Genetic and Nutritional Analysis of Indigenous Rice Cultivars of Sikkim Himalaya

A Thesis Submitted to

SIKKIM UNIVERSITY

In Partial Fulfilment of the Requirement for the

Degree of Doctor of Philosophy

By

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December 2022

CERTIFICATE

This is to certify that the Ph.D. thesis entitled **"Genetic and Nutritional Analysis of Indigenous Rice Cultivars of Sikkim Himalaya"** submitted to **Sikkim University** in partial fulfillment for the requirement of the degree of Doctor of Philosophy in Botany embodies the research work carried out by **Mr. Deepak Chettri** at the Department of Botany, Sikkim University. It is a record of bonafide investigation carried out and completed by him under my supervision. He has followed the rules and regulations prescribed by the University.

The results are original and have not been submitted anywhere else for any other degree or diploma.

It is recommended that this Ph.D. thesis be placed before the examiners for evaluation.

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CERTIFICATE

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DECLARATION

I declare that the Ph.D. thesis entitled **“Genetic and Nutritional Analysis of Indigenous Rice Cultivars of Sikkim Himalaya”** submitted for the degree of Doctor of Philosophy to Sikkim University is a bonafide research work, carrieed out by me under the supervision of **Dr. N. Sathyanarayana,** Professsor, Department of Botany, Sikkim University.

I also wish to apparise that the thesis does not bear content that has been submitted for a degree or diploma at any other University or Institution. Futher the references used to agument the research and the materials obtained have been duly acknowledged.

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"Genetic and Nutritional Analysis of Indigenous Rice Cultivars of Sikkim Himalaya"

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CONTENTS **Page No**

Abbreviations	i
Symbols and Units	ii
List of Tables	iii
List of Figures	iv
List of Plates	v
CHAPTERS	
1. INTRODUCTION	**1-15**
1.1. Origin of cultivated rice	2-4
1.2. Rice: culture and cultivation in India	4-7
1.3. Rice cultivation in northeast India	7-8
1.4. Rice landraces of northeast India	8-9
1.5. Rice landraces of Sikkim	10-11
1.6. Indigenous Traditional Knowledge (ITK)	11-12
1.7. Evaluation based on seed morphometric traits	12-13
1.8. Molecular markers and Association analysis	13-15
2. REVIEW OF LITERATURE	**16-39**
2.1. Rice: Global significance	16
2.2. Botany	16-17
2.3. Landraces in rice breeding	17-18
2.4. Rice landraces of northeast India	18-20
2.5. Germplasm collection and characterization	20
2.5.1. Physico-chemical and nutritional characteristics of the grain	20-26
2.5.2. Molecular characterization	27-29
2.5.3. Genome Wide Association Studies (GWAS)	29-30
2.6. Indigenous Technical Knowledge (ITK)	30-31
2.7. Importance of ITKs in agriculture	31-32
2.8. ITKs related to rice cultivation in India	32
2.8.1. Main field preparation	32-33
2.8.2. Seed selection and germination	33-34
2.8.3. Green leaf-manuring	34-35
2.8.4. Control of weeds, diseases and pests	35-36
2.8.5. Storage and harvesting	36-37

2.9. ITKs on rice cultivation from northeast India 37-38
2.10. Problem statement 38-39

3. MATERIALS AND METHODS 40-53
3.1. Study material 40
3.2. Cataloging and documentation of Indigenous Traditional Knowledge 40-41
3.3. Phenotypic diversity based on grain and kernel traits 41
3.3.1. Evaluation of seed traits 41-42
3.3.2. Biochemical analysis 42
3.3.2.1. Aroma content 42
3.3.2.2. Alkali digestion test and Gelatinization temperature 42-43
3.3.2.3. Amylose content 43-44
3.4. Proximate nutritional and mineral composition 44
3.4.1. Determination of Moisture content 44
3.4.2. Determination of Ash content 45
3.4.3. Determination of Crude fiber content 45
3.4.4. Determination of Crude fat content 45-46
3.4.5. Determination of Protein content 46
3.4.6. Determination of Carbohydrate content 46-47
3.4.7. Mineral content 47
3.5. Molecular characterization and Association analysis 47
3.5.1. Plant material 47-49
3.5.2. Genotyping 49-50
3.5.3. Allele scoring 51
3.5.4. Data analysis 51-52
3.5.5. Phenotyping 52-53
3.5.6. Marker-Trait Association 53

4. RESUTS 54-109
4.1. Cataloging and documentation of Indigenous Traditional Knowledge 54
4.1.1. Collection of indigenous rice landraces 54-64
4.1.2. Documentation of Indigenous Traditional Knowledge 65
4.1.2.1. Selection of seeds 65
4.1.2.2. Preparation of seed beds and land 65-70

4.1.2.3. Water management techniques 70-71
4.1.2.4. Method of rice planting 71-72
4.1.2.5. Soil fertility management 72-73
4.1.2.6. Harvesting and threshing 73
4.1.2.7. Storage and post-harvest techniques 73-74
4.2. Phenotypic diversity based on grain and kernel traits 75
4.2.1. Morphometric characterization 75-77
4.2.1.1. Grain length (mm) 77
4.2.1.2. Grain breadth (mm) . .78
4.2.1.3. Seed thickness (mm) 78-79
4.2.1.4. Grain L/B ratio or seed class (Fineness) 79
4.2.1.5. 1000 grain weight (g) 79-80
4.2.1.6. Kernel length, breadth and kernel L/B ratio 80
4.2.2. Organoleptic qualities of cooked rice (Cooking quality) 81-82
4.2.2.1. Kernel elongation ratio 82
4.2.2.2. Kernel elongation after cooking (KEaC) 83
4.2.2.3. Cooked kernel length-breadth ratio 83
4.2.3. Biochemical properties 83
4.2.3.1. Grain aroma 83
4.2.3.2. Gelatinization temperature through Alkali Spreading value (ASV) 83-85
4.2.3.3. Amylose content 85-86
4.2.4. Variability for the seed and cooking quality traits 86-90
4.2.5. Correlation between the grain and cooked rice characters 91-92
4.2.6. Principle Component Analysis (PCA) 92-94
4.3.Proximate nutritional and mineral content 94
4.3.1. Proximate composition 94-95
4.3.1.1. Determination of Moisture content 95
4.3.1.2. Determination of Ash content 95
4.3.1.3. Determination of Fat content 95
4.3.1.4. Determination of Crude fiber content 96
4.3.1.5. Determination of Protein content 96
4.3.1.6. Determination of Carbohydrate content 96
4.3.2. Mineral content 97-99

4.4. Molecular characterization and Association analysis 99
4.4.1. Marker attributes 99-102
4.4.2. Genetic diversity 102-105
4.4.3. Population structure 106
4.4.4. Marker Trait Association 107-109

5. DISCUSSION 110-132
5.1. Cataloging and documentation of Indigenous Traditional Knowledge (ITKs) 110
5.1.1. Documentation and collection of indigenous rice landraces 110-114
5.1.2. Documentation of Indigenous Traditional Knowledge 114-117
5.2. Phenotypic diversity based on grain and kernel traits 117
5.2.1. Morphometric characterization 117-119
5.2.2. Organoleptic qualities of cooked rice (Cooking quality) 119-120
5.2.3. Biochemical properties 120-122
5.3. Proximate nutritional and mineral composition 122-127
5.4. Molecular characterization and Association analysis 128-132

6. SUMMARY AND CONCLUSIONS 133-136
6.1. Cataloging and documentation of rice landraces and Indigenous Traditional Knowledge (ITKs) 133-134
6.2. Phenotypic diversity based on grain and kernel traits 134-135
6.3. Proximate nutritional and mineral composition 135-136
6.4. Molecular characterization and Association analysis 136

7. FUTURE PROSPECTS 137

8. BIBLIOGRAPHY 138-174

9. PUBLICATIONS 175-176

ABBREVIATIONS

2-AP	2-Acetyl-1-Pyrroline
AC	Amylose Content
AM	Association Mapping
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
ASV	Alkali Spreading Value
CTAB	Cetyl Trimethyl Ammonium Bromide
DArT	Diversity Aarray Technology
DUS	Distinctiveness, Uniformity and Stability
FAO	Food and Agriculture Organization
GLM	General Linear Model
GR	Green Revolution
GT	Gelatinization Temperature
GWAS	Genome-Wide Association Studies
HMT	Hindustan Machine Time
HYV	High Yielding Varieties
ICAR	Indian Council of Agricultural Research
ISSR	Inter Simple Sequence Repeats
ITK	Indigenous Traditional Knowledge
MAB	Marker Assisted Breeding
MAF	Major Allele Frequency
MCMC	Markov Chain Monte Carlo
MLM	Mixed Linear Model
MTA	Marker-Trait Association
NGS	Next-Generation Sequencing
NRRI	National Rice Research Institute
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
PPV& FR	Protection of Plant Varieties and Farmers' Right
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences Software
SRISTI	Society for Research into Sustainable Technologies and Institutions
SSR	Simple Sequence Repeats
UPGMA	Un-weighted Pair group Method with Arithmetic Mean

SYMBOLS AND UNITS

%	Percentage
CV	Coefficient of Variation
%P	Percentage of Polymorphic Loci
cm	Centimeter
g	Gram
min	Minutes
mm	Millimeter
mM	Millimolar
ng	Nanogram
mg	Milligram
ml	Millilitre
s	Seconds
SD	Standard Deviation
SE	Standard Error
Tm	Temperature
γ	Gamma
μl	Microlitre
μM	Micromolar
Mt	Million Tonnes
$t\ ha^{-1}$	Tonnes per Hectares
Mha	Million Hectares
Masl	Meter above sea level
RPM	Revolutions Per Minute

LIST OF TABLES

Sl. No.	Tables	Page No.
1	Global and Indian scenario of rice cultivation and production	1
2	Distinguishing characters of the three *Oryza sativa* subspecies	4
3	Area, productivity and production of paddy in different states of India (2019-20)	5
4	The scale used for scoring the alkali digestion and gelatinization temperature	43
5	Details of 53 rice landraces used in the study	48-49
6	Sequence information of 45 SSR markers used for genotyping	50-51
7	List of phenotypic traits evaluated among rice landraces	53
8	Rice landraces collected during the study	56-57
9	Indigenous Traditional Knowledge (ITKs) associated with rice cultivation in Sikkim	67-69
10	Accession-wise variability of seed traits in rice landraces of Sikkim	75-77
11	Classification of rice landraces based on grain length	77
12	Classification of rice landraces based on grain breadth	78
13	Classification of rice landraces based on seed thickness	78
14	Classification of rice landraces based on L/B Ratio	79
15	Classification of rice landraces based on test weight (g)	80
16	Variability for cooking quality traits among 65 landraces	81- 82
17	Classification of rice landraces based on the Alkali Spreading Value (ASV)	84
18	Classification of rice landraces based on Gelatinization temperature	85
19	Classification of rice landraces based on Amylose content	85
20	One-way ANOVA for seed and cooking quality traits in 65 rice landraces	86
21	Accession-wise variability for seed and cooking quality traits in 65 rice landraces	88-90
22	Correlations among the 14 seed-based traits	91
23	Eigen values of the five principal components (PCs) for 14 seed-based traits	94
24	Variability of proximate and mineral contents in 65 rice landraces from Sikkim	98
25	One-way ANOVA for proximate and mineral contents in 65 rice landraces	98
26	Correlations among the proximate and mineral compositions	99
27	Characteristics of 42 SSR markers used in genetic diversity analysis	101-102
28	Summary of genetic diversity statistics of 53 rice landraces	103
29	Grouping of accessions in UPGMA clusters	105
30	Marker-Trait Associations obtained from the General Linear Model (GLM) and the Mixed Linear Model (MLM)	108

LIST OF FIGURES

Sl. No.	Figures	Page no.
1	Production share of rice by region (Source: FAOSTAT 2021)	2
2	Schematic representation of the evolution of cultivated rice	3
3	District-wise rice area in proportion to the total agricultural area in India (Source: NRRI 2019)	5
4	Location map of the 12 village sites selected for the study	41
5	Correlation matrix of 14 phenotypic traits evaluated on the 65 rice landraces	92
6	Scatter plot based on 14 seed and cooking quality traits from the Principal Component Analysis (PCA)	93
7	UPGMA dendrogram depicting the relationship among 53 rice landraces of Sikkim	104
8	Principal Coordinate Analysis (PCoA) of tested germplasm	105
9	Model-based population structure analysis of 53 rice landraces (A) ΔK showing the presence of 3 hypothetical subpopulations (B) Population structure for 53 rice landraces of Sikkim sorted by kinship (Q) matrix at K = 3	106

LIST OF PLATES

Sl. No.	Plates	Page No.
1	Rice landraces collected during the field study (Plate a-f)	58-63
2	(a) Typical field view of upland rice cultivation in North Sikkim (b) Land preparation for the cultivation of upland rice (c) & (d) Seedling stage (e) Popular upland rice landraces of Sikkim	64
3	(a) Wet ploughing for seedbed preparation (b) Bunds clearing and mud slicing (c) Seedbed/Nursery (d) Terraced field (e) transplantation from nursery to the main field (f) Levelling of rice field	70
4	(a) Country plough (halo) (b) leveler (dadhey) (c) Kodalo (d) wooden hand leveler (phyauri) (e) Kachiya (f) Hasiya	71
5	(a) & (b) Threshing and winding (c) *Bhakari* (d) *Dhikuti* and (e) Tradition mill (*Dhiki*)	74
6	Types of Alkali digestion of milled rice grains	84
7	Representative gel images of markers RM 314 (a & a1) and RM 253 (c & c1). Both a & a1, b & b1 represents the banding pattern of 53 landraces	101

CHAPTER-1

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the principal food crops for more than half of the world's population (Table 1) (Pathak et al. 2019). Its cultivation covers roughly 160 million hectares (Mha), spanning 44^{o}N (North Korea) to 35^{o}S (Australia). The plant grows profusely in the irrigated and rainfed lowland regions of the tropics (Anonymous 2020). Irrigated rice system accounts for 78% of total rice production, of which 55% is concentrated in alluvial floodplains, terraces, inland valleys, and deltas in the humid and sub-humid subtropics of Asia (Pathak et al. 2020).

Table 1. Global and Indian scenario of rice cultivation and production

Parameters	World	India
-Production (Mt)	500 (milled rice), 750 (paddy), 1875 (residues)	112 (milled rice), 170 (paddy), 425 (residues)
-Feeding people (billion)	4 (56% of the population)	0.8 (65% of the population)
-Area (Mha)	166 (10% cropland)	43 (22% cropland)
-Grown by families (million)	144 (25% of farmers)	67 (56% of farmers)
-Livelihood of rural poor (million)	400 (40% of poor)	150 (40% of poor)
-Annual value (US$ billion)	206 (13% of crop value)	53 (17% of crop value)
-Irrigation water use (km^3 yr^{-1})	880 (35% of the total)	200 (29% of the total)
-Fertilizer use (Mt yr^{-1})	25 (15% of total)	6.5 (37% of total)
-Methane emission (Mt yr^{-1})	25 (12% of agriculture)	3.5 (18% of agriculture)

Source: *Pathak et al. (2019)*

In terms of global production, Asia ranks first accounting for more than 90.5% of output followed by America (5.2%), Africa (3.6%), Europe (0.6%), and Oceania (0.1 %) (Figure 1). China (206.5 Mt), India (157.2 Mt), Indonesia (70.8 Mt), Bangladesh (52.2 Mt), and Vietnam (44.9 Mt) are the top rice-producing countries (FAOSTAT 2021).

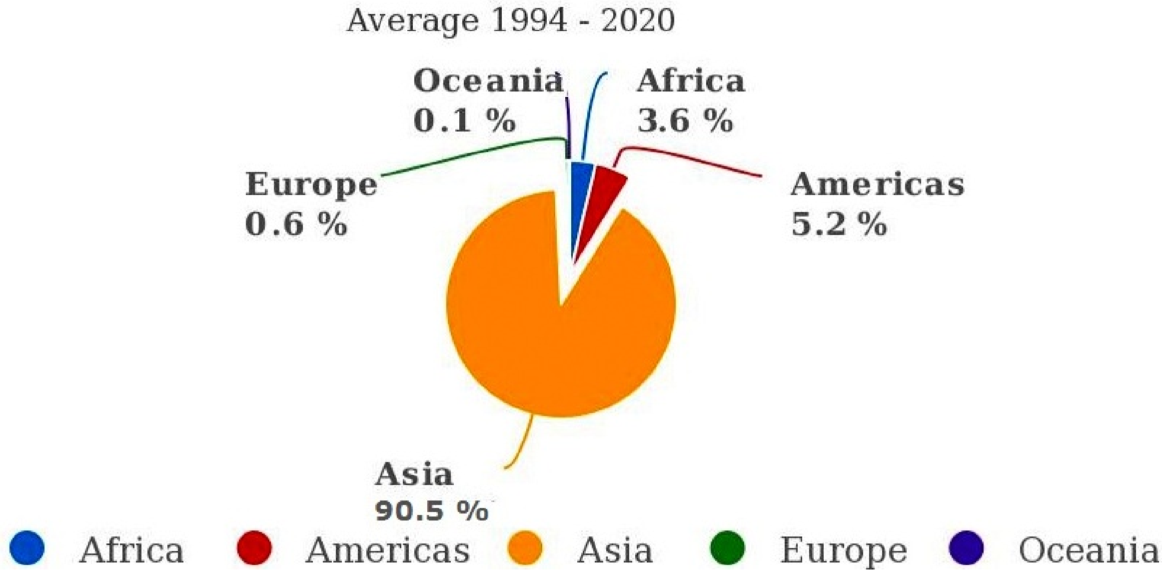


Figure 1. Production share of rice by region (Source: FAOSTAT 2021).

1.1. Origin of cultivated rice

Archaeological and historical evidenc indicates that Southeast Asia (for *O. sativa)* and Africa (for *O. glaberrima)* are the primary sites of origin of cultivated rice. *O. perennis* has been determined to be the common ancestor of both of these species. While *O. sativa* (Asian rice) appears to have descended from *O. rufipogan* (wild perennial) and *O. nivira* (wild annual), *O. glaberrima* (African rice) is thought to have arisen from *O. longistaminate* (wild perennial) and annual *O. barthii* (wild perennial) (Figure 2). Further, due to long-term adaptation under the different climatic conditions, the cultivated *O. sativa* has evolved into three geographical sub-species: *O. sativa* sub sp. *indica*, *O. sativa* sub spp. *japonica,* and *O. sativa* subspp. *javanica* with a distinct sterility barrier. It can reach up to 80% between *indica* and *japonica* crossbreedsbut only slightly between *indica* and *javanica* crossbreeds (Wan et al. 1996).

Many evolutionary biologists believe that, in India, the *indica* variant was initially cultivated in the area encompassing the eastern Himalayan foothills (including northeast India) stretching through Burma, Thailand, Laos, Vietnam, and Southern China, whereas the *japonica* variety was domesticated from the wild rice introduced to India from southern China (Ramiah 1953; Richharia 1960; Chang 1976). Morphologically, short, erect plants with compact panicles, pubescent spikelets, and oval to spherical grains are the distinct characteristics of the *japonica* subspecies, found primarily in Japan. The subspecies *javanica* is known for tall, erect plants with long panicles and awned spikelets and is mainly grown in Indonesia. The subspecies *indica* is a tall, spreading plant with a wide range of morphological features (Table 2).

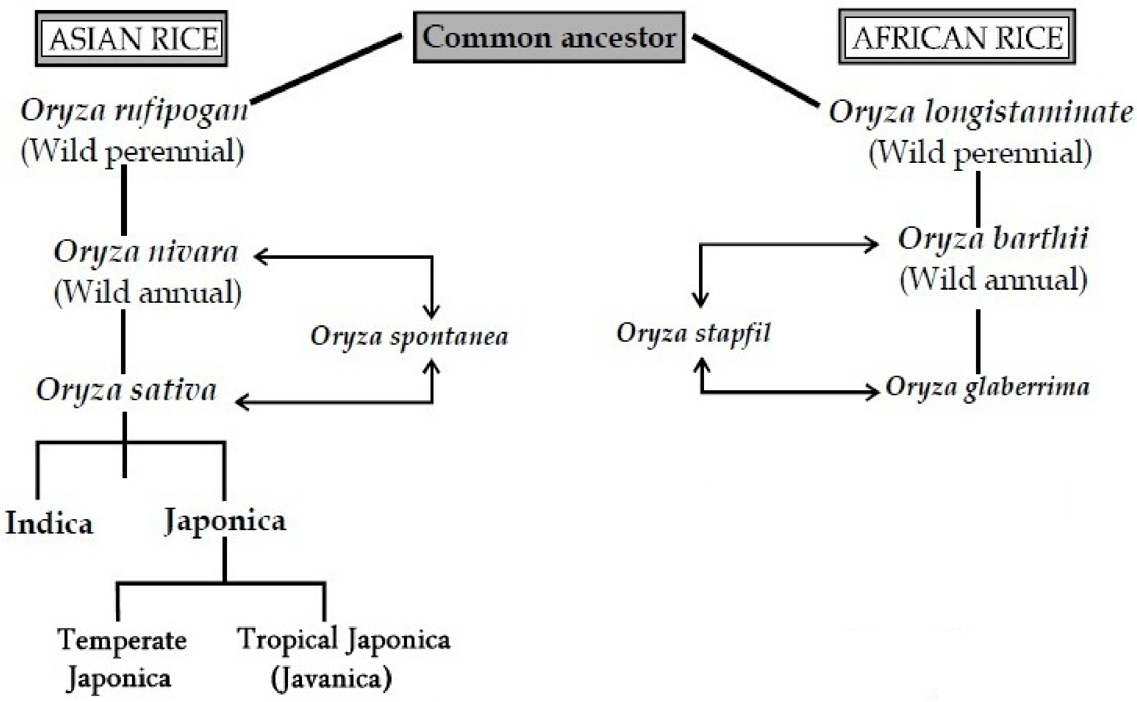


Figure 2. Schematic representation of the evolution of cultivated rice

Table 2. Distinguishing characters of the three *Oryza sativa* subspecies

Character	**Sub-species**		
	Indica	***Japonica***	***Javanica***
Leaves	Broad, light green	Narrow, dark green	Broad, stiff, light green
Tillering	Profuse	Moderate	Low
Height	Tall	Short	Tallest
Hairs on lemma and palea	Thin short hairs on the lemma	Dense, long hairs on lemma and palea	Long hairs on lemma and palea
Awns	Mostly awnless	Awnless to long awned	Awnless or long awned
Rains	Slender, somewhat flat grains	Short, roundish grains	Broad, thick grains
Grain shattering	Fast shattering	Low shattering	Low shattering
Tissues	Soft plant tissues	Hard plant tissues	Hard plant tissues
Photosensitivity	Varying sensitivity to photoperiod	Varying sensitivity to photoperiod	Low sensitivity to photoperiod

Source: *Dunna & Roy (2013).*

1.2. Rice: culture and cultivation in India

In India, about 43 Mha agricultural area is dedicated to rice cultivation which yields 112 million tonnes (Mt) of milled rice with an average productivity of 2.6 t ha^{-1} (Anonymous 2021). It is the staple food for more than 65% of the country's population, occupying a pivotal place in assuring the food and livelihood security of the farmers. Uttar Pradesh, West Bengal, Punjab, Odisha, Chhattisgarh, Bihar, and Punjab are India's leading rice-producing states (Ricepedia 2022-Table 3).

India, with its eternal growing season and the deltas of the Ganges-Brahmaputra, Kaveri, Krishna, Godavari, Indravati, and Mahanadi rivers, as well as a dense network of canal irrigation, allows the farmers to grow two and, in some pockets three crops a year (Randhawa 1980). Both western and eastern coastal strips covering all the primary deltas, the Assam plains and the surrounding low hills, foothills, and Terai region- along the Himalayas are the major rice growing regions in India (Paul 2013). Further, the mountainous terrace that stretches from Kashmir to Assam, with its age-old hill irrigational advantages, also contributes to the total rice grown in the region. District-wise rice area in proportion to the total agricultural area in India is presented in Figure 3.

Table 3. Area, productivity and production of paddy in different states of India (2019-2020)

State	Area (Mha)	Production (Mt)	Productivity t ha^{-1}
Uttar Pradesh	5.81	19.91	3.42
West Bengal	5.12	22.45	4.39
Odisha	3.77	9.83	2.61
Chhattisgarh	3.76	7.40	1.97
Bihar	3.31	12.14	3.67
Punjab	3.07	20.07	6.55
Assam	2.43	7.93	3.26
Andhra Pradesh	2.16	12.25	5.68
Madhya Pradesh	2.04	6.19	3.04
Telangana	1.96	9.39	4.79
Tamil Nadu	1.83	9.96	5.45
Jharkhand	1.74	6.12	3.53
Maharashtra	1.45	4.10	2.82
Haryana	1.42	6.79	4.77
Karnataka	0.99	4.53	4.56
Gujarat	0.86	2.84	3.31
Others	2.07	7.27	3.51
Total	**43.77**	**169.14**	**3.86**

Source: Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India (2020).

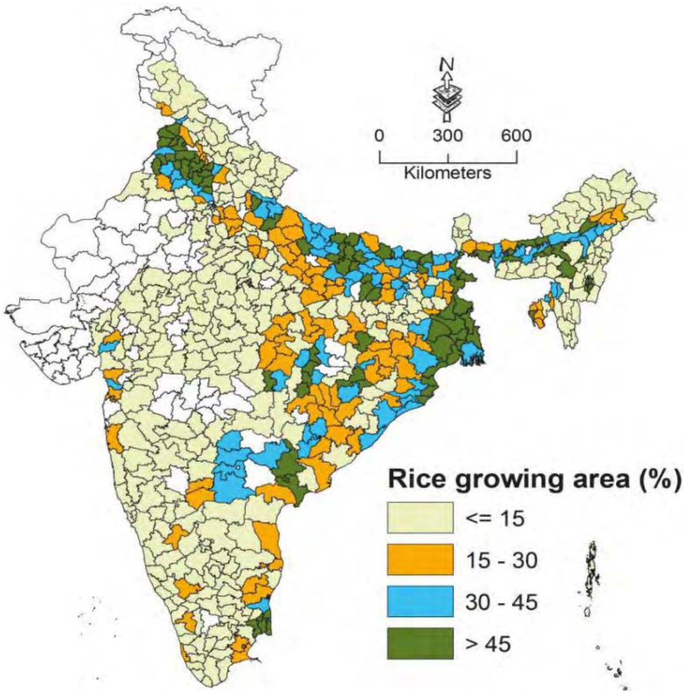


Figure 3. District-wise rice area in proportion to the total agricultural area in India (Source: NRRI 2019).

