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

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

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

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

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

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

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

Date: 24/05/2023
Place: **ICAR RC for NEH Region, Sikkim Centre, Tadong, Gangtok**

10/5/2023 Signature Name: Shaon Kumar Das Principal Investigator Dr. Shaon Kumar Das Senior Scientist (Agril. Chemistry/Soil Science) ICAR RC for NEH Region, Sikkim Centre, Tadong, Gangtok, Sikkim	Signature Name: Chief Finance Officer (Head of Finance) Sr. Finance & Accounts Officer ICAR RC for NEH Region Umiam, Meghalaya	Signature Name: Head of Institute Director (ICAR-NEH) 24/5/23 Director ICAR Research Complex for NEH Region Umroi Road, Umiam, Meghalaya-793103

EXPENDITURE STATEMENT
NATIONAL MISSION ON HIMALAYAN STUDIES

Statement showing the expenditure of the period from 1st April 2022 to 31st March 2023

Sanction No. & Date: **GBPNI/NMHS-2019-20/SG/317 dated 16.10.2019**

1. Total outlay of the project/Fellowship : 3627328.00

2. Date of Start of the Project/fellowship : 24th February, 2020

3. Duration : 03 years

4. Date of Completion : 24th February, 2023

a) Amount received during the financial year : 857048.00
b) Unspent amount carried forward from pervious Financial year : 00.00
c) Total amount available for Expenditure (a+b) : 857048.00

S. No.	Budget head	Amount Carried forward	Amount received	Amount received+ amount carried forward	Expenditure	Amount Balance/ excess expenditure
1	Salaries	00.00	333600.00	333600.00	333600.00	00.00
2	Permanent Equipment Purchased (Item-wise	00.00	00.00	00.00	00.00	00.00
3	Travel(Domestic)	00.00	115000.00	115000.00	115000.00	00.00
4	Consumables	00.00	64000.00	64000.00	64000.00	00.00
5	Contingency	00.00	65000.00	65000.00	65000.00	00.00
6	Activities & other project cost	00.00	193272.00	193272.00	193272.00	00.00
7						
8						
9						
10	Institutional charges	00.00	86176.00	86176.00	86176.00	00.00
11	Accrued bank Interest	00.00	00.00	00.00	00.00	00.00
12	**Total**	**00.00**	**857048.00**	**857048.00**	**857048.00**	**00.00**
13	Amount allowed to be Carried forward to the next financial year (2023-2024)	**00.00**				

Certified that the expenditure of **Rs. 857048.00 (Rupees eight lakh fifty seven thousand and fourty eight only)** mentioned against Sr, no.12 was actually incurred on the project/ scheme for the purpose it was sanctioned.

Date: 24/05/2023

Signature Name: Shaon Kumar Das Principal Investigator Dr. Shaon Kumar Das Senior Scientist (Agril. Chemistry/Soil Science) ICAR RC for NEH Region, Sikkim Centre,Tadong,Gangtok, Sikkim	Signature Name: Chief Finance Officer (Head of Finance) Sr. Finance & Accounts Officer ICAR RC for NEH Region Umiam, Meghalaya	Signature Name: Head of Institute Director (ICAR-NEH) Director ICAR Research Complex for NEH Region Umroi Road, Umiam, Meghalaya-793103

OUR REF. No.

ACCEPTED AND COUNTERSIGNED

Date:

COMPETENT AUTHORITY
NATIONAL MISSION ON HIMALAYAN STUDIES (GBPNIHESD)