Culturally rice has played a major role in shaping the socio-cultural life of the sub-continent. The relationship between the people and the rice through the ages has inspired the cultural expression of several forms depicted via. stories, songs and paintings. Rice is considered divine, and is an important part of festivals and religious ceremonies (Pachauri et al. 2013). Many Hindu festivals including *Onam* in Kerala, *Bihu* in Assam, *Sankranthi* in Andhra Pradesh, *Pongal* in Tamil Nadu, *Makara Sankranthi* in Karnataka, *Nabanna* in West Bengal, *Ropain* and *Dzo-tiyum* in Sikkim celebrates transplantation and harvest of Paddy. Annaprashan - an ancient Hindu feeding ceremony, celebrating the transition of an infant to solids- literally means, in Sanskrit, “feeding of the rice”. Following this ceremony, the infant is progressively fed solid meals. Annaprashana is also known as Bhaat khulai in the Garhwal hills, Mukhe Bhaat in Bengal or Choroonu in Kerala, and Bhaat khwai in Nepali (Srividya 2018).

Presenting the antiquity of rice cultivation in India, Richharia and Govindasamy (1990), in their book "Rice of India", indicate that the country has been endowed with more than two lakhs (200,000) rice varieties, rich biodiversity that no other country on this planet can boast. Today, most states have their preferred rice varieties suited to the ecotype and rooted deep in their cultures and traditional cuisines. Many of them have agricultural significance. Some are better adapted to specific soils, others require lower night temperatures or can tolerate drought and/or flooding, while some are salt tolerant. Many grow tall, providing enough hay for fodder and roofing, whereas some have awn protecting the grain from birds. Some are of short season suitable for the summer rains, whereas others mature slowly, taking 180 days. The uniqueness of these varieties does not end with the paddy plants, duration, seasonality, etc. Grains also have their unique features having specific nutritive, cooking and eating qualities

(Deb and Malhotra 2001). For example, Krishna bhog- a popular aromatic rice from Sikkim with a medium-sized grain is delicious to make kheer (payasam), *Dhakaney* or any sweet. Similarly, Gandhasaale, an aromatic rice variety grown in the Western Ghats, when it begins to cook, emits a heavenly fragrance that gently permeates throughout the house. Jeera Samba, often known as the South's Basmati rice, is slim, aromatic rice popular in Tamil Nadu and Kerala.

Further, medicinal rice is an altogether different class. Each has its property, from dealing with acne, arthritis, pregnancy-related issues, cancer, headaches, skin infections, stomach issues, heartburn and much more (Devi et al. 2008). For example, Tukmor zho, upland rice grown in the Dzongu village, North Sikkim, is used by women folk to overcome health problems ranging from gynaecological issues to joint pains. Similarly, Navara– the most well-known rice with medicinal properties is used extensively in Ayurveda for external use as *kizhis* (little bundles of medicated herbs, oils, and powders) and consumption as *kanji* (natural homemade probiotics). Similarly, Valiya Chennelu from Wayanad, Raktasali from Karela, Karibhatta from Karnataka and numerous others are the storehouse of curative properties (Krishnamurthy 1991).

1.3. Rice cultivation in northeast India

The northeastern region of India, comprising eight hilly states *viz.*, Assam, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Meghalaya, Tripura and Sikkim, represents the culturally and geographically unique landscape embodying the elements of two important biodiversity hotspots, *viz.*, Eastern Himalayas and Indo-Burma regions (Mittermeier et al. 2011). Within an altitude range of 50 to 5000 m and above, a large expanse of hills interspersed with fertile valleys offers agro-climates of exceptional diversity ranging from extreme temperate to tropical conditions (Konjengbam 2021). High rainfall (2032 mm to 10700 mm) and humidity (80-90%) characterize the region (Roy et al. 2016).

Rice is a major cereal crop which covers an area of 3.51 million hectares producing a total of 5.50 million tones of grain with an average productivity of 1.57 t/ha in this region. It is grown across the altitudes and landscapes. In Arunachal Pradesh and Sikkim, rice is grown at an altitude of 2000 m along the hilly stretches. Hilly and plain areas characterize Assam, and rice is cultivated in both. In Manipur, rice is grown in upland and lowland areas (Pattanayak et al. 2006). In Mizoram, rice is limited only to the valley and lowland areas. Rice is also the main staple in Nagaland, where more than 400 accessions have been collected. Rice in Tripura is cultivated along the hills, hillocks and flat valleys (Roy et al. 2015).

1.4. Rice landraces of northeast India

The extreme variation in altitude, topography, physical and climatic conditions along with the antiquity of cultivation, has enriched the region with an immense crop genetic diversity in the form of traditional cultivars or landraces. However, their distribution is uneven, with many being rare and grown in small areas. As the terrain offers diverse topographic conditions in terms of slopes, altitudes, and soil types, the rice varieties grown here are thought to be a treasure trove of agronomically important genes (Das et al. 2013; Choudhury et al. 2013). Selection by various ethnic groups over hundreds of years has further contributed to this unique gene pool (Rohini Kumar 2013). It is reported that an estimated 10,000 cultivars/landraces of ecological and cultural significance are conserved in this region (Hore 2005; Das et al. 2013).

However, their relevance has been continually overlooked in mainstream industrial agriculture, considering they are low-yielders. The formal agricultural institutes have paid little attention to evaluating and conserving them. Additionally, unprecedented changes brought about by the green revolution (GR) in the country's agricultural sector since 1960s, especially through the introduction high yielding varieties (HYVs), have caused significant decline in the cultivation of indigenous landraces (TAAS 2021) here as well. Further, genetic erosion has occurred rampantly due to a limited germplasm selection for the breeding efforts (Rathi et al. 2018). The introduction of HYV, fertilizers, insecticides, fungicides, and weedicides, seems to have broken the entire agricultural supply chain and the socioeconomic fabric of the farming community depending on the traditional landraces. Initially, farmers were enticed by the high grain yields, which have begun to drop over time despite the massive use of agrochemicals. With these consequences visible, farmers, institutional scientists, and extension workers are waking up to this call.

The silverline in this otherwise gloomy situation is that some progressive farmers have started planting traditional rice varieties considering their importance for holistic benefits such as soil health etc., and their ability to provide long-term yield with minimal inputs (Smale 2006). Additionally, some local farmers have always retained landraces on the small patches of their lands. These can reinvigorate conservation efforts. Additionally, they can also act as a source of novel breeding material for genetic improvement programs (Zeven 1998) for deployment in modern breeding approaches like genomic selection, as well as the discovery of untapped Qualitative Trait Loci (QTL)/genes/alleles which can pave the way for improved agricultural production, particularly in the backdrop of climate change (Huggins et al. 2019).

1.5. Rice landraces of Sikkim

Sikkim Himalaya is part of 34 globally significant biodiversity hotspots and 22 agro-biodiversity zones in India (Sharma et al. 2016). Rice has great antiquity in this region, as evident from the etymological significance of its old name, "Denzong", meaning- "*the valley of rice*". Rice farming has been an inspiring enterprise here as it has evolved in response to the dynamic needs of a multi-ethnic society of diverse food habits, limited landholding, and inhabiting difficult topographies of a harsh climate. Their enduring creative labour, for centuries, has led to the selection of an array of landraces which occupy a significant niche in the local rice production system (Rahman and Karuppaiyan 2011).

Landraces, grown traditionally on the hill slopes with and without terracing, dominate the rice production here with a 55-60% share in acreage (Rahman and Karuppaiyan 2011). They have an important influence in shaping the state's culture and customs which are maintained by the farmers for various reasons, including traditional beliefs and rituals. They are also grown for locally favorite cooking quality traits such as distinct aroma, colour and taste. Even though traditional rice cultivation is not economically viable, when expenditures for imputed personal labour and owned land cost are not factored in, it might not be a loss-making venture. Therefore, for the local farmers involved in traditional rice cultivation, the economic validations of these factors haven't mattered so much, as they held their farming as more of a cultural heritage.

There are 70 plus such landraces reported so far (Sharma et al. 2016) from the region, which awaits detailed characterization. However, barring a few scattered works (Rahman and Karuppaiyan, 2011; Das et al. 2013; Choudhury et al. 2013, Sharma et al. 2016), not much has been done in this direction. Most of these landraces suffer from poor yield, lodging, and late maturity, although they boast other superior traits such as hardiness, pest tolerance/resistance, low input requirements, and weed coexistence (Sharma et al. 2015). Even though HYVs are available (https://icar-nrri.in/released-varieties), their introduction in place of the existing landraces/cultivars is proving detrimental to the native gene pool owing to the evident genetic erosion (Rathi et al. 2018). Thus there is a need to scientifically document and characterize these genetic resources for conservation and improvement.

1.6. Indigenous Traditional Knowledge (ITK)

Indigenous Traditional Knowledge (ITK) is based on people's experience with nature and embodies deep knowledge of the natural resources developed by a given community over the years (Borthakur and Singh 2012). In agriculture, it covers a broad spectrum of understanding related to water management, farm equipment, post-harvest preservation and management, agro-forestry, biodiversity conservation, animal rearing, health care, etc. (Parkash et al. 2021). They are adapted to local culture and environment, and often tested over a long period of use, emphasizing minimum risk rather than maximizing profits (Chhetry and Belbahri 2009).

In the remote parts of Sikkim, the indigenous communities mainly depend on agriculture and animal husbandry as the primary source of their livelihood (Subba 2009). The farmers have rich traditional knowledge of agriculture, pest management, biodiversity conservation, and ethnoveterinary practices. Unfortunately, these practices, which are in vogue in rural parts, are little documented, posing a danger of extinction (Devaki and Mathialagan 2015).

Rice farming is an age-old enterprise in Sikkim. Its need-based evolution - from sowing to harvesting and preservation/storage- has instilled a pearl of unique wisdom in rice cultivation among the local farmers. As they have been acquired based on the local capacity and ecology, they all appear to stand on a sound footing. However, this heritage has witnessed a speedy extinction recently due to a lack of efforts to document and conserve them.

1.7. Evaluation based on seed morphometric traits

Evaluation of seed traits has been used as an important indicator in the characterization of the rice germplasm (Ogunbayo et al. 2005; Bajracharya et al. 2006). They provide plant breeders with the initial information on the breeding materials (Barry et al. 2007) and for their grouping.

Grain quality in rice is a combination of several physicochemical traits, which can be categorized under three broad areas: (1) physical characteristics which include shape, size, and milling (2) Physico-chemical characteristics, including amylose content, gel consistency, the volume of expansion of cooked rice, and cooking time and (3) the organoleptic qualities of cooked rice which include colour, aroma, hardness, stickiness, and consistency. The various grain types are classified according to length, width, length/width ratio, thickness and grain weight (Lu and Luh 1991). In addition, kernel dimension is the primary quality factor in many areas of processings including drying, handling of equipment, breeding, marketing and grading (Adair et al. 1973). There is an established size and shape requirement each grain type and variety must conform to meet specific market requirements. Therefore, any rice quality assessment programs usually use these indicators.

Additionally, rice's cooking and eating qualities have always been important factors for consumers. They are influenced by varietal characteristics, condition of cultivation, and post-harvest storage and processing (Meena et al. 2010). Besides, the proximate analysis, sensory attributes, gelatinization temperature, and alkali digestion aids in identifying and developing nutritionally rich rice cultivars (Dong et al. 2007).

1.8. Molecular markers and Association analysis

A genetic marker is any phenotype, gene, or DNA sequence linked to a trait with a defined chromosome position. Genetic markers act as indicators or flags (Collard et al. 2008). There are two main genetic markers: classical and DNA/molecular markers. Classical markers include morphological, cytological, and biochemical markers. In contrast, molecular markers are nucleotide sequences and can be investigated through the polymorphism revealed between the nucleotide sequences of different individuals (Perez-Sackett et al. 2011). These polymorphisms are based on insertion, deletion, point mutations, duplication, and translocation; however, they do not always influence the gene activity. DNA markers include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single-nucleotide polymorphism (SNP), and diversity arrays technology (DArT) markers (Jiang 2005).

The development of genetic markers can be considered a significant advancement in plant breeding (Muhammad et al. 2012). In crop plants, various economically important characteristics are controlled by major genes/QTLs. Such characteristics include resistance to diseases/pests (Jiang 2005), sterility and others related to the shape, colour and architecture of the whole plant or plant parts (Botstein et al. 1980). These traits are often of mono or oligogenic inheritance in nature. Even for some quality traits, one or few QTLs or genes can account for a high proportion of the phenotypic variation of the trait (Bilyeu et al. 2006). Transferring such genes to a specific line can lead to tremendous improvement of the trait in the cultivar under development (Zhang 2007).

DNA markers have been the choice tool for genetic characterization (Karkousis et al. 2010). Diverse forms of DNA markers have been discovered and successfully employed in genetics and breeding activities in various agricultural crops, but the SSRs are the most popular. SSRs are 1–6 nucleotide tandem repeat motifs found in abundance in the genomes of diverse organisms (Beckmann and Weber 1992) and preferred due to their polymorphic nature, abundance in the eukaryotic genome, and ease of generating (Tautz 1989; Morgante and Olivieri 1993). In rice, SSRs have been used earlier for the assessment of genetic diversity (Rahman et al. 2012; Choudhury et al. 2013; Singh et al. 2016; Suvi et al. 2019), conservation planning (Sharma et al. 2007), marker-assisted selection (Perez-Sackett et al. 2011; Rani and Adilakshmi 2011), cultivar identification, and hybrid purity analysis, besides gene mapping (Weising et al. 1997; Altaf-Khan et al. 2006; Sarao et al. 2010).

Further, any marker-assisted breeding (MAB) initiative entails the identification of candidate genes/QTLs for the trait of interest. Typically, linkage mapping and/or association analysis are used for this purpose. The major shortcoming in the linkage analysis is that only two alleles at any given locus can be considered in bi-parental crosses, which provides a low mapping resolution (Flint-Garcia et al. 2003). Alternatively, association mapping categorizes QTLs and identifies essential genes by utilizing natural diversity (Zhu et al. 2008). It has been successfully employed in various crop species, including Rice (Abdurakhmonov et al. 2008, Famoso et al. 2011; Huang et al. 2012; Zhou et al. 2012; Han and Huang 2013).

Hence, we have undertaken assessment of genetic relationships and population structure of the Sikkim landraces using SSR marker, in addition to Genome-Wide Association Studies (GWAS) to gain early insights into the loci controlling the key agronomic and nutritional quality traits. In terms of scope, it is hoped that our research from this Ph.D. thesis will (a) provide clues/leads for developing and promoting sustainable rice farming systems based on time-tested ITKs for the region, (b) an updated information to refine them scientifically for large-scale adaptation elsewhere, and (c) aid in the evolution of appropriate breeding and conservation strategies for preserving the valuable native rice gene pool

CHAPTER 2

2. LITERATURE REVIEW

The available literature pointing to prospects, practices and gaps related to research carried out in the present thesis is reviewed below.

2.1. Rice: Global significance

According to Maclean et al. (2002), rice accounts for 21% of global human per capita energy, 15% of per capita protein, and 21% of the fat supply. It is ranked as the world's principal human food crop. The FAO’s (2021) data shows that rice is grown in over a hundred countries producing more than 715 million tons of paddy rice annually, where ~50% of it comes from just two countries: China and India. Asian countries account for 90% of the world's total rice production (Richharia 1960). Major non-Asian rice-producing countries include Brazil, the United States, Egypt, Madagascar, and Nigeria, which account for 5% of the rice produced globally (FAO 2103). In Africa, rice is the fastest-growing staple food, the production of which has risen steadily from 9.3% in 1961 to 15.2% in 2007 (IRRI 2009). Rice, therefore, is of special importance for the nutrition of the large reaches of the population in the Asia-Pacific region, parts of Latin America and the Caribbean, and increasingly so in Africa (FAO 2015). Additionally, it provides the means of employment and income for more than 200 million households worldwide (FAO 2015).

2.2. Botany

Rice (*Oryza sativa* L.) is a cereal food crop belonging to the family Poaceae. It can grow up to 1-1.8m tall or more, depending on the variety and soil fertility. As a grass family member, the rice plant sports long, slender leaves between 50-100 cm long and

2-2.5 cm broad. It develops a central stem and many tillers, which range from 0.6 to 6m (floating rice) in height. Each panicle produces 50–300 flowers (floret or spikelet) (Britannica 2016).

Li (2003) described the rice grain as rough rice or paddy consisting of the endosperm, embryo and several thin layers of differentiated tissues- the pericarp (the ovary wall), the seed coat, and the nuclellus. The seed coat comprises six layers of cells, the innermost of which is the aleurone layer. According to Lu (1999), rice endosperm consists mainly of starch granules in a proteinaceous matrix, together with sugar, fat, crude fiber and organic matter.

Oka (1988) differentiated *O. sativa* into two partially isolated gene pools: *indica* and *japonica.* These two wide varieties, also often referred to as subspecies, are distinguished based on their morphological, physiological, biochemical and molecular traits (Vaughan et al. 2008; Lu et al. 1991; Zhang et al. 1992; Yang et al. 1994). Ma and Bennetzen (2004) and Tang et al. (2004) investigated the molecular basis of this differentiation and reported that the information from the genomic data also supported this division, with both possessing relatively distinct genomes that may have originated from a common ancestor about 200,000 to 440,000 years ago.

2.3. Landraces in rice breeding

The landraces maintained by the farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period (Cleveland et al. 2000). This aids in their adaptation to a wide agro-ecological niche and, many a time, they also have unmatched qualitative traits and medicinal properties (Gyawali et al. 2017).

Holden et al. (1993) reviewed the importance of landraces in breeding programs. They inferred that, in the agricultural system, landraces could not be denied because the improvement of the current variety depends on the beneficial genes that may only be found in landraces and wild varieties.

Hore (2005) has pointed out the adverse consequences of the large-scale spread of modern, high-yielding varieties in the Indian agricultural system leading to a reduced genetic base and increased genetic vulnerability in the traditional varieties. This trend has also been reported in several other Asian countries (Chaudhary et al. 2006).

Considering the above, several works of literature (Smale 2006; Gauchan et al. 2006) have stressed on the imperative role of the farmers and researchers in the continuation and management of these valuable genetic resources to conserve the diversity, rarity and adaptability of native rice germplasm.

2.4. Rice landraces of northeast India

Being a biodiversity hotspot, northeast India has a wealth of indigenous rice germplasm sporting a valuable gene pool (Sharma et al. 1971). The region is considered the center of genetic differentiation of *O. sativa*. As such, the whole region is a veritable treasure of rice germplasm (Morinaga 1968), as evident from the many ecotypes available (Das and Ahmed 1995).

Asthana and Majumdar (1981) pioneeringly evaluated 465 landraces of northeast India and identified various ecotypes with the help of metroglyph analysis. Based on the morphological, genetic and chemical characteristics, they divided the accessions into groups and subgroups. Gupta et al. (1995) found that rice varieties cultivated at higher elevation areas (2,500-3,000 m asl) in Arunachal Pradesh possess morphological features attributable to *O. sativa* sp. *japonica,* such as a globose grain, narrow, dark green and drooping flag leaves and a thin culm. Of the 35,000 rice germplasm collections from India, they reported that about 8,000 comes from northeast India.

Hore (2005) reported that natural populations of many wild rice species, such as *Oryza rufipogon*, *O. granulata*, *O. officinalis*, *O. nivara*, *O. meyeriana*, *Hygrorhiza aristata*, *Leersia hexandra* and *Zizenia latifolia* are also found in northeast India. According to Ramakrishnan (2006), the cultivated rice varieties in Nagaland show morphological features intermediate between the *japonica* and *indica*.

Choudhury et al. (2014), while characterizing the indigenous rice varieties of northeast India, reported that soft and sticky rice varieties are abundant in Meghalaya, and at least 20 dominant landraces are still cultivated in the Garo hills district of the state. He further reported that the state of Mizoram is rich in aromatic and sticky rice and has a few droughts and cold-tolerant varieties.

Chakraborty and Ray (2019), in their study on 171 rice landraces from six northeast states, demonstrated that the socio-cultural interaction has shaped the diversity and divergent history of the rice landraces over time and space.

Such variability in germplasm understandably reflects high levels of genetic diversity, which can serve as potential genetic stock for improving yield, resistance to pests and pathogens, and agronomic performance (Brush 1995; Hoisington et al. 1999; Mandel et al. 2011).

In Sikkim, much variation was observed in morpho-agronomic characters such as panicle type, awn, glume colour, grain type and kernel colour (Arora, 1982). In their agro-biodiversity study, Sharma et al. (2016) documented 70 landraces, enunciating the need for detailed characterization/evaluation to determine their relevance for the breeding program and conservation planning.

Kapoor et al. (2017 and 2019) have also documented and studied some Physico-chemical and micronutrient characteristics of the indigenous rice landraces of Sikkim and reported 53 rice landraces under cultivation in various agro-ecological regions of the state. Their results unveiled the potential for improving popular landraces like Attey, Kalo nunia, Birinful and Krishna bhog.

However, from the available literature (Das et al. 2013; Choudhury et al. 2013; Rahman and Karuppaiyan, 2014; Roy et al. 2015; Charkaborty et al. 2019), we could determine that not much work has been done to assess the genetic diversity, and population structure and identifying markers/genes for the key agronomic traits in rice landraces from the Sikkim Himalayan region.

2.5. Germplasm collection and characterization

2.5.1. Physico-chemical and nutritional characteristics of the grain

Grain quality characteristics include physicochemical properties *viz.*, dimension, shape, weight, fragmentation, hardness, milling properties, the chemical composition of the endosperm, aroma and nutritional factors. Phenotypic characterization based on grain quality traits is fundamental to any rice breeding programme. According to Lodh (2002), determining rice grain quality should also include parameters such as grain colour, size and shape, which are important from the consumer's viewpoint in domestic and international markets.

Patra and Dhua (2003) studied 120 upland rice germplasm of the Jaypore tract of Orissa and revealed enormous morphological and agronomical variability among the grain characters. Parray and Asif (2008), evaluating the grain characters of 66 indigenous Kashmir rice cultivars, reported that most cultivars had genes for superior rice quality. The collections were also found to have significant variability for the grain colour and aroma, suggesting a constellation of unique allelic resources.

Further, Sinha and Mishra (2013) studied 55 traditional rice varieties of West Bengal for grain morphological characters and reported wide variation. They have used several parameters such as grain appearance, nutritional value, cooking and eating quality to determine the grain quality.

As rice is the most important grain, accounting for over a fifth of all calories consumed globally (Fukaguwa 2019), its nutritional value and cooking properties have been accorded great importance (Tan et al. 2001; FAO 2004; Jiang et al. 2005; Dong et al. 2007). Rice is often consumed as a whole grain after cooking and, in a regular Asian diet, can contribute to 40 to 80% of the total calorie intake (Paramita et al. 2002; Singh et al. 2005; Hossain et al. 2009; Cai et al. 2011). Juliano and Villareal (1993) suggested that rice breeding programs prioritize micronutrient concentration. In terms of protein, according to Eggum and Juliano (1975), rice has a lesser quantity of it (8.5%) as compared to wheat (12.3%), barley (12.8%), and millet (13.4%). However, it is considered as one of the finest quality cereal proteins due to high lysine (3.8%) content. Banerjee et al. (2011) reported the protein content of milled grains in a set of 258 diverse rice landraces maintained in the germplasm section of IGKV, Raipur, and found wide variability in lysine content.

Baruah et al. (2006), working with 10 *Baodhan* (Deepwater paddy) cultivars of Assam, found that protein content varied from 8.03% to 13.20% with a mean value of 11.78%. Similar findings have been reported by Loying (2007), working with some deep water paddy landraces where protein content varied from 9.63% to 13.22% with a mean of 11.06%. However, Roy et al. (2010), working with the scented rice landraces of Assam, found low levels of protein in the range of 6.12% to 8.43%. They stated that what is palatable and attracts great demand in the market need not necessarily be nutritionally rich.

With regard to fat content, Devi et al. (2008), in their preliminary study on the physical and nutritional qualities of 15 indigenous rice cultivars of northeast India, found that local cultivars had total fat values of 1.2 to 4.2 %. Pathak et al. (2017) studied the variability in grain quality characters of Assam's local winter rice (*Sali*) and reported the crude fat content from 2.64 to 3.76 %. This is consistent with the studies from the other regions. Kitta et al. (2005), while evaluating the lipid content and fatty acid composition of Japan's major non-glutinous rice cultivars, have reported that lipid content and fatty acid composition are not influenced by temperature and edaphic factors. Notably, these factors differ considerably in the northeast region and Sikkim in particular.

Crude fiber is not digested and, as such, does not provide nourishment. However, it aids digestion, retains moisture, and, most importantly, aids excretion. Sujatha et al. (2004), while studying the two varieties of raw and parboiled rice cultivated in the coastal region of Dakshina Kannada district of Karnataka, found a higher percentage of crude fiber and ash content (1.48% and 0.88%). They stated that crude fiber content showed variation between different seasons and parboiling processes, resulting in greater retention of nutrients in milled parboiled grain (Deosthale and Pant 1970). Durgarao et al. (2017) compared the nutritional content of Basmati, HMT and Indrayani instant rice. They reported that the highest (1.70%) crude fiber content in HMT rice and the lowest (0.40%) was observed in Indrayani rice.

Further, ash content comprises total minerals, generally low in paddy and mostly restricted to the pericarp. These are phosphorus, chlorine, potassium, calcium, magnesium, sodium, etc. (Grist 1975). Deosthale and Pant (1970) studied the ash content in some red rice cultivars, and the values were found to be higher, in the range of 1.2% to 1.63%. Another research carried out by Patel and Rajani (1967) with some indigenous cultivars of Gujarat showed wide variation in the range of 0.98% to 2.08%. Kandali et al. (1995), working with some superior varieties developed by Assam Agricultural University, reported a variation in ash content in the range of 0.85% to 1.5% with a mean of 1.12 %.

Anjum et al. (2007) investigated the mineral content of Irri-6, Irri-9, Sarshar, and DR-83 coarse rice cultivars of Punjab and reported that the mineral content such as iron, zinc, manganese, and copper varied amongst cultivars. They suggested that rice breeders should pay more attention to Sarshar and Irri-6 for use in hybridization projects since these varieties contain more proteins and minerals.

Banerjee et al. (2011) analyzed the variability in grain protein, Fe and Zn content of 46 accessions of rice landraces from West Bengal. They recorded wide variations in micronutrient levels which ranged from 4.82 μg/g to 22.69 μg/g and 13.95 μg/g to 41.73 μg/g for grain Fe and Zn content, respectively. Similarly, the nutritional composition of 10 different aromatic rice samples obtained from the Agricultural Research Station, Karnataka, was evaluated by Nadiger and Kasturiba (2015). They found that Ambe mohar-1 had the highest levels of protein and iron. In contrast, Ambe mohar-2 had the highest zinc levels, suggesting their suitability as a parental line in the rice breeding programme. The nutrient profile of 32 rice landraces of Garo hills, Meghalaya, was evaluated by Longvha et al. (2020). They found substantial nutrient variability among the genotypes and reported that the studied landraces are a good source of dietary fiber. Particularly, their low phytate content is of significance for enhanced mineral bioavailability.

Giri and Vijaya (2000) stated that cooking qualities are very important for rice consumers' acceptance of a variety. It is determined in terms of amylose content (AC), gelatinization temperature (GT), cooking time, elongation ratio, water uptake ratio and aroma (Lisle et al. 2000). The cooking properties of six major Indian rice cultivars were evaluated by Yadav et al. (2007), who found no significant difference in the cooking time of different cultivars. They observed that basmati's cell wall is more compact than those of non-basmati rice, which suffered cracking and leaching out easily during cooking, resulting in a higher solid loss. Rathi et al. (2010) evaluated the nutritional and cooking characteristics of some imported and scented rice types for quality improvement. Results showed that the imported brands differed significantly ($p<0.05$) from the local varieties by cooking faster and having a high length-wise expansion. They concluded that the imported brands showed better cooking characteristics, but the local varieties were nutritionally superior. Pathak et al. (2017) also studied the *Sali* rice of Assam and reported considerable genetic variation in different cooking quality characteristics.

The temperature at which starch granules undergo an irreversible process is known as gelatinization temperature (GT), characterized by crystalline melting, loss of birefringence and starch solubilization (Correia et al. 2012). The GT affects rice cooking quality (Ghosh and Govindaswamy 1969), water intake, volume expansion, and linear kernel elongation during the cooking process (Tomar and Nanda 1982). High GT rice gets overly mushy when overcooked, elongates less and remains undercooked under regular cooking procedures. Hence it is least accepted by consumers around the world. According to Dela Cruz et al. (1989), the temperature during grain formation, impacts gelatinization temperature. He stated that when the ambient temperature is high during grain ripening, starch with a higher gelatinization temperature is produced. Vanaja and Babu (2004) showed that high-amylose rice varieties absorb more water, have low gelatinization temperature and produce more cooked material.

In terms of evaluation, out of 100 *ahu* rice genotypes of Assam, 97 genotypes showed a low GT range (55–68ºC) with ASV 5.0-7.0 (Rathi et al. 2010). Out of 99 local *Sali* rice of Assam, Pathak et al. (2017) found that 73 genotypes were found to have low GT (55–68°C, ASV: 5-7) and 22 showed intermediate GT (69-74°C, ASV: 3-5). They concluded that the intermediate Alkali spreading value (ASV) indicated the medium disintegration and classified it as intermediate GT, which is highly desirable for quality grain.

Amylose Content (AC) is the single most characteristic for predicting rice cooking and processing behavior (Juliano 1979; Webb 1985). The ratio of two types of starches in the rice grain, amylose and amylopectin, influences milled rice's cooking and eating qualities (Juliano et al. 1964). Rice cultivars are classified as waxy (0-2 %), very low (3-9 %), low (10-19 %), moderate (20-25 %), and high (>25 %) based on their amylose concentration (Dela Cruz et al. 1989).

Singh et al. (1998) reported a narrow range of amylose content in the Himachal Pradesh rice collection (19.5 to 22% in brown rice and 22 to 25% in milled rice) and found that majority of them fall within the intermediate range. Similarly, Thongbam et al. (2010) reported that medicinal rice cultivars of Manipur have amylose concentrations ranging from 14.33 to 29.47%, with the majority of the cultivars falling into the intermediate amylose content category (20- 25%). They reported a special class of rice locally known as Chokuwa rice (soft rice), indigenous to Manipur, needs no cooking and can be consumed after simply soaking in the cold to lukewarm water. This rice is characterized by its semi-glutinous character with amylose content ranging from 12 to 17%.

Pathak et al. (2017) studied the *Sali* rice of Assam. They found that out of 99 local genotypes, 78 were grouped into the waxy (glutinous) category due to very low amylose rice (0-12% amylose), 11 were considered as low amylose rice (12- 20% amylose), another six as intermediate amylose rice (20-25% amylose) and the remaining four glutinous rice genotypes as the high amylose rice (>25% amylose). Even a higher range of amylose contents (0.136-11.4%) for some glutinous rice of Assam was reported by Bhattacharjee (2006).

One of the most important factors for consumer acceptance and the economic value of rice in speciality markets is its aroma. Aromatic rice has distinct fragrances released during cooking and eating and in the field during harvesting, storage, and milling (Efferson 1985). The aroma of rice is believed to be contributed by more than 100 compounds (Tsugita 1986, Widjaja et al. 1996). Of them, the popcorn-like smell of aromatic rice is generated from 2-acetyl-1-pyrroline (2-AP) content and is considered desirable by many consumers (Buttery et al. 1988). Aroma is reported to be controlled by a single recessive gene found on chromosome 8 (Buttery et al. 1988).

Tsugita et al. (1980) conducted an odour evaluation and gas chromatography test of four different types of Japanese rice and identified 40 volatile compounds from milled rice and found a substantial difference in milled rice's odour and volatile components. They also suggested that the surface layer elements of rice grains play a vital role in developing cooked rice aroma.

Tava and Bocchi (1999) reported that 2-AP is the most important volatile component contributing to the aroma; they concluded that the differences in aroma could be due to genotype, environment and pedoclimatic conditions. Parray and Asif (2008) characterized and evaluated 66 indigenous rice cultivars adapted to agro-climatic conditions of Kashmir for their component traits and quality parameters. Significant variation in aroma in the collection revealed a constellation of elite allelic resources in this set of germplasm.

Thus considering the need for comprehensive information on the rice landraces of Sikkim, it was felt essential to determine the variability for the physicochemical and nutritional parameters.

2.5.2. Molecular characterization

Molecular characterization provides precise information on the extent of genetic diversity, which is an important pre-requisite in conceiving the pre-breeding strategies. Germplasm management, varietal identification, and DNA fingerprinting all require it. A brief review has been summarized as follows.

Molecular markers, such as RFLP, RAPD, SSRs, ISSRs, AFLP, and SNPs are powerful tools in the assessment of genetic variation and the elucidation of genetic relationships within and among the species (Joshi et al. 2000). Several of them are currently available to assess the variability and diversity in rice. Among the various PCR-based markers, SSR markers are more popular in rice because they are highly informative, mostly mono-locus, co-dominant, easily analyzable and cost-effective (Chambers and Avoy 2000; Zhang et al. 2014; Thomson et al. 2007). In many studies, a combination of morpho-agronomic and molecular analyses is also employed (Kumar et al. 2009; Ghalmi et al. 2010; Sharma et al. 2010).

Ye-Yun et al. (2005) used SSR marker analysis to test the parental lines of five superior hybrid rice varieties. A total of 144 SSR primer pairs were used across the 12 rice chromosomes, with 47 detecting polymorphism in the lines tested. Chakravarthi et al. (2006) used 30 SSR primers on chromosomes 7-12 to determine the genetic diversity and DNA fingerprinting of 15 elite rice genotypes. The findings revealed that all primers showed distinct polymorphism among the cultivars, demonstrating the power of microsatellites to detect polymorphism.

Similarly, Herrera et al. (2008) used SSR markers to assess the genetic diversity in Venezuelan rice cultivars to broaden the genetic base of rice germplasm in the country. Although the reported genetic diversity was low, this study also demonstrated the utility of SSR for determining the genetic diversity of the rice genotypes.

To investigate the genetic diversity of 25 popular rice varieties in India, Upadhyay et al. (2012) used 12 SSR markers. They discovered genotype-specific alleles in 14 varieties that can be used to characterize the specific germplasm material. Meti et al. (2013) also used SSR markers to investigate the allelic diversity and relationships among the 48 traditional indigenous aromatic rice germplasm grown in the eastern part of India. The results showed that tested primers produced distinct polymorphisms among the landraces/varieties, demonstrating the robustness of SSR markers.

Das et al. (2013) examined the population structure of aromatic rice landraces of West Bengal and other northeast states utilizing the 23 previously mapped SSR markers. Based on the model-based clustering approach, a distinct cluster consisting predominantly of northeast aromatic landraces was observed. They concluded that diversity present in aromatic rice landraces of northeast states is higher than the West Bengal germplasm.

Roy et al. (2015) analyzed the population structure and genetic diversity of 107 aromatic rice accessions collected from various states of northeast India. Their result highlighted the importance of inputs from regional studies for understanding the diversification of aromatic rice in India. Similarly, Chakraborty et al. (2019) studied 171 rice landraces for genetic diversity and population structure from six northeastern states of India using 27 standard microsatellite data. They revealed the divergent history of the aromatic rice landraces and the alternate origin of aroma in *aus* aromatic group.

Therefore, in this study, we have deployed microsatellite markers for determining the genetic diversity in rice landraces of Sikkim.

2.5.3. Genome-wide association studies (GWAS)

The advancement in next-generation sequencing technologies has resulted in a wealth of genomic sequence data previously unimaginable (Edwards et al. 2013; Van et al. 2013; Varshney and Dubey 2009). Understanding the molecular basis of the traits linked to the identifications of valuable QTLs will aid in pyramiding multiple genes to generate superior and farmer-friendly rice varieties.

To map QTLs/genes for diverse phenotypes in rice, biparental mapping and genome-wide association studies (GWAS) have been used (Collard and Mackill 2008; Norton et al. 2014; Zhang et al. 2014; Nawaz et al. 2015). A landmark GWAS was reported by Huang et al. (2010) where they investigated 14 agronomic traits (morphological, yield components, grain quality and agronomic or physiological) in a panel of 517 rice landraces and detected many novel QTLs with relatively small effects. Putative QTLs explained about 36% of the phenotypic variation, and QTLs for six traits were found close to previously characterized genes. Similarly, Nawaz et al. (2015) studied the

Association Mapping of QTLs for eight elements in brown rice and reported a total of 60 marker loci associated with 8 elements clustered into 32 genomic regions. Their result provided insights into genetic basis of rice grain elements accumulation which will be helpful in identification of genes associated with accumulation of Zn, Fe and other elements in rice. Other researchers have also successfully used GWASs for identifying the important QTLs in Rice (Huang et al. 2010; Zhao et al. 2011; Huang et al. 2012; Wang et al. 2014; Wang et al. 2015; Kumar et al. 2015).

Among other crops, the method has been employed to find out significant loci for different elemental concentrations in wheat (Velu et al. 2016; Alomari et al. 2017), Fe and Zn in barley (Mamo et al. 2014; Gyawali et al. 2017).

Thus, we have made an early attempt to determine the Marker Trait Association (MTAs) for the important agronomic traits in the rice germplasm of Sikkim.

2.6. Indigenous Technical Knowledge (ITK)

India has nurtured a remarkable civilization with a history of agriculture dating back 5,000 years. The country’s ancient scriptures, teachings, and countless proverbs contain a vast storehouse of knowledge, ideas, concepts, and practices to establish harmonious relationships between man, animals, and nature. Different researchers have given different names for it. Some commonly used terms include 'traditional knowledge,' 'indigenous technology,' ‘local knowledge system,' ‘farmers’ ingenuity and wisdom,' ‘Indigenous traditional knowledge’ and so on. Here we have used the term ITK.

According to Warren (1991), indigenous knowledge is local knowledge unique to a particular culture or society. This knowledge serves as a society's information base, facilitating communication and decision-making. Similarly, Sharma (2009) stated that indigenous practices provide invaluable knowledge and aid in using natural resources best. He advocated that ITK documentation must be part of environmental and sustainable development curricula. According to Talukdar et al. (2012), ITKs are local people's knowledge developed through close interactions with nature and natural resources for their livelihood to mitigate immediate crop environmental challenges to maintain productivity and sustainability.

2.7. Importance of ITKs in agriculture

ITKs on agriculture assume critical importance even in the modern era as they are cheaper, locally available, and have lesser side effects. Thus, it is important to study, understand, document and share them. Considerable literature is available on this aspect owing to the attention it has received from individuals, scientists, NGOs, and organizations. Nevertheless, research or reports on their practical implementation and scientific validation are scarce.

Rathi (2013) stated that many indigenous communities have deep knowledge of the ITKs related to the weather forecast, seed germination, water conservation, restoring soil fertility, managing insect pests and diseases of plants and livestock, post-harvest storage etc.

Roy et al. (2015) reported that such ITKs are still in vogue in organic agriculture and are sustainable and eco-friendly. According to Gopalakrishnan (2005), the ITKs are a central component of the daily life of millions of people in developing countries. Srinivasrao et al. (2021) opine that in recent times, Western science has become more curious about ITKs as they may provide novel clues for addressing the problems related to agriculture.

According to World Intellectual Property Organization (2017), several ITK documentation projects have been/are being conducted worldwide. For example, more than 22,000 community innovations and other TK components have been recorded by the Society for Research into Sustainable Technologies and Institutions (SRISTI) in the Honeybee Database and other systems.

2.8. ITKs related to rice cultivation in India

2.8.1. Main field preparation

The sole purpose of ploughing was to prepare well-pulverized soil to bring the field in good tilth, incorporating the organic matter in the soil, controlling the weeds and, to a certain extent, controlling pests and diseases (Das et al. 2003).

Dani (2018) reported that, generally, three ploughings are carried out before puddling. Farmers followed length or breadth-wise deep ploughing. A similar observation is reported in many other works of literature from all over the country (Ahuja and Srivastava 1995; Sharma et al. 2016; Krishnankutty et al. 2021).

According to Singh (1992), farmers of Sonapur and Hamirpur villages in Uttar Pradesh used soil from the bottom of ponds to enrich their rice fields before planting season. The nutrient-rich soil base is formed by decomposed waste products, such as leaves, twigs, and other organic components, mixed with the soil. This practice is reported to be followed even in the tank-fed areas of Tamil Nadu, Karnataka and Andhra Pradesh (Ayyavu, 1995). They reported that using this practice increased crop yields by 25-30% while fertilizer costs were reduced. Similar observations of field management were reported by Das et al. (2003), Ram et al. (2003) and Acharya et al. (2001).

Acharya et al. (2001) reported that farmers from north Goa gathered a herd of sheep/goats in the rice field before land preparation to enrich the soil. Animal excrement provides valuable nutrients to the soil. The shepherd collects a fee from the farmers for this purpose. This is a common practice in most rice-growing areas.

2.8.2. Seed selection and germination

Lakshmana (2000) documented that, in Tamilnadu, the bold grains are usually selected for seed purposes. One month before harvest, fields with healthy crop growth are chosen. Plots that are free of pests and diseases are used. While threshing manually, the bold seeds, which are easily shattered by the first two beating operations are selected, and the partially filled and diseased grains are separated accordingly. Similar observations have been recorded by Ganeshamurty (2000), Nayak et al. (2002) and Das et al. (2003) in various rice-growing states of the country. It was the best method to attain maximum performance in terms of growth and yield.

Uprooting and re-establishing the seedlings make the plants grow much more vigorously (Morachan, 1993). Rambabu (1997) reported that usually, for raising a paddy nursery, the place with a higher elevation near the water source is selected so that seedling gets enough water for growth.

Selvarani (2000) reported that farmers in Chennai soak rice seeds for 24 hours and fill them in a copper vessel called *Anda*, whose mouth is covered with paddy straw. The vessel is then placed upside down in a room corner for 24 hours. The following day, sprouted seeds will be used for planting. A similar observation of the seed sprouting process was observed by Narayanasami (2006).

Acharya et al. (2001) reported a similar seed broadcasting process on marshy land in the Bhojpur district of Bihar. It is exercised in approximately 90% of marshy and waterlogged lands, and rice yield is comparatively (20 to 30%) higher than transplanted rice. Rathi (2013) investigated the seed germination process of two tribes (Halba and Gond) and found that the Halba tribes in the southern part of the Bastar district cover the paddy seeds with moist jute bags. In contrast, the Gond tribe from the northern part of the district covers seeds with the green leaves of Saaz tree (*Terminalia chibula*).

2.8.3. Green leaf manuring

Green manuring is an old practice adopted to improve the fertility and productivity of the soil. It involves growing and burying leguminous crops *in situ*. Besides the beneficial effects of organic matter, green manure supplies a good amount of nitrogen (Morachan, 1993). For *in situ* manuring, Rautary (2002) and Rath et al. (2006) observed farmers in Tamil Nadu and Rajasthan grow khejri trees (*Prosopis cineraria*) in rice fields. According to farmers, khejri draws moisture and nutrients from the underground soil for crops.

Farmers in Arunachal Pradesh collect the weed water hyacinth (*Eichhornia crassipes*) and heap it in pits for nearly 30-40 days to decompose. The compost is sun-dried before being applied to fields and ploughed into the soil. Following this, fields are flooded in preparation for rice transplanting during the Kharif season. This compost enriches the soil with nutrients and is reported to improve the soil's physical, chemical and biological condition (Acharya et al. 2001).

Ratan et al. (2016) documented that farmers of Jharkhand add Kuda (*Hollarhena antidysenterica*), Undi (*Calophyllumino phyllum*), and Adulsa (*Adhatoda vasica*) leaves in the puddled fields. They concluded that these plants have insecticidal and antimicrobial properties, which besides adding manure, also make soil free from harmful insects and microbes.

2.8.4. Control of weeds, diseases and pests

Farmers in Arunachal Pradesh sieve rice seeds before planting to separate weed seeds as most of them are smaller than the rice seeds (Singh 1992). Similar ITKs were documented from other states by Verma et al. (1998), Tripathy (1999), Nayak and Biswal (2002).

According to Iyyappan (1997), rice farmers in Tamilnadu soak paddy seeds in diluted biogas slurry for 12 hours to increase seedling's resistance to pests and diseases and to remove weed seeds.

Using botanical-based ITKs is a critical component of the rural rice cultivation system. Chemical pesticides, though more effective, are hazardous to the environment and harmful to human health. As a result, they are being used as a last resort and only when all other control measures fail (Anonymous, 1999). Various ITKs have been used to successfully manage diseases and pests in different rice ecosystems based on the severity of insect pests.

Singh (1992) believes that using traditional rice varieties in the field is the best way to reduce the risk. According to his report, farmers in Jharkhand prefer traditional varieties because they are disease and pest-resistant and work well in existing micro-farming situations. Even in stressful conditions, these varieties produce at least some yield and pose no risk.

Nayak et al. (2002) found that the farmers use custard apples (*Annona squamosa*) for pest control in paddy crops. Insects and pests are controlled by broadcasting the custard apple leaves or seeds. The smell of leaves repels plant parasites and is toxic. The leaves are raw, whereas the seeds are ground and used as a powder. This is an age-old practice that every farmer adheres to.

Similarly, Sankharyya (2004) reported that the farmers of Narkuda village in Andhra Pradesh use custard apples. The smoke of burnt custard apple leaves is placed near rat burrows. Rats are killed, and insects are driven away from plants due to this practice.

To protect the rice field from Gundhi bug (*Leptocorsia acut*e) infestation, Prasad (1994) found that discarded rubber tyres are burnt in the field at night. Bugs are attracted towards the fire and killed. It is practiced by the farmers in Ranchi district, Jharkhand (Prakash et al. 2014).

2.8.5. Storage and harvesting

The best grains are separated and collected for seed at the threshing yard. This is done in almost all rice-growing states. Sun drying of rice crops for 4-5 days is generally followed, as the excess moisture evaporates, reducing the danger of disease and pest attack (Anonymous, 2000).

According to Dhirabhai (1997), in Tamilnadu, harvested seeds are dried in the sun. The seeds are kept in a tightly sealed container called *Kottain* smeared with cow dung paste. It is stored until the following planting season. The cow dung plastering protects the seed from insect attacks while not interfering with its biological activity. According to Sarma and Rahman (2010), farmers in Assam's Nalbari district store paddy in earthen pots by sealing the top with hay and applying cow dung to seal the pot. Farmers reported that paddy seeds stored in this manner remain viable and of good quality for two years. Santosh and Chhetry (2012) documented a similar observation from Tripura.

Ratan et al. (2016) investigated Jharkhand's rice cultivation practices and pest control techniques. They discovered that notch (*Vitexne gundo*), neem (*Azadirachta indica*), and pungam (*Pongamia pinnata*) leaves are mixed with sun-dried harvested crops during seed storage to prevent the seed from storage pests and insects. In addition to mixing, a few leaves are placed on top of the seeds.

2.9. ITKs on rice cultivation from northeast India

The indigenous communities of northeast India largely depend on agriculture and animal husbandry as their main source of livelihood. They possess rich knowledge of ITKs pertaining to agriculture, pest management, crop biodiversity conservation and ethno-veterinary practices. Unfortunately, these practices which were in vogue throughout the rural parts are little documented and are in danger of extinction.

Chhetry and Belbahri (2009) reported that people of northeast region observe the movement of insects and animals (butterflies, ants, and termites) to forecast rainfall and other climatic parameters. They claimed that indigenous practices have a high potential to address the issue of even climate change and that they should be promoted for scientific validation.

Choudhary (2003) reported that Cho crabs are used as an insect attractant by farmers of Pasighat block in Arunachal Pradesh. Farmers kill 40-50 crabs, and they are then tied with bamboo sticks and placed at random in the field. Insects are drawn to the smell of crabs and sit on the body to suck the sap. As a result, the crop is protected from insects.

Singh (1992), while studying the rice-based agro-ecosystem of the various villages of east Manipur (Khurai, Nandeibum, Leikai, Imphal), reported that farmers combine rice husk with excreta of poultry, cattle, pigs and ash, and the mixture is spread over the paddy field. Paddy yield has been reported to increase by 25-30% after applying this mixture (Santosh and Chhetry 2012).

In Sikkim, farmers build levelled terraces for better water conservation and distribution in the rice field (Sharma et al. 2016). The benches and terraces are watered through perennial springs, tapped from the higher elevation for irrigation, which also solves soil erosion.

Lamichaney et al. (2019) studied the indigenous methods related to grain storage from the Lepcha and Limboo communities of Sikkim. They reported that indigenously made bamboo baskets and mud structures from locally available materials have been successfully used to store harvested crops, protecting them from storage pests, insects and rodents. Similar studies on indigenous post-harvest practices have been reported previously, and scientists rated more than 80% of them as rational (Rambabu 1997; Somasundaram 1995 and Ganeshamurthy 2000).

2.10. Problem statement

From the foregoing review, it can be ascertained that, even though some efforts have been made to record the ITKs on different aspects, little work has been done to comprehensively document the indigenous knowledge associated with the rice cultivation practices in Sikkim. Information is only available on the fermented food beverages (Tamang et al. 1996), conservation of birds and medicinal plants used by the Lepcha community (Acharya et al. 2009), disaster management practices (Jha and Jah 2011), production of indigenous meat products (Rai et al. 2009), bioresource management (Subba 2009), indigenous methods for grain storage by Lepcha and Limboo community (Lamichaney et al. 2019) and ethnic fermented food products (Thapa and Tamang 2020).

Similarly, the collection and documentation of traditional landraces have received moderate attention from various researchers and government aided research organizations. So far, 70-plus landraces have been reported (Sharma et al. 2016), which await detailed characterization. However, barring a few scattered works (Rahman and Karuppaiyan 2011; Das et al. 2013; Choudhury et al. 2013, Sharma et al. 2016 and Chakraborty et al. 2019), not much has been done in this direction. Kapoor et al. (2017 and 2019) have made a decent effort to document the indigenous rice landraces of Sikkim. However, their detailed characterization based on the cooking qualities, biochemical traits and genetic characteristics is still awaited. In particular, no studies have focused on determining the genetic structure and its utility in molecular breeding programs.

Thus, through this Ph.D. thesis, an effort has been made to address this gap by investigating the indigenous traditional knowledge associated with the rice cultivation practices in Sikkim besides characterizing their nutritional, biochemical and molecular attributes for use in the breeding programs.

The specific objectives of the Ph.D. work were as follows:

1. To map, catalogue and document traditional knowledge of indigenous rice cultivars of Sikkim Himalaya.
2. To determine phenotypic diversity among the rice genotypes based on grain and kernel traits.
3. To determine proximate nutritional and mineral compositions of collected germplasm.
4. To undertake molecular characterization using DNA markers.

CHAPTER 3

3. MATERIALS AND METHODS

3.1. Study material

Totally 74 rice landraces were collected from different parts of Sikkim during the field trips carried out from 2016-2018. Following the preliminary screening, 65 landraces were retained after removing the duplicate accessions. Analysis of seed traits, cooking quality traits, proximate composition and mineral levels were carried out on these 65 landraces. Further, shortlisting based on the phenotypic characterization was done to select 53 landraces for molecular characterization and Genome-Wide Association Mapping studies.

3.2. Cataloging and documentation of Indigenous Traditional Knowledge

The study was conducted during 2016-19 in the rice-growing villages of all the four districts of Sikkim (Figure. 4). After careful mapping, the locations were selected based on (a) the long history of rice cultivation and (b) the availability of farmers with experience in rice farming and familiarity with the indigenous knowledge. From each of the selected locations, elderly and experienced farmers were contacted, and a total of 37 of them were interviewed randomly as respondents.

The data was collected through a structured interview and group discussions. The information gathered via. questionnaire included: the name of landrace, cultivation practice, utilization including speciality and organoleptic traits, traditional uses etc. In addition, village-level workers (VLWs) of the state agriculture department were also consulted for information on cultivation practices. Apart from these primary sources, information on rice cultivation, etc., was also gathered from various secondary sources, i.e., magazines and journals.

For germplasm collection and laboratory analysis, freshly harvested rice grains were collected after obtaining informed consent from the farmers. The collection strategy involved capturing maximum variability through minimum representative accessions, which was arrived using the structured interview data. In addition, the samples were obtained from the rice farms maintained by the agriculture department.

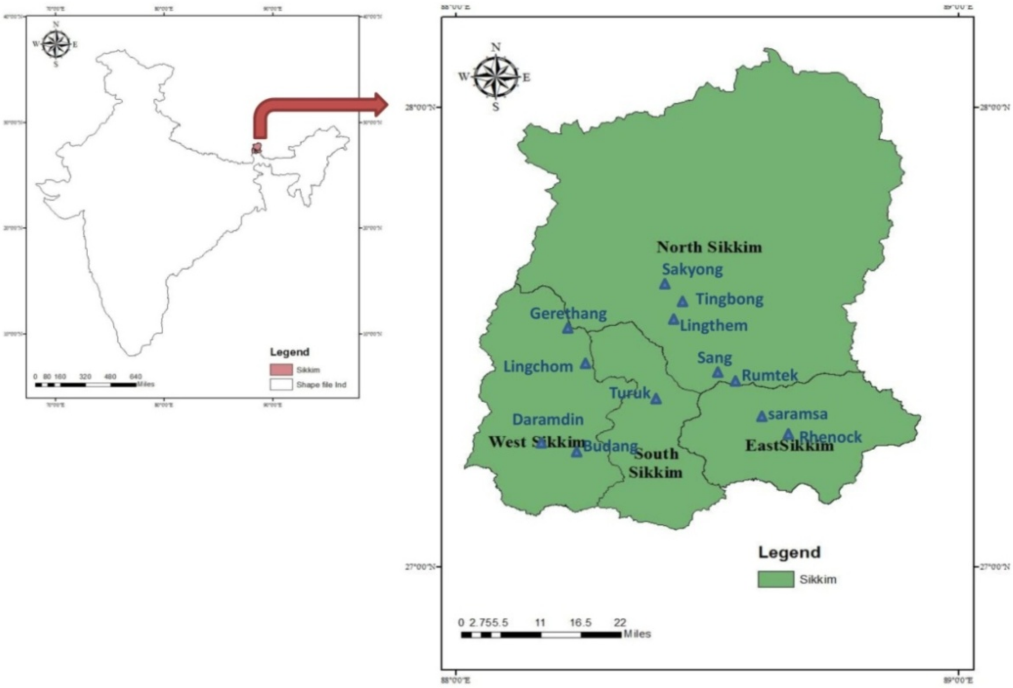


Figure 4. Location map of the 12 village sites selected for the study

3.3. Phenotypic diversity based on the grain and kernel traits

3.3.1. Evaluation of seed traits

Quality traits like grain length, width, thickness, kernel width, and kernel lengths were measured using the methods prescribed by the National test guidelines for DUS (PPV & F.R. Act 2007). The data from the ten randomly chosen grains were measured in millimeters (mm) using a digital Vernier caliper. In the case of cooked kernels, five grams of the de-husked samples were soaked in 15 ml water for 10 min and cooked for 20 min. After cooling, the grains were measured using a digital Vernier caliper. For 1000-grain weight, three replicates of 100 whole grains, dried to 13% moisture content, were chosen randomly and weighed in grams (g), and then the average was multiplied by 10 (Nalladulai et al. 2002).

All statistical analyses, such as correlation, Principal Component Analysis (PCA), and one-way analysis of variance (ANOVA) were performed using the R program (R Core Team 2014) and the Statistical Package for the Social Sciences software, SPSS v. 17 (SPSS Inc. 2008).

3.3.2. Biochemical analysis

3.3.2.1. Aroma content

The aroma was evaluated based on the method described by Sood and Siddiq (1978) with minor modifications. Two grams of rice kernels were treated with 10 ml of 1.7% (w/v) potassium hydroxide (KOH) kept at room temperature (~25°C) for about 10 min in a closed condition and then smelled. Since aroma is a subjective trait, a panel of five members was used to obtain consistent data. The aroma content was rated on a scale of 0-3 (0 for no, 1 for less, 2 for moderate, and 3 for high aroma).

3.3.2.2. Alkali digestion test and gelatinization temperature

The Alkali digestion test was conducted as per the method described by Dela-Cruz and Khush (2000). Six milled kernels of each experimental rice group were separately immersed in 10 ml of 1.5% KOH solution in a Petri dish and arranged so that the grains did not touch each other. The dishes were covered and incubated for 24 h at 30°C. Starchy endosperm was rated visually based on a 7-point numerical spreading scale (Dela-Cruz and Khush 2000).

Table 4. The scale used for scoring the alkali digestion and gelatinization temperature

Score	Observation of treated kernels	Alkali Digestion	Gelatinization Temperature
1	Kernel not affected	Low	High
2	Kernel swollen	Low	High
3	Kernel swelled, collar complete or narrow	Low or intermediate	High-intermediate
4	Kernel swelled; collar complete and wide	Intermediate	Intermediate
5	Kernel split or segregated, collar complete and wide.	Intermediate	Intermediate
6	Kernel dispersed; merging with collar	High	Low
7	Kernel completely dispersed and intermingled	High	Low

3.3.2.3. Amylose content

Amylose content was determined following the protocol of Sompong et al. (2011). One ml of 95% ethanol and 9 ml 1N NaOH were added to 100 mg of rice flour and mixed properly. The samples were then heated for 10 min in a water bath at 45°C to gelatinize the starch. Samples were cooled down and transferred to a 100 ml volumetric flask, and 5 ml of starch solution and 1 ml acetic acid (1 N) were added. After adding 0.4 ml of Lugol’s solution, the volume was adjusted to 100 ml with distilled water. The absorbance was recorded at 620 nm with a UV-Vis Spectrophotometer (Thermo Fisher Scientific). The amylose content was determined from the standard calibration curve prepared from the Potato amylose. The absorbance vs amylose content graph was plotted, where the resulting regression equation was $Y = 0.0217x - 0.0161$ (r= 0.9999). The result was expressed as a percentage by mass in the milled rice on a dry basis (Juliano 1971).

Subsequently, rice landraces were classified into five groups according to their amylose content: (i) waxy (1–2%), (ii) very low (2–9%), (iii) low (10–20%), (iv) intermediate (20–25%), and (v) high (25–33%) based on the methods adopted by IRRI (2009).

3.4. Proximate nutritional and mineral composition

The grains were harvested after maturity, sun-dried and then de-husked. Rice flour was prepared with a blender. The complete grain and rice flour were maintained at room temperature before the analysis. All experiments were done in triplicates for authentication.

3.4.1. Determination of Moisture content

Moisture content was determined by the standard protocol of AOAC (1990) (method 14:004).

The crucible was thoroughly washed with distilled water, dried in an oven at 100°C for 30 minutes, and allowed to cool. After cooling, weight (W1) was recorded. Afterwards, 2.0 g of the finely ground rice flour was put into the crucibles and weighed to get W2. A crucible with a sample was dried at 100°C for four hours, then cooled down for 30 minutes, and the sample weights were then taken (W3). The moisture content (%) of the sample was calculated as,

$$\textbf{\%Moisture} = \frac{\textbf{W2-W3}}{\textbf{W2-W1}} \textbf{ x100}$$

Where, W1 = initial weight of the empty crucible

W2 = weight of crucible + sample before drying and

W3 = final weight of crucible + sample after drying

3.4.2. Determination of Ash content

The total ash content was determined as described by AOAC (1990) (method 14:006). About one gram of finely ground dried sample was taken into a porcelain crucible and incinerated at 600°C for six-hour in a muffle furnace until the ash was obtained. The ash was cooled in and reweighed. The % of ash in the tested rice sample was calculated as follows:

$$\textbf{\%Ash} = \frac{\textbf{Weight of Ash}}{\textbf{Weight of original sample}} \textbf{ x100}$$

3.4.3. Determination of Crude fiber content

The crude fiber was measured using the method of AOAC (1990) (method 14:020). About 2.0 g of the rice sample, hydrolyzed in a beaker with petroleum ether, was boiled under reflux for 30 min with a 200 ml solution of 1.25% H_2SO_4. The solution was then filtered, and the filtrates were washed with boiled water until they were no longer acidic. The residue was then transferred onto a beaker and boiled for another 30 min with 200 ml of a solution containing 1.25% NaOH per 100 ml. The boiled samples were washed with boiled distilled water. The residues were filtered, dried at 100°C for 2 hr, cooled and washed. The crude fiber percentage was calculated as per the following formula,

$$\textbf{\%Crude Fibre} = \frac{\textbf{Weight after drying}}{\textbf{Weight of sample}} \textbf{ x100}$$

3.4.4. Determination of Crude fat content

Total fat was determined by the soxhlet extraction method (AACC 2000). Approximately 2.0 g of rice samples was weighed accurately into a thimble. The dried boiling flasks were weighed and filled with about 300 ml of petroleum ether (boiling point 40 - 60°C). The extraction thimble was plugged in tightly. Then the soxhlet apparatus was assembled and allowed to reflux for six hours. The thimble was removed carefully, and petroleum ether was collected. The flask was dried then at 105 – 110°C until the complete removal of petroleum ether. It was cooled and weighed. The percentage of fat was computed using the formula below,

$$\%Fat = \frac{\text{Weight of Fat}}{\text{Weight of sample}} \times 100$$

3.4.5. Determination of Protein content

The protein content was determined by the method described by Lowry et al. (1951). Ten milligrams of the sample was ground in 50 ml of 10% NaCl, appropriately mixed and centrifuged at 2000 rpm. The supernatant was heated in a boiling water bath for 15 minutes, then cooled and centrifuged at 2000 rpm for 10 minutes. The volume of the supernatant collected was adjusted to 50 ml with 10% NaCl solution.

Standard BSA at various concentrations (0.2 µg, 0.4 µg, 0.6 µg, 0.8 and 1.0 µg) was taken, and the volumes were adjusted to 1 ml with water. The final volume to be assayed was adjusted to 10 ml by adding 5 ml each of alkaline solution of Sodium-Potassium Tartrate and Copper Sulfate and 0.5 ml of Folin reagent, and a standard curve was prepared. The seed extract (0.1 ml) was mixed with 0.9 ml distilled water, and the final volume was made up to 6.5 ml with the reagents. Three independent tests were performed for the sample. The absorbance was measured at 750 nm using a UV-Vis Spectrophotometer (Thermo-Fisher Scientific, Model 201).

3.4.6. Determination of Carbohydrate content

The total percentage of carbohydrate content in the rice sample was determined by the method reported by Onyeike et al. (1995). This method involved adding the samples' total values of moisture, ash, fat, crude fiber and protein contents, then subtracting it from 100. The value obtained is the percentage carbohydrate content of the sample. Thus:

% Carbohydrate =100 - (% moisture + % crude fiber + % protein + % lipid + % ash)

3.4.7. Mineral content

The mineral contents were estimated according to the standard method using Atomic Absorption Spectrophotometer (Perkin Elmer). Seeds from 65 landraces were de-husked gently. One gram of oven-dried ground de-husked seed samples was placed in a 150 ml conical flask. To this, a 25–30 ml diacidic mixture (HNO_3:$HClO_4$; 5:1 v/v) was added and kept overnight. The next day, it was digested by heating till a clear white precipitate settled down at the bottom. The crystals were dissolved by diluting in double-distilled water. The contents were filtered through Whatman No. 42 filter paper. The filtrates were made to 50 ml with double distilled water and used to determine Iron, Zinc, Copper and Manganese contents. Concentration was expressed in mg/100 g of rice against the standard curve.

3.5. Molecular characterization and Association analysis

3.5.1. Plant material

From the collection of 74 landraces, 53 were used for the phenotypic and genetic characterization (Table 5) and the association mapping. The plants were raised during the Kharif season at the experimental farm situated at Nandok, east Sikkim (27^{o}17’36” N and 88^{o}36'18") at an elevation of 972 m above sea level. Initially, seeds were sown in a raised bed nursery, and after 30-days, seedlings were transplanted in the field. The experiment was laid out in a randomized block design with three replications.

Each entry was transplanted in three rows with 20 cm spacing between rows and 14 cm between plants. The standard agronomic practice was followed.

Table 5. Details of 53 rice landraces used in the study

Sl. No.	Local name	Collection Location	Grain type	Aroma status	Altitude (ft)
1	Auti dhan	Dalapchand (E.S.)	LB	NS	3323
2	Bhagey tulashi	Saureni (E.S.)	SB	NS	3241
3	Bhotangey	Bering (E.S.)	SB	NS	2089
4	Dhansey	Kaiyathang (E.S.)	SB	NS	4563
5	Doodh kalami	32 Mile (E.S.)	LS	NS	2293
6	Doodh katey	32 Mile (E.S.)	LS	MS	2293
7	Dorakhey	Dalapchand (E.S.)	MS	NS	3300
8	Fauryal	Lingthem (N.S.)	MS	NS	4533
9	Fourey	Bering (E.S.)	LS	NS	2080
10	Jasuda	Rhenock (E.S.)	MS	NS	1614
11	Jhapaka	Rhenock (E.S.)	MS	NS	1614
12	Jogi dhan	Dalapchand (E.S.)	SB	NS	3316
13	Kaanchi	Dentam (W.S.)	SB	MS	4842
14	Kagey	Reshi (E.S.)	LB	NS	3940
15	Kalo dhan	Tintek (E.S.)	SB	SS	4744
16	Kalo nuniaI	Daramdin (W.S.)	SB	SS	3146
17	Kaltura	Reshi (E.S.)	LB	MS	3933
18	Kataka	Bering (E.S.)	MS	SS	2089
19	Krishna bhog	Rumtek (E.S.)	MS	SS	5089
20	Phudungey	Assam Lingzey (E.S.)	SB	NS	4314
21	Rudhua	Saramsa (E.S.)	SB	MS	3001
22	Sano attey	Rumtek (E.S.)	SB	NS	5133
23	Sirkey	Tintek (E.S.)	SB	NS	4980
24	Taprey	Rumtek (E.S.)	SB	NS	4523
25	Thulo attey	Bering (E.S.)	SB	NS	2085
26	Thulo tulashi	Saureni (E.S.)	SB	NS	3243
27	Tulashi	Bering (E.S)	SB	MS	2089
28	Chinizho	Pentong (N.S.)	SB	NS	5781
29	Dharmali	Pentong (N.S.)	SB	SS	5781
30	Marbonzho	Pentong (N.S.)	SB	NS	5781
31	Mumpupzho	Pentong(N.S.)	SB	NS	5781
32	Tukmorzho	Pentong (N.S.)	LB	NS	5781
33	Basmati	Gerethang (W.S.)	SB	MS	3723

34	Birinful	Gerethang (W.S.)	MS	MS	3973
35	Champa	Daramdin (W.S.)	MS	NS	3136
36	Charingrey	Chota-Singtam (E.S.)	LB	NS	4258
37	Japani	Malbasey (W.S.)	LS	NS	2384
38	Kalo nunia II	Budang (W.S.)	MS	SS	2143
39	Khampti	Budang (W.S.)	LB	NS	2143
40	Lalbaachi	Daramdin (W.S.)	LB	NS	3146
41	Manipuri	Malbasey (W.S.)	LB	NS	2384
42	Marshi	Daramdin (W.S.)	SB	NS	3146
43	Panbhara	Daramdin (W.S.)	LB	NS	3146
44	Raja bara	Budang (W.S.)	LS	MS	2384
45	Ram jeera	Gerethang (W.S.)	SB	SS	3922
46	Shyam jeera	Daramdin (W.S.)	SB	NS	3146
47	Thamba	Melli (S.S.)	LS	NS	2434
48	Timburey	Daramdin (W.S.)	SB	NS	3146
49	Champasari	Turuk (S.S.)	LS	NS	2650
50	Chirakhey	Bering (E.S.)	SB	NS	2089
51	Mansarey	Daramdin (W.S.)	LS	NS	3146
52	Masuley	Melli (S.S.)	MS	NS	2434
53	Ram bhog	Tareythang (E.S.)	SB	SS	2086

** E.S. East District of Sikkim, W.S. West District of Sikkim, N.S. North District of Sikkim, S.S. South District of Sikkim, S.B.: Short bold, L.B.: Long Bold, MS: Medium Slender, L.S.: Long Slender, NS: Non-Scented, MS: Mild Scented, S.S.: Strongly Scented*

3.5.2. Genotyping

Genomic DNA was isolated from fresh young leaves (14-20 days) following the modified Cetyl-trimethylammonium bromide method (Doyle and Doyle 1987). For genotyping, 45 SSR primers pairs (Table 6) used previously for the association analysis (Islam et al. 2018) were downloaded from Gramene's marker database (http://www.gramene.org), and primers were synthesized. These markers offered coverage on all 12 chromosomes of the rice genome. DNA amplification was carried out in a BIORAD T100 thermal cycler (USA) with a reaction mixture containing 1 µl of genomic DNA (50 ng), 0.5 µl of each primer (10 pmol/µl), 6 µl of PCR master mix (Nucleo Spin, Takara) and 2 µl of PCR grade water. PCR amplification was performed as follows; initial denaturation at 94ºC for 4 min followed by 35 cycles of denaturation, annealing, and extension at 94ºC for 1 min, 55ºC for 1 min, 72ºC for 1 min followed by a final extension at 72ºC for 5 min. The PCR products were resolved on a 3% agarose gel using 1X Tris-boric acid-EDTA (TBE) buffer. After staining with the ethidium bromide, bands were visualized using the gel documentation system (BIORAD-Chemi Doc XRS+, USA). All PCR reactions were repeated at least twice before the data analysis.

Table 6. Sequence information of 45 SSR markers used for genotyping

Marker	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
RM5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
RM495	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC
RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
RM312	GTATGCATATTTGATAAGAG	AAGTCACCGAGTTTACCTTC
RM283	GTCTACATGTACCCTTGTTGGG	CGGCATGAGAGTCTGTGATG
RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG
RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
RM322	CAAGCGAAAATCCCAGCAG	GATGAAACTGGCATTGCCTG
RM489	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG
RM338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC
OSR13	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC
RM514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
RM307	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
RM537	CCGTCCCTCTCTCTCCTTTC	ACAGGGAAACCATCCTCCTC
RM551	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG
RM178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC
RM413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
RM170	TCGCGCTTCTTCCTCGTCGACG	CCCGCTTGCAGAGGAAGCAGCC
RM510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
RM454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
RM190	GCATTGTCATGTCGAAGCC	CTAGCAGGAACTCCTTTCAGG
RM253	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC
RM314	CTAGCAGGAACTCCTTTCAGG	AACATTCCACACACACACGC
RM455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
RM118	CCAATCGGAGCCACCGGAGAGC	CACATCCTCCAGCGACGCCGAG
RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
RM10	TTGTCAAGAGGAGGCATCG	CAGAATGGGAAATGGGTCC

RM408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC
RM44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
RM284	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG
RM342	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC
RM515	TAGGACGACCAAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
RM316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
RM215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG
RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC
RM287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
RM536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
RM144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTA GTGCATG
RM19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA
RM20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG
RM277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG

3.5.3. Allele scoring

Post amplification, a cluster of two to five distinct bands was visible in the stained gel for most markers. Using a standard 50 bp DNA ladder, the size of the most intensely amplified band of each microsatellite marker was determined. For individual markers, the presence of an allele was recorded as “1” and the absence as “0”.

3.5.4. Data Analysis

Power Marker V.3.25 (Liu and Muse 2005) was used to calculate the major allele frequency (M_{AF}) and the polymorphism information content (PIC). The marker characteristics such as observed heterozygosity (*Ho*), expected heterozygosity (*He*), the total number of alleles (Na), effective number of alleles (Ne), and percentage of polymorphic loci (%P) were computed using GenAlEx V.6.5 (Peakall and Smouse 2012). The genetic diversity indices like total genetic diversity (*Ht*), genetic diversity within populations (*Hs*), genetic differentiation (*Gst*), Shannon’s information index (*I*), and Nei's gene diversity (*h*) were determined using POPGENE V.1.32

(Yeh et al. 1999). The distance-based clustering was performed with an Un-Weighted Pair Group Method with Arithmetic mean (UPGMA) using FreeTree software (Pavlicek et al. 1999). The principal coordinate analysis (PCoA) plot was constructed using DARwin V.6.0 (Perrier and Jacquemound-Collet 2006). The robustness of the UPGMA dendrogram was tested with a bootstrap analysis using 1000 iterations.

Using STRUCTURE V. 2.3.4 software (Pritchard et al. 2000), the Bayesian model was implemented to ascertain the optimal genetic clusters within the 53 rice accessions. The admixtures model and correlated allele frequencies were applied for each run with 100,000 burn-in periods and 200,000 Markov Chain Monte Carlo (MCMC) simulations. The optimum K value was determined from 10 replicate runs for each value of K (Evanno et al. 2005). The ΔK was based on the change in the log probability [LnP(D)] of the data between successive K values. To obtain a clear peak at the ΔK, the output from the structure analysis was loaded onto STRUCTURE HARVESTER V. 6.0 (Earl and Von Holdt 2011). Analyses of molecular variance (AMOVA) and pairwise F_{ST} were carried out by GenAlEx V. 6.5.

3.5.5. Phenotyping

Twenty-one quantitative and qualitative traits of agronomic (7), quality (9), and nutritional (5) significance were evaluated (Table 7). For the seven agronomic traits such as days to 50% flowering, flag leaf length (cm), flag leaf width (cm), stem thickness (cm), plant height (cm), panicles per plant, number of effective tillers per plant, and days to 50% flowering, observations were recorded on five randomly selected plants as per the method prescribed by the National test guidelines for DUS (PPV & F.R. Act, 2007).

Table 7. List of phenotypic traits evaluated among rice landraces

Sl. No.	Phenotypic Traits	Sl. No.	Phenotypic Traits
1	Days to 50% flowering	12	Number of panicles per plant
2	Grain width	13	Flag leaf length
3	Grain length	14	Flag leaf width
4	Grain thickness	15	Stem thickness
5	100-grain weight	16	Plant height
6	Kernel width	17	Protein
7	Kernel length	18	Carbohydrate
8	Kernel thickness	19	Crude fat
9	Kernel width after cooking	20	Crude fiber
10	Kernel length after cooking	21	Amylose value
11	Number of effective tillers		

3.5.6. Marker-Trait Association

Software TASSEL V. 5.0 (Bradbury et al. 2007) was used to perform the marker-trait association analysis. Both the models: the general linear and the mixed linear models, were tested. The major limitation of the GLM is that it does not incorporate the kinship matrix (K matrix). Instead, it uses population structure (Q matrix) for determining the marker-trait association, which sometimes results in false positives. This limitation is overcome by the MLM, which incorporates both Q and K matrix reducing the spurious association (Yu et al. 2006). The significance of the marker-trait association was tested at $P < 0.05$, and the correlation coefficient ($R^2 \geq 10\%$) was used to determine the phenotypic variation explained by each marker-trait association.

CHAPTER 4

4. RESULTS

4.1. Cataloging and documentation of Indigenous Traditional Knowledge

4.1.1. Collection of indigenous rice landraces

We collected 74 traditional rice landraces from four districts of Sikkim (Table 8, Plate 1a- 1f). They represent a significant portion of the previously reported landraces from the state.

We attempted to record informal material about the rice varieties and their cultivation practices at the time of collection/survey. We found that their local nomenclature is mainly based on the morphological characters and/or growing locations. Thus, the Nepali name not only indicates their dominant attribute but also identification. For instance, landraces with small-sized grains were known by the names: Sano, Kaanchi and Masino (all meaning small) dhans (rice), while the long types were called Thulo or Lamo (big/large) dhan. Landraces like Kalimpongey denotes Kalimpong-district in West Bengal. Kalo nunia and Kalo dhan denoted the grain's black (kalo=black) lemma. Taprey denoted that the awns are inseparable from rice grains.

In terms of cultivation, the agroecological conditions primarily influenced the farmer's decision on the variety to be planted with altitude and the irrigation source being the two most essential variables. Most irrigation sources were found in low-lying areas where farmers grew two or more types of rice landraces. Even the land holding decided their numbers. Farmers believe height, biomass, disease resistance, and grains with medium amylose content are the most sought-after traits. The varietal mixture in the field is another important feature we observed in the local seed systems. Farmers deliberately mixed the seeds of different landraces before sowing. Some prominent combinations included: Birinful + Attey, Tulashi + Attey, Sano attey + Thulo attey, Kataka + Krishna bhog etc.

In terms of agronomic attributes, majority of the landraces are of late maturing type (150- 175 days). Also, half of the total landraces were of short grain type. Basmati II, Danasay and Sirkey are of broad grain type. Seeds of Ram jeera and Shyam jeera were the smallest, resembling cumin seeds. Kalo nunia, Tulasi, Kataka and Kalo dhan are aromatic types mostly used as powdered rice, stuffed rice and Sathua. Landraces like Fourey, Japani, Mansarey, Panbhara, Marshi and Attey are used for preparing a sale roti (a local sweet dish); Dhanase and Chewrey dhan for chewda (puffed rice). Krishna bhog, Kalo nunia and Birinful are known for their aroma and quality. The most popular rice landraces widely cultivated are Krishna bhog, Thulo attey, Kalo nunia, and Tulashi. Many of the surveyed farmers informed that Sano attey is known for the lodging resistance.

The rice cultivation practice in Sikkim can be broadly categorized into two types: (i) direct sowing method and (ii) transplanting method. The latter is popular and widely practised, while the former is still used in a few patches at high altitudes. The rice landraces which are directly sown are upland type (Ghaya dhan). They are predominantly cultivated by the primitive Lepcha tribe native to remote Lepcha villages (protected) of Pentong, Sakyong, and Tingvong of upper Dzongu in North Sikkim, where an age-old Jhum system of agriculture is still in practice (Plate 2a-2d). Popular upland rice includes: Kalo tukmar, Seto tukmar, Tukmorzho, Mumpupzho, Marbonzho, Chinizho, and Kalo dharmali. Among the transplanted landraces, the most commonly cultivated landraces under irrigated conditions are Thulo attey, Sano attey, Krishna bhog, Kalo nunia, Birinful, Doodhkatey, Kataka, Tulashi, Kalo dhan, etc., which are mostly grown below 1200 m asl. The cultivation of fine aromatic rice varieties like Krishna bhog, Kataka, Tulashi, Kalo nunia (types I, I and III) etc., are confined to the low-hill areas where temperature and humidity are high. Paddy cultivated in the middle hills primarily are short to medium grain types like Attey, Phudungey, Sirkey, Taprey etc.,

Table 8. Rice landraces collected during the study

Aromatic rice

Sl. No.	Local name	Place of collection	Sl. No.	Local name	Place of collection
1	Basmati	Gerethang (W.S.)	12	Krishna bhog I	Rumtek (E.S.)
2	Basmati I	Nandok (E.S)	13	Krishna bhog II	Tareythang (E.S.)
3	Basmati II	Rumtek (E.S)	14	Rajabara	Budang (W.S.)
4	Bhagey tulashi	Saureni (E.S.)	15	Rambhog	Tareythang (E.S.)
5	Birinful	Gerethang (W.S.)	16	Ramjeera	Gerethang (W.S.)
6	Kalimpongey	Saureni (E.S.)	17	Rudhua	Saramsa (E.S.)
7	Kalo nunia I	Saramsa (E.S.)	18	Sano tulashi	Aho (E.S)
8	Kalo nuniaII	Tintek (E.S.)	19	Shyam jeera	Daramdin (W.S.)
9	Kalo nuniaIII	Daramdin (W.S.)	20	Timburey	Daramdin (W.S.)
10	Kalo dhan	Budang (W.S.)	21	Tulasi	Bering (E.S)
11	Kataka	Bering (E.S.)			

Non-aromatic rice

22	Auti dhan	Dalapchand (E.S.)	45	Lama dhan	Ramthang (N.S)
23	Bhotangey	Bering (E.S.)	46	Mailey attey	Tintek (E.S)
24	Champa	Daramdin (W.S.)	47	Manipuri	Budang (W.S.)
25	Champasari	Turuk (N.S.)	48	Mansarey	Daramdin (W.S.)
26	Charinangre	Chota-Singtam	49	Marshi	Daramdin (W.S.)
27	Chewrey dhan	Budang (W.S)	50	Masari	Daramdin (W.S.)
28	Chirakhey	Bering (E.S.)	51	Masino	Daramdin (W.S.)
29	Damrojho	Ramthang (N.S)	52	Masuley	Melli (S.S.)
30	Dhansey	Rumtek (E.S.)	53	Namphokey	Sripatam (S.S)
31	Doodhkalami	32 Mile (E.S.)	54	Nepalzho	Lingthem (N.S)
32	Doodhkatey	32 Mile (E.S.)	55	Nepalia	Phodong (N.S)
33	Dorakhey	Dalapchand (E.S.)	56	Panbhara	Daramdin (W.S.)
34	Fauryal	Dzongu (N.S.)	57	Phudungey	Assam Lingzey (E.S.)
35	Fourey	Bering (E.S.)	58	Pountain	Daramdin (W.S.)
36	Japani	Budang (W.S.)	59	Sano attey	Rumtek (E.S.)
37	Jasuda	Rhenock (E.S.)	60	Sano tulashi	Aho (E.S)
38	Jhapaka	Rhenock (E.S.)	61	Seto dharmali	Sang (E.S)
39	Jogi dhan	Dalapchand (E.S.)	62	Sirkey	Tintek (E.S.)
40	Kaanchi	Dentam (W.S.)	63	Taichung	Ramthang (N.S)
41	Kagey	Reshi (E.S.)	64	Taprey	Rumtek (E.S.)

42	Kaltura	Reshi (E.S.)	65	Thamba	Melli (N.S.)
43	Khampti	Budang (W.S.)	66	Thulo attey	Bering (E.S.)
44	Lal baachi	Daramdin (W.S.)	67	Thulo tulashi	Saureni (E.S.)

Upland rice

Sl. No.	Local name	Place of collection	Sl. No.	Local name	Place of collection
68	Chinizho	Dzongu (N.S.)	72	Marbonzho	Dzongu (N.S.)
69	Dharmali	Pentong, (N.S.)	73	Seto tukmar	Sakyong (N.S)
70	Kalo tukmar	Sakyong (N.S)	74	Tukmorzho	Dzongu (N.S.)
71	Mumpupzho	Dzongu (N.S.)			

E.S. East District of Sikkim, W.S. West District of Sikkim, N.S. North District of Sikkim, S.S. South District of Sikkim

Plate 1 (a) Rice landraces collected during the field study

Plate 1 (b) Rice landraces collected during the field study

Plate 1 (c) Rice landraces collected during the field study

Plate 1 (d) Rice landraces collected during the field study

Plate 1 (e) Rice landraces collected during the field study

Plate 1 (f) Rice landraces collected during the field study

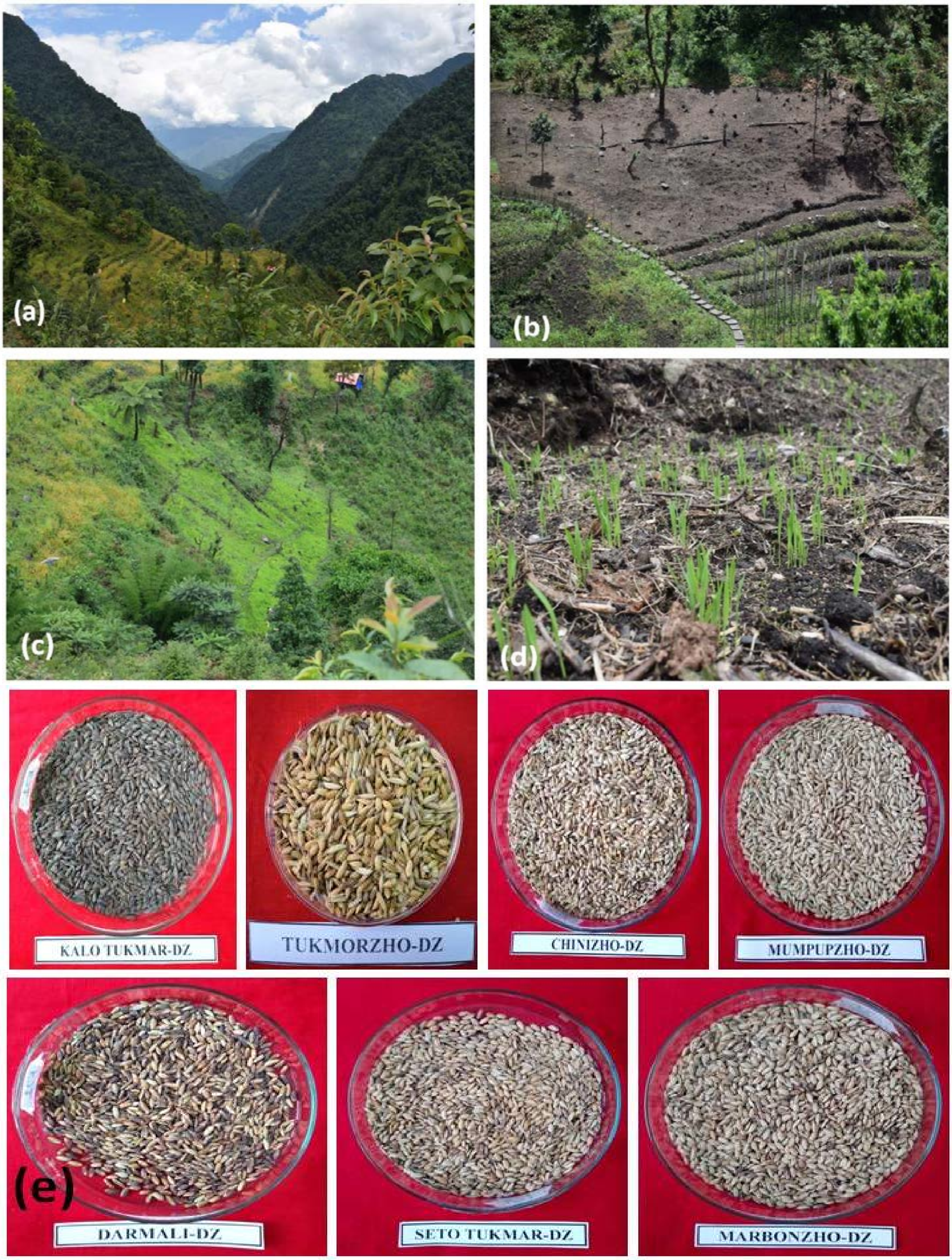


Plate 2. (a) Typical view of upland rice cultivation in North Sikkim (b) Land preparation for the cultivation (c) & (d) Seedling stage (e) Popular upland rice landraces of Sikkim

4.1.2. Documentation of the Indigenous Traditional Knowledge (ITKs)

We documented 35 indigenous traditional knowledge associated with rice cultivation in Sikkim. The rationale behind their uses is depicted in Table 9. Following are some of the important ITKs documented during our study.

4.1.2.1. Selection of seeds

For selecting and preserving the healthy seeds for sowing, the plant possessing bold and healthy grains is selected in advance, and proper care is provided to protect it from insect pests and birds. The selected plants are sorted and subjected to manual threshing during the harvesting. The threshing yard (khalegara) is prepared by uniform spreading of a mixture of cow dung and mud paste. The selection of seeds is made on the threshing floor. While threshing, bold seeds, shattered by the first two beatings, are collected. This process is effective in separating the partially filled and diseased grains. Farmers here believe that the grains selected by this method exhibit better vigor.

4.1.2.2. Preparation of the seed beds and land

The nursery sites are selected near the assured source of irrigation. The beds are ploughed twice in dry condition; terrace walls are cut to incorporate the debris, and unwanted plant materials are dumped into the field (Plate 3a and 3b). The debris gradually decomposes to provide nutrients to the soil. The first ploughing is followed by the second after nearly two weeks. During this interval, the weeds sprout in the field. Hence during the second ploughing, farmers expect to eliminate all of them.

The suitable time for the land preparation for the nursery/seedbed (Biyaar) (Plate 3c) is determined based on the onset of the southwest monsoon in June and during the harvesting time of the maize planted in the rice field. It is common knowledge that timely sowing is the best non-monetary input. If sowing is delayed by one week after July, the flowering and maturity phases are delayed or extended by another month. The ideal time determined for nursery rising are: (i) High hills: first week of May to the first week of June, (ii) Mid hills: second week of May to the second week of June, and (iii) Low hills June to early August. The nursery area needed to generate seedlings for transplanting in one hectare is roughly 1/10 hectare, and the seed rate is 50kg/ha. To ensure rapid and uniform germination, seeds are soaked for 24 hrs in clean water, drained after that, and the seeds are incubated in a warm, moist place for 36-48 hrs to let them sprout for sowing. The farmers of Lingthem, Dzongu (Lepcha settlement), give hot water (50-52^{o}C) treatment to render the seeds disease-free. The Chettri community of the lower Bering has a traditional way of soaking the seed with an gold ornament overnight, which they believe will yield a good harvest.

Table 9. Indigenous Traditional Knowledge (ITKs) associated with rice cultivation in Sikkim

Sl. No.	Description of ITKs	The rationale behind the use
Seed selection, treatment, and germination		
1	Fields having healthy growth are selected in advance, and proper care is given to protect the selected plot from animal and pest attacks.	Healthy grains will be selected for next year's sowing; the seeds will have high vigour.
2	Seeds collected from the first two thrashings by the manual method are used to select next year's seeds.	To remove partially filled unfertile as well as diseased grains.
3	Hot water treatment to seeds at 50-52ºC for 10-12 minutes.	It enhances germination and renders seeds disease-free.
4	Overnight soaking of seeds with gold ornament.	Believed to yield a good harvest.
Rising of seedlings		
5	During seedbed preparation, the land is pulverized along with the stubbles and weeds and allowed to decompose.	Seedlings were grown this way to facilitate easy uprooting, as they do not penetrate deep into the soil. Uprooted seedlings contain very little amount of soil in their roots.
6	Ash sprinkled over the nursery bed before the broadcasting of seeds.	Facilitates quick germination and easy uprooting of seedlings.
7	In the case of upland rice like Tukmar and Tukmorzho, seeds are directly sown into the cleared hill slopes.	The hardy seeds are expected to germinate and survive based on natural resources.
8	Most of the landraces from low hills are grown on nursery beds in wet conditions.	Growing seedlings in wetlands facilitate quick and vigorous growth as seedlings do not suffer water stress due to drought.
9	Chinizho (upland rice) collected from Pentong village is cultivated in the marshy fields where the field is ploughed to overturn the marshy vegetation, and seeds are directly sowed in a field.	The seed germinates and grows using natural water availability as the field always remains wet.
Main field preparation		
10	Clearing, slashing and burning of vegetation from the sloppy hills with the initiation of monsoon rain in case of upland rice cultivation.	These practices allow the ash to be fertile and disinfect the soil before planting.
11	Ploughing with initial irrigation in case of low land types well ahead of final puddling and keeping the field for 15-20 days after levelling until some excreta of earthworm are seen on the soil surface.	This practice allows the growth of earthworms which facilitates the decomposition of stubbles and weeds, thereby making the soil more fertile with decomposed materials and earthworm excreta.
12	Cutting/slicing the bund edges and walls of the terraced field with local hoes.	Lessons weed growth around the borders and reduce the pest population by disease-spreading insect vectors.

13	Bunds are plastered with mud paste from the wet field during land preparation.	Weed growth is suppressed, and runoff rainwater, as well as irrigated water, is reduced.
Fertility management		
14	Burning of stubbles followed by hand ploughing in case of upland rice.	The ashes produced from the burning of stubbles add to soil fertility. Besides, the pest and its eggs get burnt, and its population in the subsequent crop becomes less.
15	Intercropping with a common rice bean along the bunds of the terrace field.	During field preparation, topsoil from those bunds containing the nitrogen-fixing *Rhizobium* bacteria is sliced back to rejuvenate the fertility and improve the soil's nitrogen level.
16	Rearing of cattle herds in the field after harvest.	Since there is enough biomass and forage for cattle to feed, the cattle dung and urine provide the essential nutrients and possible disinfection to the soil.
Transplanting method		
17	In upland rice cultivation, seeds are sown directly into furrows instead of transplanting.	To provide the required temperature for germination and to avoid runoff losses of seeds in torrential rains.
18	Some upland rice varieties are transplanted directly to the seedbeds prepared at hill slopes to terraced fields.	The varieties like Marbonzho and Chinizho are cultivated in dryland and can be transplanted even in wet fields.
19	Uprooting and transplanting of 25-30 days old seedlings from the nursery to the main field during the month of Asar (June –July).	This practice is called Ropain in the local language. It allows the seedlings to attain height to suit the water depth in the main field. Also, the rice transplanted during this time caters to the timely initiation of panicles at a suitable time and temperature, reducing the infestation of insects and pests.
20	Instead of discarding the leftover seedlings, they are kept in a cluster in one of the corners of the main field immersed in water.	In the event of the death of seedlings immediately after transplanting, these seedlings can be used for gap filling.
Water management		
21	Water is channelized to the rice field from nearby perennial spring water tapped from the higher elevation.	This practice will provide a continuous water supply to the terraced field on hill slopes. Water generally drains out through the single outlet of the field.
22	Irrigation with bamboo pipes by splitting the bamboo into two halves vertically and removing the nods act as a pipe for carrying water.	Since bamboo is widely available, bamboo pipes are so prepared to reduce the loss of irrigation water during transit, and they are cheaper than poly pipes.
Plant protection.		
23	Bamboo sticks and other trees are cut and erected in the nursery and the main field.	Birds perch on the branches and sticks and act as predators of insects present in the crops.

24	Plantation of drumsticks trees (*Moringa olifera*) along the rice field.	The leaves, barks, etc., of the drumsticks tree are known to have high pesticidal properties and check the infestation of stem borer
25	Spray fresh cow dung solution (approximately 1 kg raw cow dung in 10-12 L of water).	Cow dung solution controls bacterial leaf blight to some extent. Further, the crop is protected from cattle as they do not graze upon cow-dung-treated crops.
26	Crabs, frogs, or toads are killed and hung from bamboo sticks erected in a crop field in different places.	The peat, especially bugs, attracts and crowds over the dead crabs/frogs instead of sucking soft grains.
27	Burning of firewood straw etc., in and around the field at night.	Various pests, particularly bugs and hoppers, jump onto the fire and get destroyed.
28	Hanging of damaged videos and audiotapes over the nursery bed of rice just after broadcasting seeds and in the main field in the crop's maturity.	The glittering/shining property of tape drives away the birds and prevents the birds from feeding the grains.
29	Keeping scarecrows made up of thatch in the nursery and the main field.	It frightens birds and prevents the birds from eating up the grains.
30	Hot water treatment of seeds at 50-52ºC for 10-12 minutes.	Reduces the spreading of brown spot diseases of rice.
31	Field sanitation, burning straw and stubbles in the field, and early sowing of seeds.	Reduces the spreading of blast diseases.
32	Use of Tulsi (*Ocimum* sp.) leaf extract prepared by boiling approx. 25 gm of leaves in 1 L of water.	Spray reduces the spreading of blast diseases.
Post-harvest practices		
33	Leaves of neem patti (*Azadirachta indica* A. Juss.), hardi [*Curcuma longa* (L.)], ginger (*Zingiber officinale* Roscoe), Bhojo (*Acorus calamus* L.*)*,bakainu leaf (*Melia azedarach* L.), and curry leaf [*Murraya koenigii* (L.) Spreng.]are kept with rice grains in granaries and other storage structures.	The disagreeable odour and insecticidal properties of these leaves keep away the storage insect pests from causing damage to the grains. All the mentioned plants have proven pesticide and insecticidal properties.
34	Storing grains in locally made structures called Bhakhari and Kothe prepared from bamboo and plastered with raw cow dung and mud mixed paste.	Grains stored in these structures are not easily damaged because these structures prevent most of the pests from reaching grain from outside.
35	Grains for seed purposes are stored in gunny bags placed over the fireplace.	The smoke from burning firewood prevents the infestation of any pests and insects.

Plate 3. (a) Wet ploughing for seedbed preparation (b) Bund clearing and mud slicing (c) Seedbed/nursery (d) Terraced field (e) Transplantation from nursery to the main field (f) Levelling of rice field

4.1.2.3. Water management techniques

Seedlings raised in the nurseries are transplanted onto the irrigated terraces. The field is allowed to remain submerged throughout the growing season, and water is drained out through a single field outlet. For better water conservation and management, farmers build levelled terraces depending on the slope, preventing the soil erosion. The benches and terraces are watered through perennial springs tapped from the higher elevation (Plate 3d). The water is collected into small gullies commonly known as 'Kulo' in the local Nepali language. It is used to irrigate rice fields. Often times, a single kulo has to be used by many farmers and needs to be maintained regularly for uninterrupted flow from distant places. The surface flow of water from one terrace to another is managed so that suspended solid particles remain in the terraces only (Plate 3e). However, for upland paddy, no irrigation is required. In this case, paddy is cultivated on the inclined hills utilizing rain water.

4.1.2.4. Method of rice planting

All the respondents reported that the transplantation from nursery to field occurs during June-July (Aasar as per the local Hindu calendar). Transplanted rice is cultivated on meticulously designed terrace fields. Proper levelling of the field is done with the help of a wooden beam (Plate 3f) (Dadhey) after two wet ploughings with a country plough (Plate 4a and 4b) to prepare the land for transplanting of 25-30 days old seedlings.

Plate 4. (a) Country plough (halo) (b) Leveler (dadhey) (c) Kodalo (d) Wooden hand leveler (phyauri) (e) Kachiya (f) Hasiya.

The final levelling of the field is done by a wooden hand leveler (Plate 4c and 4d) (Kodalo and Phyauri) to maintain the water level in the field throughout the crop season.

After attaining the optimum stage of seedling, i.e. four leaf stages or about 21 to 25 days after sowing in short-duration varieties and 30-35 days after sowing in case of long-duration varieties, they are lifted carefully to minimize the root injury and kept in the shade to avoid the water losses. If a nursery's soil becomes hard, seedlings are uprooted in the presence of water. Transplanting is done manually; row and plant spacing are not maintained. Farmers inter-crop rice with a traditional soybean variety [*Glycine max* (L.) Merrill], rice bean [*Vigna umbellata* (Thunb.) Ohwi et Ohasi] and black gram (*Vigna mungo* L.) on the bunds. A dedicated rice plantation festival of "Aasar Pandra" or "*Ropain*" is celebrated to mark the beginning of the cropping season. The festival has great significance for the local farmer as it is the time of year when farmers plant new seedlings in their fields wishing to grow quintals of rice from kilos. It was found that the rainfall pattern is also one of the critical factors for determining rice productivity in hills. Accordingly, local names have been assigned to the rainfall pattern called *Jhari* (continuous rainfall for two to several days). Such jharis are very crucial for almost all rain-dependent agriculture. A total of nine Jharis have been documented. They are Titey jhari, Naurethey jhari, Sisney jhari, Bhadaurey jhari, Sauney jahri, Makurey jhari, Bhangeri jhari, Kartikey jhari and Sohrasaradey jhari.

4.1.2.5. Soil fertility management

To manage the soil fertility, two traditional approaches are followed alongside the previous cropping (i) intercropping of rice beans in the ridges/bunds of terraced fields and (ii) rearing the cattle herds in the field. The purpose is to provide nutrients to the soil. During the field preparation stage, the topsoil from these bunds containing the nitrogen-fixing *Rhizobium* bacteria is sliced back to the terraces for rejuvenating the fertility, and new soil is put on the bunds for planting the rice bean crops every year. Rice bean is a non-determinate type of legume that enriches the soil by adding a large amount of foliage and fixing more nitrogen than other legumes.

4.1.2.6. Harvesting and threshing

The upland rice is harvested using a sickle (Plate 4e and 4f) (Kachiya and Hasiya in Nepali) panicle by panicle. Handfuls are bundled for initial storage and drying in the farm hut. When convenient, the rice bunches are taken down and thrashed. In the case of lowland rice, the crop is harvested using sickles and left in the field for 2-3 days for drying, which is tied into bundles. These bundles are carried to threshing yards or khalegara, stacked on a wooden platform, locally called Kunieu, for 2 to 2.5 months. They are then threshed to obtain grains by beating against a wooden plank (Plate 5a), followed by removing unfilled grains by winding with the help of Nanglo (Plate 5b). The straw is also trodden under the feet of bullocks to obtain the remaining grains from the panicle.

4.1.2.7. Storage and post-harvest techniques

Most farmers have minimal land holdings sufficient only for household consumption and planting for the next sowing. Hence, indigenous knowledge of storage aids structures is the most important. Further, due to heavy rainfall (about 2731 mm annually), the storage of grains is a huge challenge. Some of the indigenously prepared bamboo storage structures, such as (i) Bhakari (Plate 5c) (ii) Kotha/Kothe, are used for this purpose. Farmers also have “Dhikutis” (Plate 5d), which means storage in Nepali. Dhikuti is made up of four side boxes prepared with wood having the capacity of more than 400 kg of grains. It is the most preferred grain storage method. Bhakari and Kothe are woven bamboo structures prepared by rolling the closely knitted bamboo mat smeared with cow dung and mud to seal the holes for safe storage of seeds and grains.

In some cases, mustard seed pressed cakes (Peena) are also used for plastering the bamboo mat, thereby increasing the durability of the structures. Many locally available additives are also used to enhance the life of the stored seeds. Most common among them are the neem (Azadirachta indica A. Juss.), hardi [Curcuma longa (L.)], adhuwa (Zingiber officinale Roscoe), bhojo (Acorus calamus L.), bakainu (Melia azedarach L.), and curry patta [Murraya koenigii (L.) Spreng.]. All the storage structures are easy to use and proved to be efficient. The traditional mill called Dikhi, constructed using big wooden trunks (Plate 5e), now rarely exists. Farmers now prefer to take their paddy to electric mills where the milling cost is about 2-5 Rs./Kg of paddy.

Plate 5. (a) & (b) Threshing and winding; (c) *Bhakari;* (d) *Dhikuti*; (e) Traditional mill (*Dhiki*)

4.2. Phenotypic diversity based on grain and kernel traits

From the total of 74 rice landraces collected, 65 were retained for further studies after removing the duplicate collections. This comprised aromatic, non-aromatic, low land and upland landraces, of which seven were upland types collected from the upper Dzongu, and the remaining were cultivated in mid and low hills.

The collected germplasm was evaluated based on the following two broad categories of characteristics.

- Morphometric characterization based on grain and kernel traits
- Organoleptic and Cooking characteristics

4.2.1. Morphometric characterization

The following phenotypic traits were evaluated in this study: grain length (GL), grain breadth (GB), grain length/breadth (G-L/B), grain size, grain shape, kernel length (KL), kernel breadth (KB), kernel length/breadth (K-L/B), kernel size, kernel shape, and 1000 grain weight (1000 GW). The results are presented in Table 10.

Table 10: Accession-wise variability of seed traits in rice landraces

Name of Rice	GL	GB	GT	GLBR	KL	KB	KLBR	KT	GW
Authi dhan	9.09	2.64	1.96	3.44	6.37	2.43	2.62	1.82	23.40
Basmati I	8.33	2.44	1.76	3.41	6.24	1.94	3.22	1.65	22.13
Basmati II RU	8.32	3.53	2.34	2.36	5.24	3.15	1.66	2.23	24.70
Bhagey tulashi	6.84	3.15	2.05	2.17	4.46	2.66	1.68	1.96	20.17
Bhotangey	5.87	3.02	2.13	1.94	4.65	2.94	1.58	2.05	19.50
Birinful	8.84	2.44	2.07	3.63	6.26	2.34	2.68	1.74	23.83
Champa	10.27	2.96	2.14	3.47	6.84	2.57	2.66	1.94	26.43
Charenangre	8.46	2.37	1.82	3.56	6.47	2.24	2.89	1.74	24.30
Chewrey dhan	10.25	2.37	2.06	4.32	7.25	2.05	3.53	1.73	19.70
Chini zho	8.66	3.23	2.07	2.68	5.44	2.57	2.13	1.93	28.07
Chirakey	6.86	2.75	2.16	2.49	5.06	2.46	2.06	1.95	22.03
Danasay	7.35	3.60	2.25	2.05	5.34	2.76	1.93	2.05	24.23
Darmali	7.96	2.96	2.07	2.69	5.35	2.86	1.87	1.93	25.67
Dhood kalami	10.11	2.66	1.97	3.80	7.23	2.26	3.19	1.75	26.57
Doodhkatey	10.63	2.61	2.27	4.07	8.46	2.24	3.77	1.85	25.97

Dorakhey	7.23	2.42	1.95	2.99	5.23	2.04	2.56	1.72	23.93
Fauryal	9.85	2.67	2.14	3.69	5.55	2.24	2.48	1.73	23.77
Fourey	8.94	2.26	2.05	3.96	6.14	2.04	3.01	1.84	21.07
Japani	10.62	2.66	2.05	3.99	7.52	2.26	3.33	1.86	26.00
Jasuda	8.92	2.31	2.05	3.86	5.17	2.04	2.54	1.84	23.80
Jhapaka	8.94	2.34	2.05	3.82	5.24	2.06	2.55	1.84	23.50
Jogi dhan	6.06	2.95	2.36	2.06	4.34	2.34	1.86	1.95	19.27
Kagey	8.54	2.67	2.26	3.20	6.25	2.35	2.65	1.95	19.30
Kalo dhan	7.04	3.06	2.05	2.30	5.74	2.86	2.00	1.96	24.03
Kalo nunia I RU	7.85	2.24	1.64	3.50	4.24	2.16	1.96	1.54	21.27
Kalo nunia II GE	8.04	2.54	1.85	3.17	6.25	2.26	2.76	1.82	22.80
Kalo nunia III DA	8.36	2.41	1.95	3.46	4.25	2.27	1.87	1.82	23.13
Kalo nunia IV	7.67	2.37	2.04	3.24	4.54	2.14	2.12	1.94	26.87
Kaltura	7.35	2.85	2.06	2.58	6.25	2.54	2.46	1.85	18.27
Kataka	8.14	2.13	1.76	3.83	5.24	2.03	2.58	1.65	18.23
Khampti	9.46	2.54	1.95	3.73	6.75	2.46	2.75	1.76	24.57
Krishna bhog I TA	9.05	2.53	2.06	3.58	6.36	2.15	2.96	1.76	21.13
Krishna bhog II	8.24	2.85	1.93	2.89	6.84	2.44	2.81	1.93	22.47
Lal bachi	9.62	2.44	1.95	3.97	6.76	2.34	2.90	2.04	26.43
Mailey attey	6.85	3.05	2.16	2.25	5.05	2.81	1.80	1.95	24.67
Manipuri	9.15	2.64	1.94	3.47	6.34	2.35	2.69	1.86	29.43
Mansaray	9.60	2.66	2.06	3.61	6.95	2.27	3.07	1.85	22.23
Marbon zho	7.24	3.34	2.44	2.17	5.52	3.15	1.75	2.05	26.10
Marshi	6.96	3.25	2.25	2.14	5.04	2.74	1.84	1.84	24.57
Masari	8.35	2.75	1.95	3.04	5.96	2.52	2.36	1.84	22.70
Masino	8.04	2.24	2.04	3.60	5.23	2.04	2.57	1.86	20.33
Masule	8.64	2.65	1.96	3.26	6.27	2.26	2.78	1.76	21.67
Mumpup zho	7.34	3.43	2.24	2.14	5.44	3.26	1.67	2.05	26.90
Panbhara	9.74	2.68	2.15	3.64	7.04	2.34	3.01	1.97	29.70
Phudungey	7.26	3.16	2.45	2.30	5.55	3.04	1.83	2.06	24.70
Pountain	9.74	2.45	2.06	3.97	6.95	2.25	3.08	1.87	27.17
Raja bara	8.17	2.26	1.95	3.61	6.19	2.04	3.04	1.86	19.83
Ram bhog	6.44	3.05	2.15	2.11	4.35	2.98	1.46	2.04	21.50
Ram jeera	5.95	2.94	2.05	2.03	4.34	2.55	1.70	1.85	15.73
Sanu attey	6.25	3.14	2.25	1.99	4.25	2.17	1.96	1.55	18.53
Shyam jeera	5.96	3.04	2.35	1.96	4.23	2.56	1.65	2.12	20.87
Sirkey	7.64	3.55	2.36	2.16	5.25	3.17	1.66	2.25	28.40
Tapray	8.26	3.12	2.05	2.65	5.55	2.74	2.03	1.91	24.03
Thamba	9.15	2.46	1.94	3.72	6.65	2.20	3.02	1.75	22.27
Thulo attey	6.85	3.03	2.15	2.26	5.05	2.75	1.83	2.07	22.80
Timburey	6.45	2.91	2.13	2.22	3.63	2.65	1.37	2.04	20.50
Tukmorzho	8.26	2.54	1.94	3.25	6.34	2.35	2.69	1.83	27.50
Tulashi	6.06	2.96	2.15	2.05	4.07	2.16	1.89	1.95	18.40
Champasari	9.84	2.54	2.05	3.88	6.75	2.36	2.86	1.91	21.70
Nepali zho	8.75	2.53	2.14	3.46	5.42	2.31	2.34	2.04	22.53
Thulo tulashi	7.96	3.26	1.95	2.44	5.82	2.80	2.08	2.04	24.53
Venten	8.86	2.54	2.03	3.49	6.54	2.35	2.78	1.84	26.80
Rudhuwa	7.05	3.22	2.15	2.19	5.03	2.81	1.79	1.92	22.47
Kaanchi	6.14	3.04	2.24	2.02	4.35	2.84	1.53	2.05	19.53

Sano tulashi	6.04	2.92	2.03	2.07	4.05	2.16	1.87	1.85	20.30
Minimum	**5.87**	**2.13**	**1.64**	**1.94**	**3.63**	**1.94**	**1.37**	**1.54**	**15.73**
Maximum	**10.63**	**3.60**	**2.45**	**4.32**	**8.46**	**3.26**	**3.77**	**2.25**	**29.70**
Average	**8.10**	**2.77**	**2.08**	**3.00**	**5.67**	**2.45**	**2.36**	**1.89**	**23.15**
SD	**1.29**	**0.36**	**0.16**	**0.73**	**1.02**	**0.33**	**0.58**	**0.14**	**3.02**
SE	**0.16**	**0.05**	**0.02**	**0.09**	**0.13**	**0.04**	**0.07**	**0.02**	**0.37**
CV	**15.93**	**13.12**	**7.62**	**24.35**	**17.97**	**13.55**	**24.42**	**7.43**	**13.02**

GL, grain length; GB, grain breadth; GT, grain thickness; GL, GLBR, grain length-breadth ratio; KL, kernel length; KB, kernel breadth; KLBR, kernel length-breadth ratio; KT, kernel thickness, GW, 1000 grain weight

4.2.1.1. Grain length(mm)

In the case of grain length, good variability was noticed between different landraces (Table 11). The higest value was recorded in Doodhkatey (10.63 mm) and the lowest in Bhotangey (5.87 mm). Four types of grain lengths were obtained: one very long type (>10.5 mm), 15 long type (9-10.5 mm), 26 medium type (7.5-9 mm) and 23 short type (<7.5 mm). Both values of grain length and breadth were highly significant among the landraces (p value <0.001).

Table 11: Classification of rice landraces based on grain length

Seed length(mm)				
Very short (<5.0 mm)	Short (5.0 -7.5 mm)	Medium (7.5-9 mm)	Long (9- 10.5 mm)	Very long (>10.5 mm)
	Bhagey Tulashi, Bhotangey, Chirakey, Danasay, Dorakhey, Jogi dhan, Kalo dhan, Kaltura, Mailey Attey, Marbon zho, Marshi, Mumpup zho, Phudungey, Ram Bhog, Ram Jeera, Sanu Attey, Shyam jeera, Thulo attey ,Timburey , Tulashi, Rudhuwa, Kaanchi, Sano tulashi	Basmati I, Basmati II RU, Birinful, Charenangre,Chini zho, Darmali, Fourey, Jasuda, Jhapaka, Kagey, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Kalo nunia IV, Kataka, Krishna bhog II, Masari, Masino, Masule, Raja bara, Sirkey, Tapray, Tukmorzho, Nepali zho, Thulo tulashi, Venten	Authi dhan, Champa, Chewrey dhan, Dhood kalami, Fauryal, Japani, Khampti, Krishna bhog I TA, Lal bachi,Manipuri, Mansaray, Panbhara, Pountain, Thamba, Champasari	Doodhkatey

4.2.1.2. Grain breadth (mm)

The results revealed little variation for this trait among the collected rice landraces. The highest grain breadth was observed in Danasay (3.60 mm), and the lowest was obtained in Kataka (2.13 mm) (Table 12).

Table 12: Classification of rice landraces based on grain breadth

Grain breadth(mm)				
Very Narrow(<2.0 mm)	Narrow (2.1-2.5 mm)	Medium (2.6-3.0 mm)	Broad (3.1-3.5 mm)	very broad (>3.5 mm)
	Basmati I, Birinful, Charenangre, Chewrey dhan, Dorakhey, Fourey, Jasuda, Jhapaka, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Kalo nunia IV, Kataka, Lal bachi, Masino, Pountain, Raja Bara, Thamba, Champasari, Nepali zho, Venten	Authi dhan, Champa, Chirakey, Darmali, Dhood Kalami, Doodhkatey, Fauryal, Japani, Jogi dhan, Kagey, Kalo dhan, Kaltura, Khampti, Krishna bhog I TA, Krishna bhog II, Manipuri, Mansaray, Masari, Masule, Panbhara, Ram jeera, Shyam jeera, Timburey, Tukmorzho, Tulashi, Sano tulashi.	Bhagey tulashi, Bhotangey, Chini zho, Mailey Attey, Marbon zho, Marshi, Mumpup zho, Phudungey, Ram bhog, Sanu Attey, Tapray, Thulo attey, Thulo tulashi, Rudhuwa, Kaanchi	Basmati II RU, Danasay, Sirkey

4.2.1.3. Seed thickness (mm)

Table 13: Classification of rice landraces based on seed thickness

	Seedthickness(mm)	
Low (<1.50 mm)	Medium (1.51-1.99 mm)	Bold (>2.0 mm)
	Authi dhan, Basmati I, Charenangre, Dhood Kalami, Dorakhey, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Kataka, Khampti, Krishna bhog II, Lal bachi, Manipuri, Masari, Masule, Raja Bara, Thamba, Tukmorzho, Thulo tulashi	Basmati II RU, Bhagey tulashi, Bhotangey, Birinful, Champa, Chewrey Dhan, Chini zho, Chirakey, Danasay, Darmali, Doodhkatey, Fauryal, Fourey, Japani, Jasuda, Jhapaka, Jogi Dhan, Kagey, Kalo dhan, Kalo nunia IV, Kaltura, Krishna bhog I TA, Mailey attey, Mansaray, Marbon zho, Marshi, Masino, Mumpup zho, Panbhara, Phudungey,Pountain, Ram bhog, Ram jeera, Sanu attey, Shyam Jeera, Sirkey, Tapray, Thulo attey, Timburey, Tulashi, Champasari, Nepali zho, Venten, Rudhuwa, Kaanchi, Sano tulashi

Significant variation in seed thickness was noticed among the rice landraces (Table 13). The maximum seed thickness was recorded in Phudungey (2.45 mm) and less in Kalo nunia I RU (1.64 mm). Based on the seed thickness, the genotypes were grouped into low (<1.50 mm), medium (1.51 – 1.99 mm) and bold (>2.0 mm).

4.2.1.4. Grain L/B ratio or seed class (Fineness)

A significant difference for L/B ratio was registered among the landraces (Table 14). The L/B values were found to be ranging between 1.37 (Timburey) to 3.77 (Doodhkatey) with an average of 2.36, suggesting the highest variation based on the coefficient of variation (CV = 24.42%). Based on the ratio of kernel length and breadth, three types of grain sizes were recorded, 37 slender types (>3.1), 27 medium type (2.1-3.0) and 4 bold type (1.1-2.0).

Table 14: Classification of rice landraces based on L/B Ratio

L/B Ratio		
Bold (1.1-2.0)	(2.1-3.0) Medium	Slender (>3.1)
Danasay, Jogi dhan, Sanu attey, Shyam jeera	Basmati II RU, Bhagey Tulashi, Bhotangey, Chini zho, Chirakey, Darmali, Dorakhey, Kalo dhan, Kaltura, Krishna bhog II, Mailey Attey, Marbon zho, Marshi, Mumpup zho, Phudungey, Ram bhog, Ram jeera, Sirkey, Tapray, Thulo attey, Timburey, Tulashi, Thulo tulashi, Rudhuwa, Kaanchi, Sano tulashi	Authi dhan, Basmati I, Birinful, Champa, Charenangre, Chewrey dhan, Dhood kalami, Doodhkatey, Fauryal, Fourey, Japani, Jasuda, Jhapaka, Kagey, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Kalo nunia IV, Kataka, Khampti, Krishna bhog I TA, Lal Bachi, Manipuri, Mansaray, Masari, Masino, Masule, Panbhara, Pountain, Raja bara, Sanu attey, Shyam jeera, Thamba, Tukmorzho, Champasari, Nepali zho, Venten.

4.2.1.5. 1000 grain weight (g)

The test weight among collected rice varied significantly (Table 15). The highest test weight was recorded in Panbhara (29.70 g), followed by Manipuri (29.43 g), which was followed by Sirkey (28.40 g). The lowest test weight was recorded in Ram jeera (15.73 g). Based on the 1000 grain weight, 16 high types (25.1-30 g), 38 medium types (20. 1-25 g) and 11 low types (15.1-20 g) were recorded.

Table 15: Classification of rice landraces based on test weight (g)

Test weight (g)				
Very low (<15)	Low (15.1-20 g)	Medium (20.1-25g)	High (25.1-30 g)	Very high (>30.1g)
	Bhotangey, Chewrey dhan, Jogi dhan, Kagey, Kaltura, Kataka, Raja bara, Ram jeera, Sanu attey, Tulashi, Kaanchi	Authi dhan, Basmati I Basmati II RU, Bhagey tulashi, Birinful, Charenangre, Chirakey, Danasay, Dorakhey Fauryal, Fourey, Jasuda, Jhapaka, Kalo dhan, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Khampti, Krishna bhog I TA, Krishna bhog II, Mailey Attey, Mansaray, Marshi, Masari, Masino, Masule, Phudungey, Ram bhog, Shyam jeera, Tapray, Thamba, Thulo attey Timburey,Champasari, Nepali Zho, Thulo tulashi, Rudhuwa, Sano tulashi.	Champa, Chini zho, Darmali, Dhood kalami, Doodhkatey, Japani, Kalo nunia IV, Lal bachi, Manipuri, Marbon zho, Mumpup zho, Panbhara, Pountain, Sirkey, Tukmorzho, Venten.	

4.2.1.6. Kernel length, breadth and kernel L/B ratio

The highest kernel length among the collected rice was recorded in Doodhkatey (8.46 mm) and the lowest in Timburey (3.63mm). The rest of them showed kernel length in the range of 8.46 to 3.63 mm.

Kernel breadth among the collected different aromatic rice landraces is presented in table 12. The highest breadth of the kernel was recorded in Timburey (3.26 mm), and the lowest kernel breadth was recorded in Basmati-I (1.94 mm). A large number of varieties ranged between 2-2.5 mm. Similarly, the highest L/B ratio of the kernel was recorded from Doodhkatey (3.77 mm) and the lowest in Timburey (1.37 mm), and the remaining ones exhibited KL/KB in the range of 2-3 mm respectively (Table 14).

4.2.2. Organoleptic qualities of cooked rice (Cooking quality)

The data on the cooking characteristics of 65 rice landraces evaluated during the study is presented in Table 16.

Table 16: Variability for cooking quality traits among 65 landraces

Name of Rice	CRL (mm)	CRB (mm)	LBR	ER	EI
Authi dhan	8.07	3.85	2.09	1.27	1.65
Basmati I	8.14	4.04	2.01	1.31	1.54
Basmati II RU	7.05	4.31	1.64	1.35	1.22
Bhagey tulashi	7.93	4.05	1.96	1.78	1.10
Bhotangey	6.95	3.34	2.08	1.50	1.39
Birinful	7.24	4.14	1.75	1.16	1.51
Champa	7.05	3.84	1.84	1.03	1.78
Charenangre	8.15	3.95	2.06	1.26	1.64
Chewrey dhan	7.95	3.55	2.24	1.10	2.04
Chini zho	8.05	4.15	1.94	1.48	1.31
Chirakey	6.24	3.95	1.58	1.23	1.28
Danasay	7.94	4.24	1.87	1.49	1.26
Darmali	8.15	4.85	1.68	1.52	1.10
Dhood kalami	9.15	3.95	2.32	1.27	1.83
Doodhkatey	10.75	3.95	2.72	1.27	2.14
Dorakhey	7.15	4.53	1.58	1.37	1.15
Fauryal	8.94	4.03	2.22	1.61	1.38
Fourey	9.04	4.02	2.25	1.47	1.53
Japani	9.74	3.44	2.84	1.30	2.19
Jasuda	7.24	3.94	1.84	1.40	1.31
Jhapaka	7.34	3.85	1.90	1.40	1.36
Jogi dhan	6.26	4.13	1.52	1.44	1.05
Kagey	7.76	3.15	2.46	1.24	1.98
Kalo dhan	7.04	4.25	1.66	1.23	1.35
Kalo nunia I RU	8.44	4.25	1.99	1.99	1.00
Kalo nunia II GE	8.34	4.15	2.01	1.33	1.50
Kalo nunia III DA	7.91	4.05	1.95	1.86	1.05
Kalo nunia IV	6.17	2.95	2.09	1.36	1.54
Kaltura	7.04	3.15	2.24	1.13	1.99
Kataka	8.24	4.04	2.04	1.57	1.30
Khampti	7.61	3.95	1.93	1.13	1.71
Krishna bhog I TA	8.04	3.24	2.48	1.26	1.96
Krishna bhog II	8.17	3.54	2.31	1.19	1.93
Lal bachi	8.22	4.34	1.89	1.21	1.56
Mailey attey	9.65	3.95	2.45	1.91	1.28
Manipuri	8.64	3.94	2.19	1.36	1.61
Mansaray	9.13	3.93	2.33	1.31	1.77
Marbon zho	8.14	4.16	1.96	1.47	1.33
Marshi	6.04	4.06	1.49	1.20	1.24

Masari	8.84	4.86	1.82	1.48	1.23
Masino	8.01	4.06	1.98	1.53	1.29
Masule	8.14	3.95	2.06	1.30	1.59
Mumpup zho	8.36	4.94	1.69	1.54	1.10
Panbhara	8.92	4.05	2.21	1.27	1.74
Phudungey	8.14	4.04	2.01	1.47	1.37
Pountain	9.06	3.81	2.38	1.30	1.82
Raja bara	8.16	3.96	2.06	1.32	1.56
Ram bhog	6.67	4.04	1.65	1.53	1.08
Ram jeera	5.96	4.05	1.47	1.37	1.07
Sanu attey	6.15	3.14	1.96	1.45	1.35
Shyam jeera	6.16	3.95	1.56	1.46	1.07
Sirkey	7.26	4.85	1.50	1.38	1.08
Tapray	7.05	4.05	1.74	1.27	1.37
Thamba	8.94	3.51	2.54	1.34	1.89
Thulo attey	7.15	4.85	1.47	1.42	1.04
Timburey	6.26	3.96	1.58	1.72	0.92
Tukmorzho	8.24	4.85	1.70	1.30	1.31
Tulashi	6.15	3.25	1.89	1.51	1.25
Champasari	8.74	4.64	1.88	1.30	1.45
Nepali zho	7.24	4.06	1.79	1.34	1.34
Thulo tulashi	8.05	4.24	1.90	1.38	1.37
Venten	8.15	3.55	2.30	1.25	1.84
Rudhuwa	7.05	3.85	1.83	1.40	1.31
Kaanchi	6.06	3.15	1.92	1.39	1.38
Sano tulashi	7.53	3.24	2.33	1.86	1.25
Minimum	**5.96**	**2.95**	**1.47**	**1.03**	**0.92**
Maximum	**10.75**	**4.94**	**2.84**	**1.99**	**2.19**
Average	**7.78**	**3.97**	**1.98**	**1.39**	**1.45**
SD	**1.03**	**0.45**	**0.31**	**0.19**	**0.31**
SE	**0.13**	**0.06**	**0.04**	**0.02**	**0.04**
CV	**13.20**	**11.45**	**15.71**	**13.80**	**21.42**

CRL, cooked rice length; CRB, cooked rice breadth; LBR, length/breadth ratio; ER, elongation ratio; EI, elongation index.

4.2.2.1. Kernel elongation ratio

The average elongation ratio of landraces varied significantly ($p<0.001$) (CV = 13.80%). The elongation ratio of cooked rice was recorded in the range of 1.03 to 1.99, with a mean value of 1.39. Kalo nunia –I exhibited the highest kernel elongation ratio in cooking (1.99), whereas the minimum elongation ratio was found in Champa (1.03) (Table 16).

4.2.2.2. Kernel elongation after cooking (KEaC)

A highly significant difference ($P<0.001$) was observed for the cooked grain quality traits among 65 landraces used in the study. Doodhkatey has the highest cooked kernel length (10.75 mm), while Ramjeera has the least (5.96 mm) with a mean value of 7.78 mm. Similarly, other genotypes had relatively high values (Table 16).

4.2.2.3. Cooked kernel length breadth ratio

The average cooked length-breadth ratio was significantly different among the landraces ($p<0.001$), with the highest in Japani (2.84) and lowest in Thulo attey (1.47). Landraces Marshi and Sirkey showed 1.49 and 1.50, respectively. The overall mean has been computed to be 1.98 (Table 16). High variability (CV = 15.71%) was observed for this trait among the landraces.

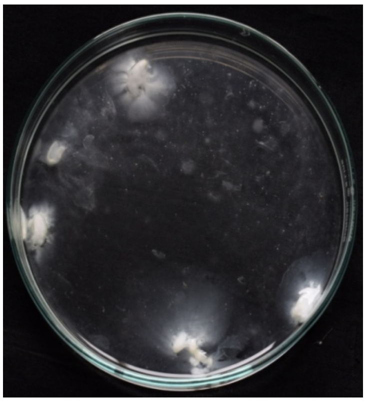
4.2.3. Biochemical properties

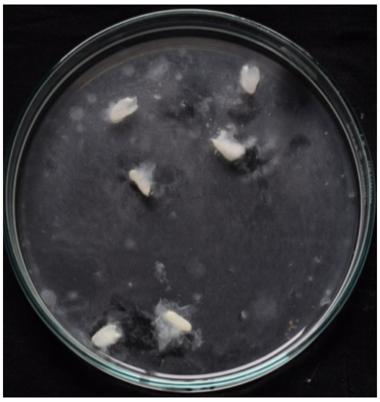
4.2.3.1. Grain aroma

About 35% of the studied landraces showed aroma character identified by testing the grains using the sensory test. Out of a total of 65, nearly 42 landraces were found to be non-aromatic, and about 23 showed aromatic traits. Further, the aromatic rice landraces were grouped into two categories based on the intensity of the aroma *viz*., strongly scented and moderately scented, where 13 were found strongly aromatic, and 10 were found moderately aromatic.

4.2.3.2. Gelatinization temperature through alkali spreading value (ASV)

Gelatinization temperature (GT) was estimated based on the alkali spreading value of the milled rice (Table 17, Plate 6). The alkali spreading values ranged from 3 - 7 for all the landraces studied with the majority showing high alkali scores.


Low Alkali Digestion


Intermediate Alkali Digestion

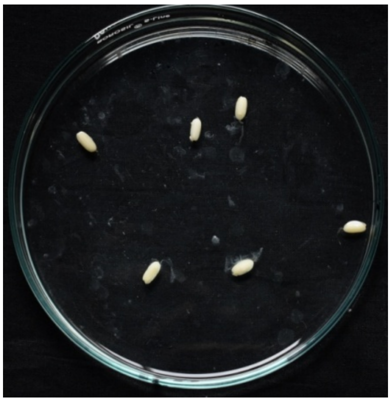

High Alkali digestion

Plate 6. Types of Alkali digestion of milled rice grains

A high alkali digestion value corresponds to a low gelatinization temperature. The low alkali digestion value with high gelatinization temperature was recorded in Phudungey and Tukmorzho. In contrast, a high alkali digestion value with low gelatinization temperature was recorded in the remaining 49 landraces (Table 18). About 14 of them recorded intermediate alkali digestion and moderate gelatinization temperature. The high alkali spreading value recorded for most landraces denotes low gelatinization temperature (55^{o} to 69 ºC).

Table 17: Classification of rice landraces based on the Alkali Spreading Value (ASV)

ASV of traditional rice landraces			
1-2 (Low)	3 (Low - intermediate)	4-5 (Intermediate)	6-7 (High)
	Phudungey, Tukmorzho	Bhotangey, Chewrey dhan, Chirakey, Dhood kalami, Jhapaka Kaltura, Kataka, Lal bachi, Manipuri, Mansaray, Mumpup zho Sirkey, Thulo tulashi, Sano tulashi	Authi dhan, Basmati I, Basmati II RU, Bhagey tulashi, Birinful, Champa, Charenangre, Chini zho, Danasay, Darmali, Doodhkatey, Dorakhey, Fauryal, Fourey, Japani, Jasuda, Jogi dhan, Kagey, Kalo dhan Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Kalo nunia IV, Khampti, Krishna bhog I TA, Krishna bhog II, Mailey attey, Marbon zho, Marshi, Masari, Masino, Masule, Panbhara, Pountain, Raja Bara, Ram bhog, Ram jeera, Sanu attey, Shyam jeera, Tapray, Thamba, Thulo attey, Timburey, Tulashi, Champasari, Nepali zho, Venten, Rudhuwa, Kaanchi.

Table 18: Classification of rice landraces based on Gelatinization temperature

Gelatilization Temperature			
High	High to Intermediate	Intermediate	Low
	Phudungey, Tukmorzho	Bhotangey, Chewrey dhan, Chirakey, Dhood kalami, Jhapaka, Kaltura, Kataka, Lal bachi, Manipuri,,Mansaray, Mumpup zho, Sirkey, Thulo tulashi, Sano tulashi	Authi dhan, Basmati I, Basmati II RU, Bhagey tulashi, Birinful, Champa, Charenangre, Chini zho, Danasay, Darmali, Doodhkatey, Dorakhey, Fauryal, Fourey, Japani, Jasuda, Jogi dhan, Kagey, Kalo dhan, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia III DA, Kalo nunia IV, Khampti, Krishna bhog I TA, Krishna bhog II, Mailey attey, Marbon zho, Marshi, Masari, Masino, Masule, Panbhara, Pountain, Raja bara, Ram bhog, Ram jeera, Sanu attey, Shyam jeera, Tapray, Thamba, Thulo attey, Timburey, Tulashi, Champasari, Nepali zho, Venten, Rudhuwa, Kaanchi.

4.2.3.3. Amylose Content

Amylose content ranged from 13.85 to 28.25%. The highest value was recorded in Phudungey with 28.25%, followed by Mumpup zho with 27.29%.

Table 19: Classification of rice landraces based on Amylose content

Amylose content				
Waxy (0–2%)	Very low amylose (2–9%)	Low amylose (10–20%)	Intermediate amylose (20–25%)	High amylose (above 25%)
		Authi dhan, Bhotangey, Dorakhey, Fauryal, Jogi dhan, Masari, Pountain, Ram bhog, Thamba, Venten, Sano tulashi, Bhagey Tulashi, Champa, Chewrey dhan, Doodhkatey, Japani, Kagey, Kalo dhan, Lal bachi, Mailey attey, Sirkey, Thulo attey, Timburey, Tulashi, Thulo tulashi, Rudhuwa, Kaanchi, Fourey, Khampti, Manipuri, Mansaray Marshi, Masino, Ram jeera, Shyam jeera.	Basmati I, Basmati II, Birinful, Charenangre, Chini zho, Danasay, Darmali, Jasuda, Kalo nunia III DA, Kaltura, Kataka, Masule, Raja Bara, Sanu Attey, Tapray, Chirakey, Dhood kalami, Jhapaka, Kalo nunia I RU, Kalo nunia II GE, Kalo nunia IV, Krishna bhog I TA, Krishna bhog II, Tukmorzho, Champasari, Nepali zho, Panbhara.	Marbon zho, Mumpup zho, Phudungey.

On the other hand, the lowest values were recorded in Chewrey dhan, with 13.85%, followed by Jogi dhan, with 14.77%. Almost 41% (27 landraces) of the tested rice was found to possess intermediate amylose content (<20-25%); about 53% (35 landraces) presented low amylose content (12-20%) and 3 landraces contained high amylose content (Table 19).

4.2.4. Variability for the seed and cooking quality traits

The one-way ANOVA revealed significant variability (p< 0.05) for the 14 seeds and coking traits. The results are depicted in Table 20.

Table 20. One-way ANOVA for seed and cooking quality traits in 65 rice landraces

Traits	F	F crit	*P*-value	Significance
Grain length (mm)	1084.38	1.41	6.39E-153	***
Grain breadth (mm)	110.61	1.41	3.69E-89	***
Grain thickness	81.31	1.41	9.72E-81	***
Grain length breadth ratio	334.34	1.41	7.02E-120	***
Kernel length (mm)	2929.96	1.41	6.24E-181	***
Kernel breadth (mm)	179.98	1.41	1.30E-102	***
Kernel-length breadth ratio	525.61	1.41	1.47E-132	***
Kernel thickness	56.94	1.41	4.09E-71	***
1000 grain weight (gm)	129.24	1.41	1.92E-93	***
Cooked rice length (mm)	2596.097	1.41	1.60E-177	***
Cooked rice breadth (mm)	548.3926	1.41	9.40E-134	***
L/B ratio	772.0584	1.41	2.30E-143	***
Elongation ratio	808.3739	1.41	1.20E-144	***
Elongation index	1285.926	1.41	1.00E-157	***

F, calculated F value; F crit, F critical value; ns, not significant; ***significant at p< 0.001.

The coefficient of variation (CV) for the 14 seed traits varied from a minimum of 7.43% to a maximum of 24.42% with an average of 15.26%. Three types of grain lengths were obtained, 15 long type (9-10.5 mm), 26 medium type (7.5-9 mm) and 23 short type (<7.5 mm). The grain length and breadth variability were highly significant among the studied landraces (p<0.001). The ratio of kernel length and breadth ranged from 1.37

(Timburey) to 3.77 (Doodhkatey) with an average of 2.36 and showed the highest variation (based on the coefficient of variation, CV = 24.42%). Based on the above trait, three types of grain sizes were recorded, slender (11 nos), medium (29 nos.) and bold type (23). Kernel thickness, which varied from 1.54 (Kalo nunia I) to 2.25 (Sirkey) with an average of 1.89, revealed the lowest variation (CV = 7.43%).

A highly significant difference ($p<0.001$) was observed for the cooked grain quality traits among the 65 landraces. Doodhkatey showed the highest cooked kernel length (10.75 mm), while Ramjeera showed the least (5.96 mm) with a mean value of 7.78 mm. Similarly, other genotypes had relatively high values. The average elongation ratio among the landraces varied significantly ($p<0.001$), as revealed by the high CV value (CV = 13.80%). Kalo nunia –I exhibited the highest kernel elongation ratio in cooking (1.99), whereas the minimum elongation ratio was obtained in Champa (1.03). The average cooked length-breadth ratio was significantly different among the landraces ($p<0.001$), with the highest in Japani (2.84) and lowest in Thulo attey (1.47). In the cooked length-breadth ratio, high variability (CV = 15.71%) was observed among the landraces (Table 21).

Table 21: Accession-wise variability for seed and cooking quality traits in 65 rice landraces

Name of Rice	GL	GB	GT	GLBR	KL	KB	KLBR	KT	GW	CRL	CRB	LBR	ER	EI
Authi dhan	9.09	2.64	1.96	3.44	6.37	2.43	2.62	1.82	23.4	8.07	3.85	2.09	1.27	1.65
Basmati I	8.33	2.44	1.76	3.41	6.24	1.94	3.22	1.65	22.13	8.14	4.04	2.01	1.31	1.54
Basmati II RU	8.32	3.53	2.34	2.36	5.24	3.15	1.66	2.23	24.70	7.05	4.31	1.64	1.35	1.22
Bhagey tulashi	6.84	3.15	2.05	2.17	4.46	2.66	1.68	1.96	20.17	7.93	4.05	1.96	1.78	1.10
Bhotangey	5.87	3.02	2.13	1.94	4.65	2.94	1.58	2.05	19.50	6.95	3.34	2.08	1.5	1.39
Birinful	8.84	2.44	2.07	3.63	6.26	2.34	2.68	1.74	23.83	7.24	4.14	1.75	1.16	1.51
Champa	10.27	2.96	2.14	3.47	6.84	2.57	2.66	1.94	26.43	7.05	3.84	1.84	1.03	1.78
Charenangre	8.46	2.37	1.82	3.56	6.47	2.24	2.89	1.74	24.30	8.15	3.95	2.06	1.26	1.64
Chewrey dhan	10.25	2.37	2.06	4.32	7.25	2.05	3.53	1.73	19.70	7.95	3.55	2.24	1.10	2.04
Chini zho	8.66	3.23	2.07	2.68	5.44	2.57	2.13	1.93	28.07	8.05	4.15	1.94	1.48	1.31
Chirakey	6.86	2.75	2.16	2.49	5.06	2.46	2.06	1.95	22.03	6.24	3.95	1.58	1.23	1.28
Danasay	7.35	3.6	2.25	2.05	5.34	2.76	1.93	2.05	24.23	7.94	4.24	1.87	1.49	1.26
Darmali	7.96	2.96	2.07	2.69	5.35	2.86	1.87	1.93	25.67	8.15	4.85	1.68	1.52	1.10
Dhood kalami	10.11	2.66	1.97	3.80	7.23	2.26	3.19	1.75	26.57	9.15	3.95	2.32	1.27	1.83
Doodhkatey	10.63	2.61	2.27	4.07	8.46	2.24	3.77	1.85	25.97	10.75	3.95	2.72	1.27	2.14
Dorakhey	7.23	2.42	1.95	2.99	5.23	2.04	2.56	1.72	23.93	7.15	4.53	1.58	1.37	1.15
Fauryal	9.85	2.67	2.14	3.69	5.55	2.24	2.48	1.73	23.77	8.94	4.03	2.22	1.61	1.38
Fourey	8.94	2.26	2.05	3.96	6.14	2.04	3.01	1.84	21.07	9.04	4.02	2.25	1.47	1.53
Japani	10.62	2.66	2.05	3.99	7.52	2.26	3.33	1.86	26.00	9.74	3.44	2.84	1.30	2.19
Jasuda	8.92	2.31	2.05	3.86	5.17	2.04	2.54	1.84	23.80	7.24	3.94	1.84	1.40	1.31
Jhapaka	8.94	2.34	2.05	3.82	5.24	2.06	2.55	1.84	23.50	7.34	3.85	1.9	1.40	1.36
Jogi dhan	6.06	2.95	2.36	2.06	4.34	2.34	1.86	1.95	19.27	6.26	4.13	1.52	1.44	1.05
Kagey	8.54	2.67	2.26	3.20	6.25	2.35	2.65	1.95	19.30	7.76	3.15	2.46	1.24	1.98
Kalo dhan	7.04	3.06	2.05	2.30	5.74	2.86	2.00	1.96	24.03	7.04	4.25	1.66	1.23	1.35
Kalo nunia I RU	7.85	2.24	1.64	3.50	4.24	2.16	1.96	1.54	21.27	8.44	4.25	1.99	1.99	1.00
Kalo nunia II GE	8.04	2.54	1.85	3.17	6.25	2.26	2.76	1.82	22.80	8.34	4.15	2.01	1.33	1.50
Kalo nunia III DA	8.36	2.41	1.95	3.46	4.25	2.27	1.87	1.82	23.13	7.91	4.05	1.95	1.86	1.05

Kalo nunia IV	7.67	2.37	2.04	3.24	4.54	2.14	2.12	1.94	26.87	6.17	2.95	2.09	1.36	1.54
Kaltura	7.35	2.85	2.06	2.58	6.25	2.54	2.46	1.85	18.27	7.04	3.15	2.24	1.13	1.99
Kataka	8.14	2.13	1.76	3.83	5.24	2.03	2.58	1.65	18.23	8.24	4.04	2.04	1.57	1.3
Khampti	9.46	2.54	1.95	3.73	6.75	2.46	2.75	1.76	24.57	7.61	3.95	1.93	1.13	1.71
Krishna bhog I TA	9.05	2.53	2.06	3.58	6.36	2.15	2.96	1.76	21.13	8.04	3.24	2.48	1.26	1.96
Krishna bhog II	8.24	2.85	1.93	2.89	6.84	2.44	2.81	1.93	22.47	8.17	3.54	2.31	1.19	1.93
Lal bachi	9.62	2.44	1.95	3.97	6.76	2.34	2.90	2.04	26.43	8.22	4.34	1.89	1.21	1.56
Mailey attey	6.85	3.05	2.16	2.25	5.05	2.81	1.80	1.95	24.67	9.65	3.95	2.45	1.91	1.28
Manipuri	9.15	2.64	1.94	3.47	6.34	2.35	2.69	1.86	29.43	8.64	3.94	2.19	1.36	1.61
Mansaray	9.60	2.66	2.06	3.61	6.95	2.27	3.07	1.85	22.23	9.13	3.93	2.33	1.31	1.77
Marbon zho	7.24	3.34	2.44	2.17	5.52	3.15	1.75	2.05	26.10	8.14	4.16	1.96	1.47	1.33
Marshi	6.96	3.25	2.25	2.14	5.04	2.74	1.84	1.84	24.57	6.04	4.06	1.49	1.20	1.24
Masari	8.35	2.75	1.95	3.04	5.96	2.52	2.36	1.84	22.70	8.84	4.86	1.82	1.48	1.23
Masino	8.04	2.24	2.04	3.60	5.23	2.04	2.57	1.86	20.33	8.01	4.06	1.98	1.53	1.29
Masule	8.64	2.65	1.96	3.26	6.27	2.26	2.78	1.76	21.67	8.14	3.95	2.06	1.30	1.59
Mumpup zho	7.34	3.43	2.24	2.14	5.44	3.26	1.67	2.05	26.90	8.36	4.94	1.69	1.54	1.10
Panbhara	9.74	2.68	2.15	3.64	7.04	2.34	3.01	1.97	29.70	8.92	4.05	2.21	1.27	1.74
Phudungey	7.26	3.16	2.45	2.30	5.55	3.04	1.83	2.06	24.70	8.14	4.04	2.01	1.47	1.37
Pountain	9.74	2.45	2.06	3.97	6.95	2.25	3.08	1.87	27.17	9.06	3.81	2.38	1.30	1.82
Raja bara	8.17	2.26	1.95	3.61	6.19	2.04	3.04	1.86	19.83	8.16	3.96	2.06	1.32	1.56
Ram bhog	6.44	3.05	2.15	2.11	4.35	2.98	1.46	2.04	21.50	6.67	4.04	1.65	1.53	1.08
Ram jeera	5.95	2.94	2.05	2.03	4.34	2.55	1.70	1.85	15.73	5.96	4.05	1.47	1.37	1.07
Sanu attey	6.25	3.14	2.25	1.99	4.25	2.17	1.96	1.55	18.53	6.15	3.14	1.96	1.45	1.35
Shyam jeera	5.96	3.04	2.35	1.96	4.23	2.56	1.65	2.12	20.87	6.16	3.95	1.56	1.46	1.07
Sirkey	7.64	3.55	2.36	2.16	5.25	3.17	1.66	2.25	28.40	7.26	4.85	1.50	1.38	1.08
Tapray	8.26	3.12	2.05	2.65	5.55	2.74	2.03	1.91	24.03	7.05	4.05	1.74	1.27	1.37
Thamba	9.15	2.46	1.94	3.72	6.65	2.20	3.02	1.75	22.27	8.94	3.51	2.54	1.34	1.89
Thulo attey	6.85	3.03	2.15	2.26	5.05	2.75	1.83	2.07	22.80	7.15	4.85	1.47	1.42	1.04
Timburey	6.45	2.91	2.13	2.22	3.63	2.65	1.37	2.04	20.50	6.26	3.96	1.58	1.72	0.92
Tukmorzho	8.26	2.54	1.94	3.25	6.34	2.35	2.69	1.83	27.50	8.24	4.85	1.70	1.3	1.31
Tulashi	6.06	2.96	2.15	2.05	4.07	2.16	1.89	1.95	18.40	6.15	3.25	1.89	1.51	1.25

Champasari	9.84	2.54	2.05	3.88	6.75	2.36	2.86	1.91	21.70	8.74	4.64	1.88	1.3	1.45
Nepali zho	8.75	2.53	2.14	3.46	5.42	2.31	2.34	2.04	22.53	7.24	4.06	1.79	1.34	1.34
Thulo tulashi	7.96	3.26	1.95	2.44	5.82	2.80	2.08	2.04	24.53	8.05	4.24	1.90	1.38	1.37
Venten	8.86	2.54	2.03	3.49	6.54	2.35	2.78	1.84	26.80	8.15	3.55	2.30	1.25	1.84
Rudhuwa	7.05	3.22	2.15	2.19	5.03	2.81	1.79	1.92	22.47	7.05	3.85	1.83	1.4	1.31
Kaanchi	6.14	3.04	2.24	2.02	4.35	2.84	1.53	2.05	19.53	6.06	3.15	1.92	1.39	1.38
Sano tulashi	6.04	2.92	2.03	2.07	4.05	2.16	1.87	1.85	20.30	7.53	3.24	2.33	1.86	1.25
Minimum	**5.87**	**2.13**	**1.64**	**1.94**	**3.63**	**1.94**	**1.37**	**1.54**	**15.73**	**5.96**	**2.95**	**1.47**	**1.03**	**0.92**
Maximum	**10.63**	**3.60**	**2.45**	**4.32**	**8.46**	**3.26**	**3.77**	**2.25**	**29.70**	**7.78**	**3.97**	**1.98**	**1.39**	**1.45**
Average	**8.10**	**2.77**	**2.08**	**3.00**	**5.67**	**2.45**	**2.36**	**1.89**	**23.15**	**7.78**	**3.97**	**1.98**	**1.39**	**1.45**
SD	**1.29**	**0.36**	**0.16**	**0.73**	**1.02**	**0.33**	**0.58**	**0.14**	**3.02**	**1.03**	**0.45**	**0.31**	**0.19**	**0.31**
CV(%)	**15.93**	**13.12**	**7.62**	**24.35**	**17.97**	**13.55**	**24.42**	**7.43**	**13.02**	**13.2**	**11.45**	**15.71**	**13.8**	**21.42**

GL, grain length; GB, grain breadth; GT, grain thickness; GLBR, grain length breadth ratio; KL, kernel length; KB, kernel breadth; KLBR, kernel-length breadth ratio; KT, kernel thickness; GW, 1000 grain weight; CRL, cooked rice length; CRB, cooked rice breadth; LBR, length/breadth ratio; ER, elongation ratio; EI, elongation index.

4.2.5. Correlation between the grain and cooked rice characters

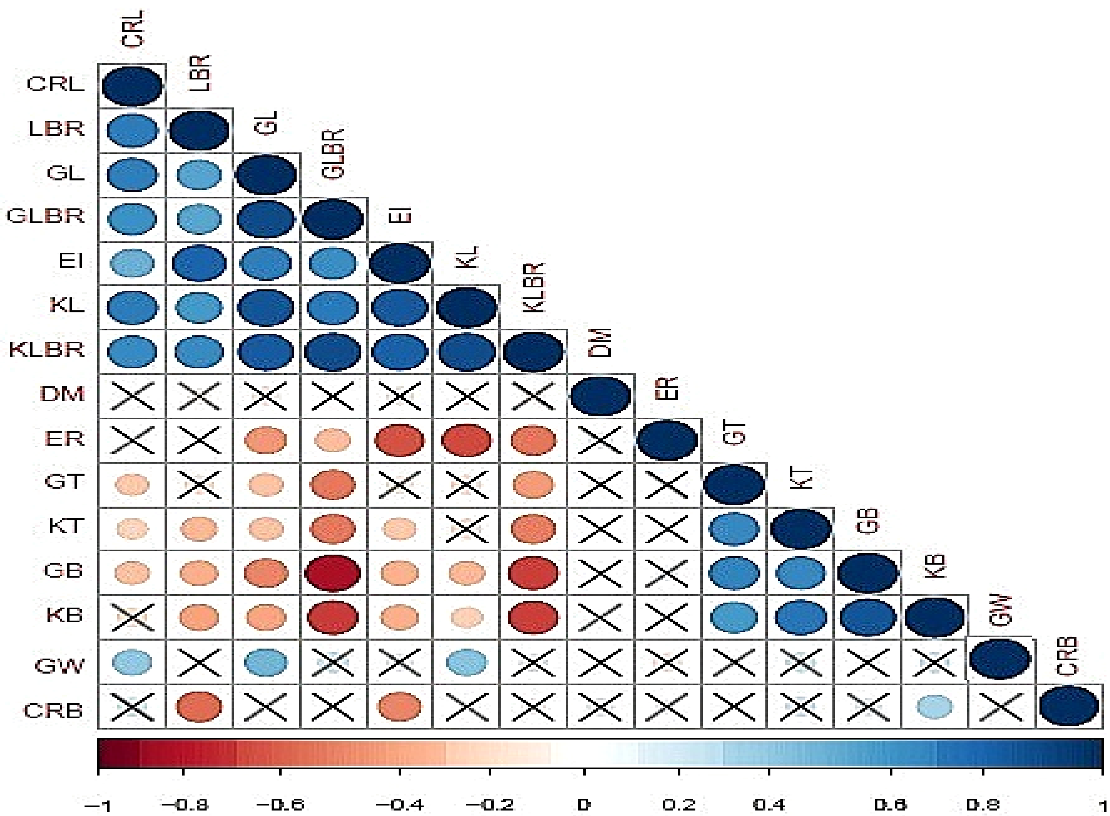
The Pearson correlation coefficient coupled with a two-tailed T-test was performed to determine the relationships among the quantitative characteristics of seeds and cooked rice (Table 22).

Table 22. Correlations among 14 seed-based traits.

	GL	**GB**	**GT**	**GLBR**	**KL**	**KB**	**KLBR**	**KT**	**GW**	**CRL**	**CRB**	**LBR**	**ER**	**EI**
GL	1	**	*	**	**	**	**	*	**	**	Ns	**	**	**
GB	-0.49	1	**	**	**	**	**	**	ns	*	Ns	**	ns	**
GT	-0.31	0.67	1	**	ns	**	**	**	ns	*	Ns	ns	ns	ns
GLBR	**0.89**	-0.83	-0.53	1	**	**	**	**	ns	**	Ns	**	**	**
KL	**0.85**	-0.34	-0.22	0.71	1	*	**	ns	**	**	Ns	**	**	**
KB	-0.41	**0.86**	0.58	-0.70	-0.26	1	**	**	ns	ns	**	**	ns	**
KLBR	**0.83**	-0.68	-0.44	**0.88**	**0.88**	-0.68	1	**	ns	**	Ns	**	**	**
KT	-0.31	0.65	0.65	-0.53	-0.22	0.73	-0.52	1	ns	*	Ns	**	ns	*
GW	0.47	0.12	0.07	0.24	0.42	0.23	0.20	0.22	1	**	**	ns	ns	ns
CRL	**0.69**	-0.32	-0.29	0.61	0.70	-0.22	0.64	-0.25	0.40	1	Ns	**	ns	**
CRB	0.05	0.17	-0.02	-0.05	0.03	0.34	-0.14	0.22	0.37	0.20	1	**	ns	**
LBR	0.54	-0.38	-0.21	0.53	0.58	-0.42	0.64	-0.35	0.08	0.70	-0.56	1	ns	**
ER	-0.45	0.12	-0.03	-0.33	-0.66	0.10	-0.54	0.02	-0.20	0.06	0.12	-0.05	1	**
EI	**0.70**	-0.37	-0.17	**0.62**	**0.84**	-0.38	**0.82**	-0.29	0.16	0.49	-0.51	**0.80**	-0.64	1

GL, grain length; GB, grain breadth; GT, grain thickness; GLBR, grain length breadth ratio; KL, kernel length; KB, kernel Breadth; KLBR, kernel-length breadth ratio; KT, kernel thickness; GW, 1000 grain weight; CRL, cooked rice length; CRB, cooked rice breadth; LBR, length/breadth ratio; ER, elongation ratio; EI, elongation index; **correlation is significant at the 0.01 level (2-tailed); *correlation is significant at the 0.05 level (2-tailed); NS, non-significance

The results showed a significant positive correlation between grain length with the grain L/B ratio, kernel L/B ratio, cooked rice length and elongation index at 0.01 levels. A significant positive correlation was also observed among the kernel L/B ratio, cooked RL, kernel and grain LBR, and elongation index. However, a significant negative correlation was observed between the grain L/B ratio and GB and KB. Similarly, a negative correlation was observed between kernel L/B ratio and GB and KB (Figure 5).



GL, grain length; GB, grain breadth; GT, grain thickness; GLBR, grain length breadth ratio; KL, kernel length; KB, kernel breadth; KLBR, Kernel-length breadth ratio; KT, Kernel thickness; GW, 1000 grain weight; CRL, cooked rice length; CRB, cooked rice breadth; LBR, length/breadth ratio; ER, elongation ratio; EI, elongation index. × = non-significant

Figure 5. Correlation matrix of 14 phenotypic traits evaluated on the 65 rice landraces.

4.2.6. Principle component analysis (PCA)

To understand the pattern of diversity among the seed and cooking quality traits, principal component analysis (PCA) was performed (Figure 6). The first two components (PCs) explained 54.62% of the total variance. The PC1 contributed 38.29% of the total variation and revealed the highest positive correlation with kernel length breadth ratio (0.96), grain length breadth (0.92), grain length (0.86), kernel length (0.83), elongation ratio (0.82), and cooked rice breadth (0.73). The PC2 contributed 16.33% of the variation, which was moderately correlated with cooked rice length (0.39), and negatively correlated with the kernel thickness (-0.04). Similarly, PC3 accounted for 14.30% of the variation, which was positively correlated with 1000 GW (0.70) and kernel breadth (0.60). All the grain and organoleptic traits were found to be the major contributors to PC1 and PC2.

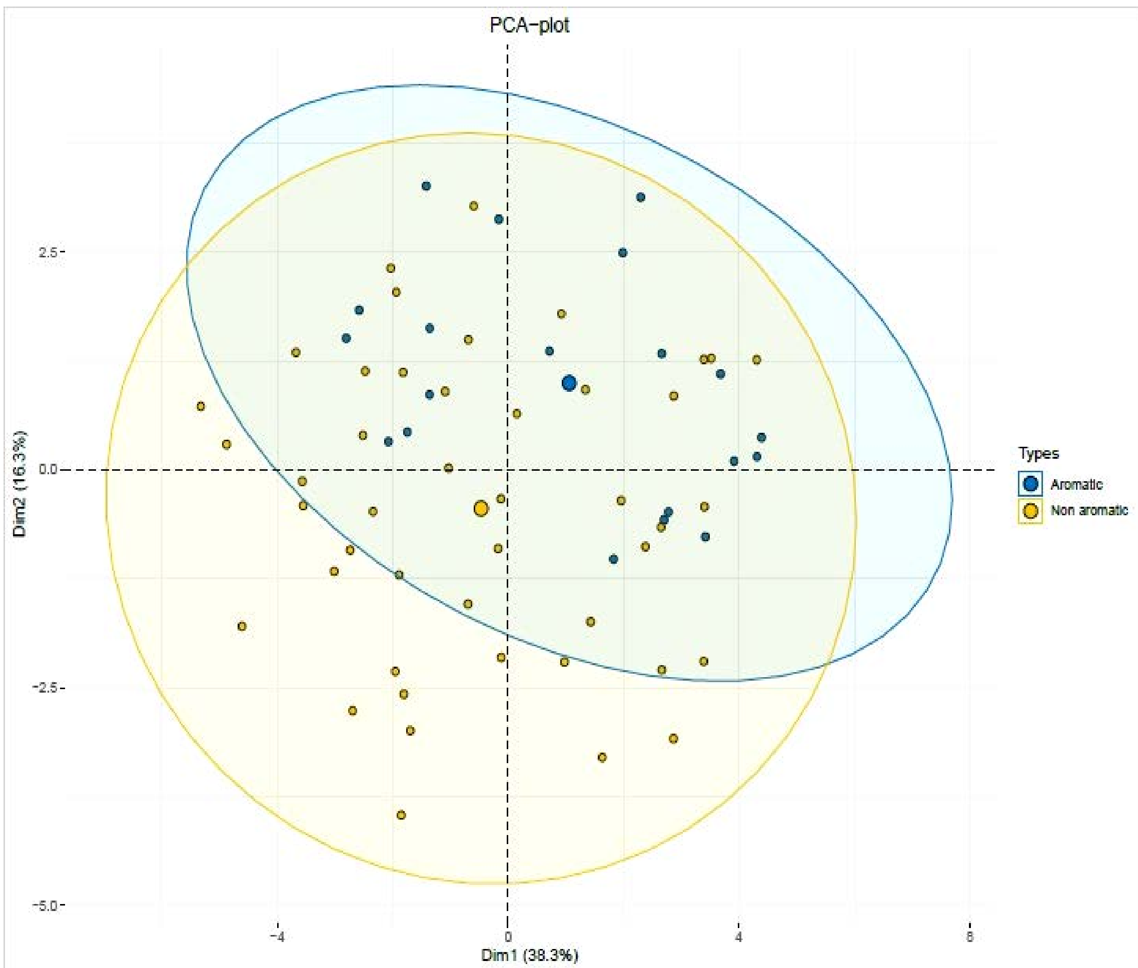


Figure 6. Scatter plot based on 14 seed and cooking quality traits from the principal component analysis (PCA). Note: blue and yellow circles represent aromatic and non-aromatic landraces, respectively.

PCA scatter biplot revealed a significant difference between the aromatic and non-aromatic rice genotypes with respect to seed traits and cooking quality attributes. The PCA scatter plot and eigenvalues of five PCs for 14 seed and cooking quality traits are provided in Table 23.

Table 23. Eigen values of the five principal components (PCs) for 14 seed-based traits

Traits	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Grain length	0.863	0.117	0.34	-0.131	-0.045
Grain breadth	-0.734	-0.124	0.513	0.157	0.148
Grain thickness	-0.515	-0.295	0.513	0.3	0.071
Grain length-breadth ratio	0.92	0.16	-0.048	-0.165	-0.1
Kernel length	0.835	0.036	0.49	0.034	-0.09
Kernel breadth	-0.696	-0.007	0.607	0.019	0.112
Kernel length-breadth ratio	0.968	0.043	0.069	0.022	-0.115
Kernel thickness	-0.589	-0.047	0.602	0.108	0.027
1000 grain weight	0.202	0.254	0.703	0-.280	0.047
Cooked rice length	-0.228	0.393	0.345	-0.753	-0.183
Cooked rice breadth	0.731	-0.126	0.023	0.314	0.586
Length/breadth ratio	-0.435	0.141	-0.41	-0.327	0.694
Elongation ratio	0.828	-0.181	0.248	0.435	0.026
Eigen values	**6.893**	**2.939**	**2.575**	**1.558**	**1.225**
% of Variance	**38.292**	**16.33**	**14.306**	**8.658**	**6.803**
Cumulative %	**38.292**	**54.622**	**68.928**	**77.586**	**84.389**

4.3. Proximate nutritional and mineral content

4.3.1. Proximate composition

The nutritional profile is generally analyzed as percentage or weight in the sample by looking at their proximate composition. This parameter is important to have an understanding of the nutritional value of food. Table 24 shows the proximate composition of 65 rice samples used in this investigation. Significant differences were found across the rice landraces studied.

4.3.1.1. Determination of Moisture content

The moisture content of the grain is an important factor in influencing the shelf life of rice and varies between 7.86 % to 14.24 % in our landraces. Maximum moisture content was observed in Manipuri and minimum in Phudungey. The overall mean was 10.88%, with significant variation observed between the landraces.

4.3.1.2. Determination of Ash content

The total mineral was estimated in terms of ash content which varied in the range of 0.67% to 4.29%, with an overall mean of 2.61%. Ash content showed the highest variation among the 65 studied landraces (CV = 39.73%). The lowest ash content was recorded in Danasay with 0.67%, followed by Rudhuwa with 0.82%. On the other hand, the highest ash content was recorded in Bhotangey with 4.29 % followed by Panbhara with 4.02%. All the other landraces showed similar values.

4.3.1.3. Determination of Fat content

Lipid content was recorded within the range of 0.63% to 3.83%. The lowest lipid contents were recorded in Jhapaka with 0.63 % followed by Chirakhey and Kalo nunia I both with 0.99%. On the other hand, the highest lipid contents were found in Bhagey tulashi with 3.83% followed by Thulo attey with 2.99%. The overall mean was found to be 1.88%. Statistically, the intraspecific variation was significant (CV=34.38).

4.3.1.4. Determination of Crude fiber content

Lipid content and dietary fiber were comparatively low and varied within the narrow range of 0.61% to 1.13%. The lowest dietary fiber was recorded in Masule with 0.61 %, followed by Rudhuwa with 0.66%. On the other hand, the highest fiber content was recorded in Venten with 1.13% followed by Basmati -I with 0.95%. The overall mean was found to be 0.82%, making it one of the smallest fractions among the major nutritional components. However, significant intraspecific variation was observed among the landraces (CV=10.45).

4.3.1.5. Determination of Protein content

Protein content determines the nutritional quality of the rice landraces. In this study, high protein content (>6%) was observed for all the tested landraces, which ranged between 6.45% to 11.28%. The lowest crude protein was recorded in Mailey attey and Jogi dhan, both having an identical protein content of 6.45% followed by Chirakhey with 6.67%. On the other hand, the highest crude protein was recorded in Doodhkatey with 11.28%, followed by Sirkey with 11.13%. The overall mean has been computed to be 8.57% (Table 24).

4.3.1.6. Determination of Carbohydrate content

Carbohydrate is the most dominant fraction among the major nutritional components of rice. The total carbohydrate content of all the rice landraces was significantly higher. The highest content was recorded in Chirakhey with 82.97%, followed by Danasay with 80.11%, and the lowest content was observed in Krishna bhog with 70.25%. The overall mean was 75.23%.

4.3.2. Mineral content

Minerals were estimated with respect to micro mineral viz. Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn).

Among the microminerals, zinc was found in a relatively high amount in the range of 1.56 mg/100 g to 2.82 mg/100 g, with a mean value of 2.10 mg/100 g. The Highest Zn content was recorded in Chini zho and Sirkey with 2.82 mg/100 g and 2.60 mg/100 g, respectively. On the other hand, the lowest value was recorded in Dorakhey with 1.56 mg/100 g. After Zn, Fe was present in the next highest amount in the range of 0.74 mg/100 g to1.29 mg/100 g with a mean value of 1.03 mg/100 g. The lowest value was recorded in Kalo nunia IV with 0.74 mg/100 g, followed by Lal bachi with 0.76 mg/100 g.

Among all the minerals, Cu was found in the lowest amount. Cu content varied within the narrow range of 0.16 mg/100 g in Basmati-II to 0.92 mg/100 g in Kalo dhan with a mean of 0.11 mg/100 g. Similarly, for Mn, the lowest value was found in Dorakhey with 1.73 mg/100 g, and the highest was recorded in Tukmor zho with 4.87 mg/100 g.

Descriptive statistics and one-way ANOVA for proximate and mineral contents in 65 rice landraces (Table 25) suggest the existence of significant variability among the studied germplasm. The highest (CV = 39.73%) and the lowest coefficient of variation (CV = 3.74%) were observed for ash content and carbohydrate content.

Table 24. Variability for proximate and mineral contents in 65 rice landraces from Sikkim

Traits	Minimum	Maximum	Average	SE	CV
Moisture	7.86	14.24	10.88	1.75	16.06
Ash	0.67	6.02	2.61	1.04	39.73
Fat	0.63	3.83	1.88	0.65	34.38
Fiber	0.61	1.13	0.82	0.09	10.45
Protein	5.45	11.28	8.57	1.49	17.40
Carbohydrate	70.25	82.97	75.23	2.81	3.74
Fe	0.74	1.29	1.03	0.15	14.78
Zn	1.56	2.82	2.10	0.27	12.65
Mn	1.73	4.87	3.26	0.90	27.50
Cu	0.16	0.92	0.59	0.23	38.89

SE, Standard error; CV, Coefficient of variation

Table 25. One-way ANOVA for proximate and mineral contents in 65 rice landraces

Traits	F	F crit	P-value	Significance
Moisture	2665.51	1.41	3E-178	***
Ash	460.86	1.41	7E-129	***
Fat	206.92	1.41	2E-106	***
Fiber	10.69	1.41	1.2E-29	***
Protein	2159.52	1.41	2E-172	***
Carbohydrate	1098.00	1.41	3E-153	***
Fe	79.67	1.41	3.5E-80	***
Zn	66.08	1.41	4.1E-75	***
Mn	47.82	1.41	1.9E-66	***
Cu	409.21	1.41	2E-125	***

*F, calculated F value; F crit, F critical value; Sig, significance; ***significant at $P < 0.001$; **significant at $P < 0.01$.*

The relationship between the ten nutritional traits among 65 rice landraces was further analyzed by correlation coefficients coupled with a two-tailed t-test to determine the strength of the correlation. Table 26 shows the correlation coefficients among the proximate values for carbohydrate, moisture, fat, protein, fiber, ash, and mineral contents.

Table 26. Correlations among the proximate and mineral composition

	Moi	**Ash**	**Fat**	**Fib**	**Prt**	**Crb**	**Fe**	**Zn**	**Mn**	**Cu**
Moi	1									
Ash	0.19	1								
Fat	0.14	-0.01	1							
Fib	-0.07	0.13	0.13	1						
Prt	0.05	-0.16	0.15	0.08	1					
Crb	-0.75**	-0.41**	-0.40**	-0.12	-0.54**	1				
Fe	-0.05	-0.05	0.06	0.12	0.07	-0.01	1			
Zn	-0.18	-0.04	0.06	-0.14	-0.16	0.21	.32*	1		
Mn	-0.13	0.08	0.07	0.14	-0.02	0.04	0.11	0.55**	1	
Cu	-0.15	-0.07	0.04	-0.04	0.02	0.10	0.16	0.50**	0.43**	1

A significant positive correlation ($p<0.05$) was observed among all the studied seed traits except between the carbohydrate and all other proximate composition parameters, implying that carbohydrate composition is highly correlated with other traits. Similarly, the relationship between Zn with Mn and Cu content was in a positive direction, which indicates that rice landraces high in Mn and Cu may likely be high in Zn value. Cu content was also significantly correlated with Mn ($r = 0.43$), though the observed values were low (Table 26).

4.4. Molecular characterization and Association analysis

4.4.1. Marker attributes

Totally 45 SSR primer pairs were used for genotyping 53 rice landraces, of which 42 (93.33%) were polymorphic, two (RM 25 and RM455) were monomorphic, and one (RM 170) failed to amplify. These 42 polymorphic SSR primers pairs were used in all the subsequent analyses.

A total of 227 alleles were detected, which ranged from 2 (in many) to 11 (RM 20), with an average of 5.262 alleles per locus. The major allele frequency (M_{AF}) was high, with a mean value of 0.679 and ranged from 0.460 to 0.930. The number of different alleles (Na) per locus varied from 1 to 9 with a mean value of 4.721, while the number of effective alleles (Ne) per locus varied from 1.210 to 6.384 with a mean of 3.344. The fact that Na > Ne indicates that only a handful of alleles contributed to the overall diversity. The expected heterozygosity (*He*) ranged from 0.175 (RM 322) to 0.842 (RM 284), with an average of 0.632. The observed heterozygosity (*Ho*) had a mean value of 0.222, and ranged from 0.00 (RM 237, RM 513, RM 178, RM 277, RM 271, RM 44) to 0.98 (RM 514).

The value of polymorphism information content (PIC), which measures the extent of polymorphism revealed by the markers based on the number of alleles per locus ranged from 0.121 (RM 322) to 0.375 (RM 44), with an average of 0.323. While the major portion of the micro satellite markers (34) was moderately polymorphic with PIC values ranging between 0.25 to 0.50, eight markers generated low polymorphism with PIC<0.25. The details of the marker attributes are provided in Table 27. The representative gel images for SSR marker RM 314 (a and a1) and RM 253 (b and b1) are provided in Plate 7.

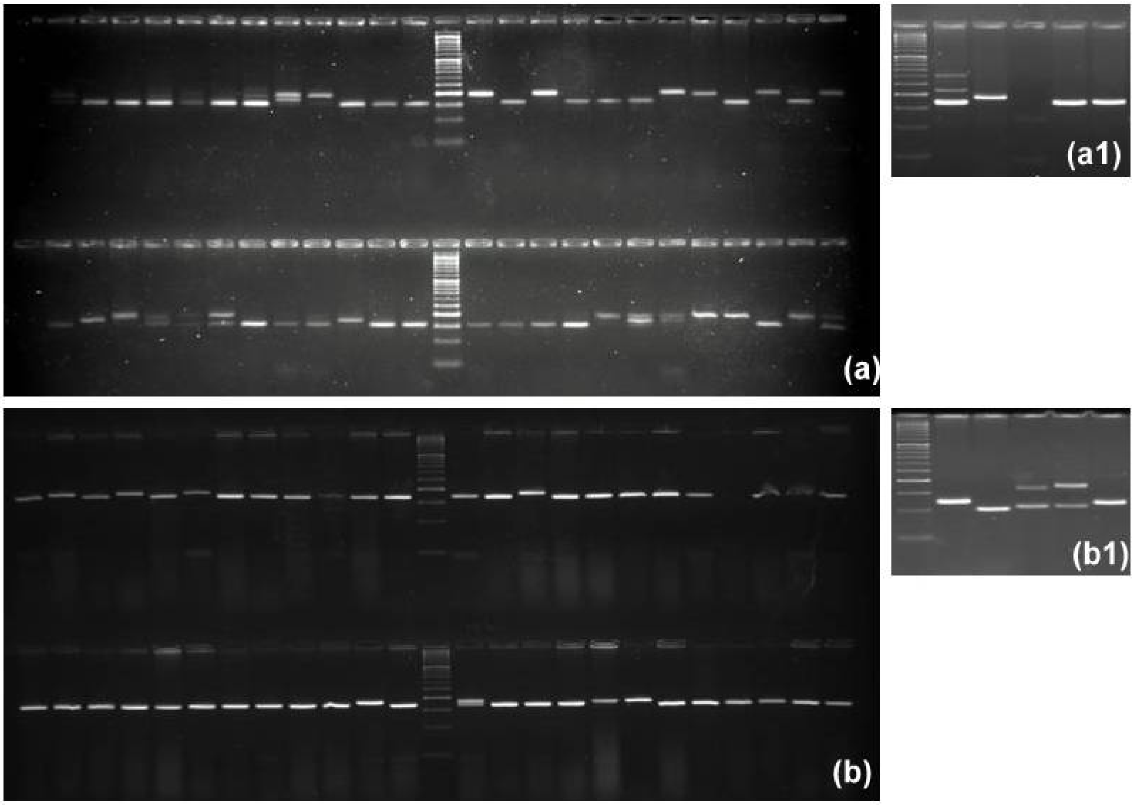


Plate 7. Representative gel images of markers RM 314 (a & a1) and RM 253 (c& c1). Both a & a1, b & b1 represent the banding pattern of 53 landraces

Table 27. Characteristics of 42 SSR markers used in genetic diversity analysis

Locus	Chr. No.	Position (cM)	No. of Alleles	M_{AF}	PIC	Na	Ne	*Ho*	*He*
RM5	1	94.9	4	0.667	0.334	3.5	2.807	0.094	0.641
RM495	1	2.8	4	0.667	0.340	3.5	3.006	0.145	0.667
RM431	1	178.3	6	0.726	0.317	6	4.994	0.336	0.799
RM237	1	115.2	3	0.625	0.357	2.5	2.115	0	0.524
RM312	1	71.6	7	0.694	0.334	6.5	4.96	0.189	0.791
RM283	1	31.4	5	0.588	0.36	4.5	2.737	0.025	0.63
RM452	2	58.4	5	0.632	0.352	4	2.786	0.188	0.641
RM6	2	154.7	5	0.711	0.315	4.5	3.783	0.058	0.732
RM322	2	49.7	3	0.93	0.121	2.5	1.216	0.085	0.175
RM489	3	29.2	3	0.63	0.355	2.5	2.038	0.192	0.507
RM338	3	108.4	7	0.745	0.307	5.5	4.276	0.091	0.761
OSR13	3	53.1	8	0.644	0.333	8	4.95	0.679	0.789
RM514	3	216.4	8	0.579	0.366	7.5	5.989	0.549	0.832

RM307	4	0	5	0.789	0.277	5	4.159	0.392	0.758
RM537	4	8.5	3	0.75	0.305	2	1.42	0.036	0.228
RM551	4	8.5	8	0.764	0.293	7	4.919	0.15	0.793
RM178	5	118.8	4	0.767	0.293	3	2.522	0	0.542
RM413	5	26.7	5	0.548	0.372	4	2.62	0.118	0.601
RM510	6	20.8	5	0.676	0.319	5	3.816	0.283	0.735
RM454	6	99.3	7	0.66	0.325	6.5	3.128	0.12	0.653
RM190	6	7.4	4	0.632	0.356	3.5	2.099	0.016	0.515
RM253	6	37	3	0.647	0.346	3	2.308	0.318	0.563
RM314	6	33.6	6	0.755	0.294	6	4.714	0.182	0.786
RM118	7	96.9	5	0.686	0.334	5	3.771	0.284	0.734
RM125	7	24.8	3	0.649	0.293	2.5	1.575	0	0.339
RM10	7	63.5	6	0.731	0.310	5.5	3.848	0.08	0.739
RM408	8	0-1.1	4	0.698	0.319	4	3.525	0.4	0.716
RM44	8	60.9	7	0.5	0.375	5.5	3.011	0.984	0.668
RM284	8	83.7	9	0.74	0.310	8.5	6.384	0.359	0.842
RM447	8	124.6	3	0.708	0.325	3	1.818	0.15	0.45
RM223	8	80.5	3	0.663	0.347	2.5	1.954	0.064	0.488
RM342	8	78.4	8	0.703	0.316	7.5	4.908	0.864	0.796
RM515	8	80.5	2	0.668	0.340	1	1.210	0	0.190
RM316	9	1.8	7	0.704	0.320	6	3.585	0.153	0.713
RM215	9	99.4	9	0.668	0.324	8	5.156	0.867	0.801
RM271	10	59.4	6	0.774	0.286	6	5.029	0.024	0.801
RM287	11	68.6	5	0.653	0.334	4	2.762	0.199	0.638
RM536	11	55.1	6	0.73	0.320	5.5	4.559	0	0.78
RM144	11	60.9	4	0.767	0.292	3.5	2.151	0.212	0.529
RM19	12	20.9	2	0.587	0.359	2	1.939	0	0.484
RM20	12	0	11	0.615	0.349	9	4.246	0.396	0.764
RM277	12	57.2	4	0.46	0.354	3.5	2.397	0.048	0.583
Mean	**-**	-	**5.262**	**0.679**	**0.323**	**4.721**	**3.344**	**0.217**	**0.632**
SE.	**-**	-	**0.332**	**0.013**	**0.006**	**0.307**	**0.21**	**0.038**	**0.029**

Chr. No., Chromosome Number; cM, centiMorgan; M_{AF}, Major Allele Frequency; PIC, Polymorphism Information content; Na, Number of Different alleles; Ne, Effective No. of Alleles; Ho, Observed heterozygosity; He, Expected heterozygosity; SE; Standard Error

4.4.2. Genetic diversity

The mean values of Nei's gene diversity (h) and Shannon's information index (I) were 0.217 and 0.354 respectively, signifying moderate genetic diversity in our collection. The mean value of total gene diversity (Ht = 0.322) further supported this. Two populations were assumed based on the presence/absence of aroma trait, of which the first population (population-1) comprised of 16 aromatic accessions, while the second population (population-2) represented 37 non-aromatic accessions. In population-1, about 97.67% of SSR markers were polymorphic, while in population-2 96.51% were polymorphic. Among the two groups, the aromatic group with I = 0.365, and h = 0.223 was slightly more diverse vis-a-vis a non-aromatic group with I = 0.343 and h = 0.209. The summary of genetic diversity is presented in Table 28.

Table 28. Summary of genetic diversity statistics of 53 rice landraces

	Na	**Ne**	***H***	***I***	***Ho***	***He***	**%P**
Population 1	5.047	3.473	0.223	0.365	0.209	0.648	97.67%
Population 2	4.395	3.215	0.209	0.343	0.225	0.616	96.51%
Mean	**4.721**	**3.344**	**0.217**	**0.354**	**0.217**	**0.632**	**97.09%**
SE.	**0.326**	**0.129**	**0.137**	**0.186**	**0.008**	**0.016**	**0.580**

Na: Total Number of Alleles per locus, Ne: Number of Effective Alleles per locus, I: Shannon's Information Index, Ho: Observed Heterozygosity, He: Expected Heterozygosity, SE: Standard Error.

The UPGMA dendrogram resolved 53 landraces, with significant bootstrap support, into two major clusters (Figure 7) - CL-I, and CL-II with a single outlier accession. Of these, CL-I included 48 accessions belonging to both aromatic and non-aromatic groups and was further divided into two smaller groups- sub-cluster-IA (SCL-IA) and sub-cluster-IB (SCL-IB). Interestingly, all the aromatic accessions such as Raja bara, Ram jeera, Ram bhog, Rudhua, Shyam jeera, Krishna bhog, Kalo dhan, Kalo nunia-I, and Kalo nunia-II, Kaanchi, Dharnali, and Birinful grouped within SCL-IB with a few non-aromatic rice accessions, while SCL-IA was entirely composed of non-aromatic rice accessions (Table 29).

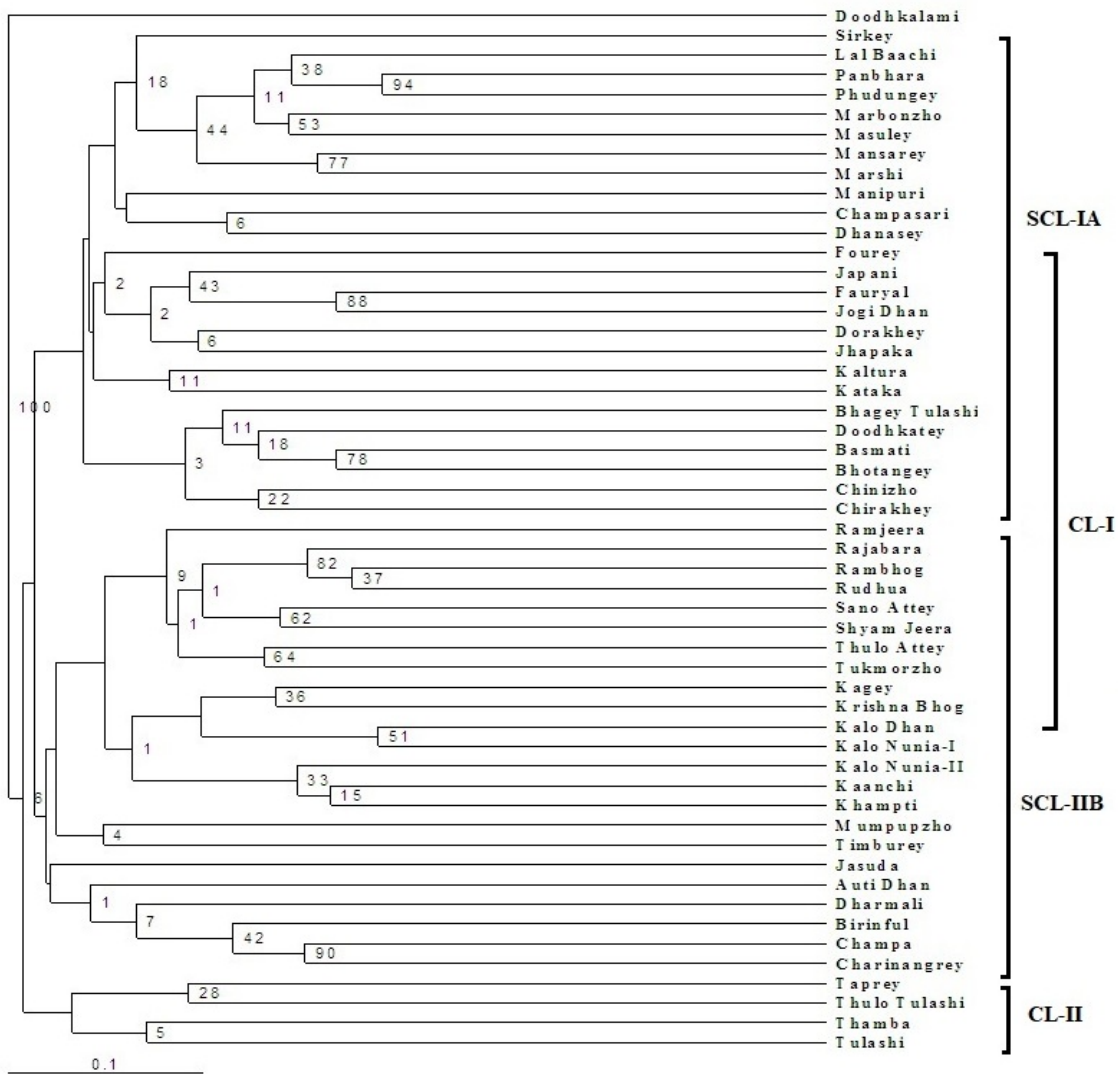


Figure 7. UPGMA dendrogram depicting the relationship among 53 rice landraces of Sikkim. The number at the node of each branch indicates the bootstrap value.

The CL-II encompassed only four non-aromatic accessions, namely Taprey, Thamba, Thulo tulashi, and Tulashi. Doodh kalami was an outlier in the UPGMA cluster. Overall, the grouping didn't fully correspond to either the geographical origin or aroma trait except for the noticeable influence of aroma in the grouping of SCL-IB accessions. A similar dispersion was observed in the PCoA (Figure 8) plot, where the first two axes accounted for 14.82% and 8.86% of the variation, respectively.

Table 29. Grouping of accessions in UPGMA clusters

Cluster	Sub-Cluster	No. of accessions	Name of the landraces
I	IA	25	Sirkey, Lalbaachi, Panbhara Phudungey, Marbonzho, Masuley, Mansarey, Marshi, Manipuri, Champasari, Dhanasey, Fourey, Fauryal, Japani, Jogi dhan, Dorakhey, Jhapaka, Kaltura, Kataka, Bhagey tulashi, Doodh katey, Basmati, Bhotangey, Chinizho, Chirakhey
	IB	23	**Aromatic:**Raja bara, Ram jeera, Ram bhog, Rudhua, Shyam jeera, Krishna bhog, Kalo dhan, Kalo nunia-I and II, Kaanchi, Dharmali, and Birinful **Non-aromatic:** Sano Attey, Thulo Attey, Tukmorzho, Kagey, Timburey, Jasuda, Auti dhan, Dharmali, Champa, Khampti, Mumpupzho, Charinanagrey
II		4	Taprey, Thulo tulashi, Thamba, Tulashi

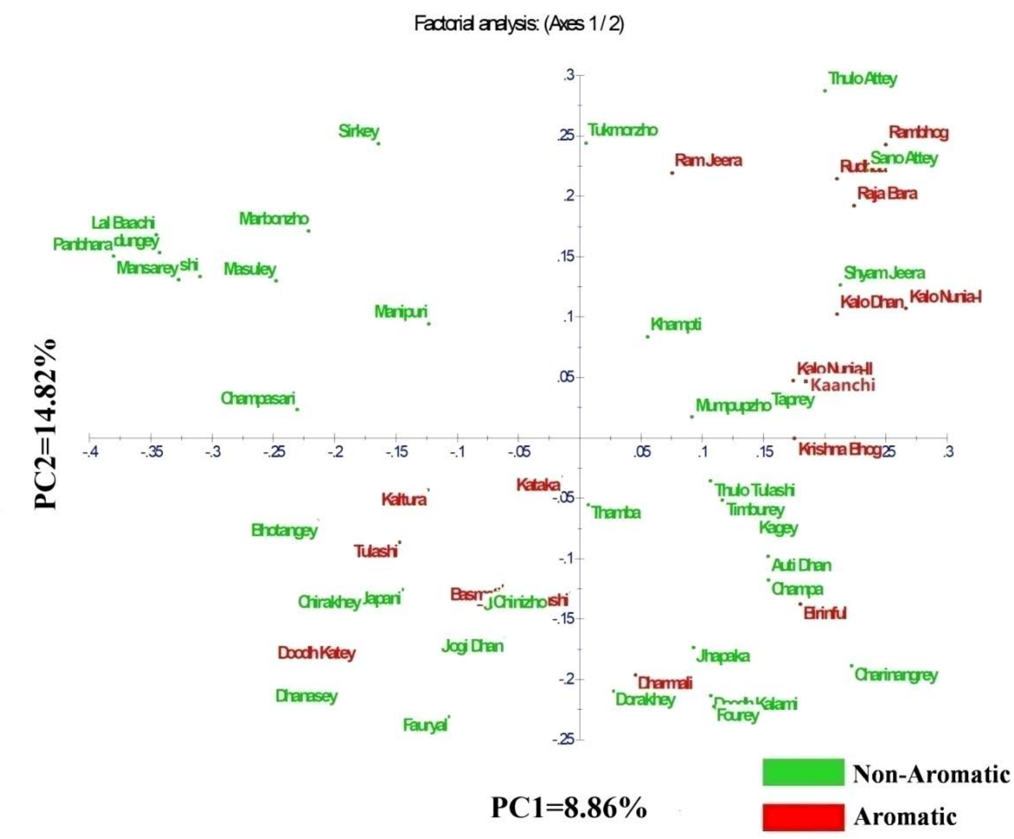


Figure 8. Principal Coordinate Analysis (PCoA) of tested germplasm

4.4.3. Population structure

The population structure analysis using STRUCTURE software revealed K=3 as the highest log-likelihood value signifying three genetic groups within our landrace collection (Figure 9). These were named RC-I, RC-II, and RC-III, respectively. Based on the Q-value (membership proportions), accessions were further designated as pure (Q-value ≥ 80%) and admixtures (Q-value < 80%), which revealed 39 pure and 14 admixtures in our collection. Out of the 39 pure accessions, 19 were grouped with RC-I with five aromatic accessions, 13 aligned in RC-II with nine aromatic accessions, and RC-III included seven non-aromatic accessions only. The analysis of molecular variance (AMOVA) showed that 99% of the molecular variance was contributed by within-population differences, while only 1% was due to variation between the populations. The results of AMOVA displayed highly significant genetic differences (P<0.001) among the individuals and within the individuals.

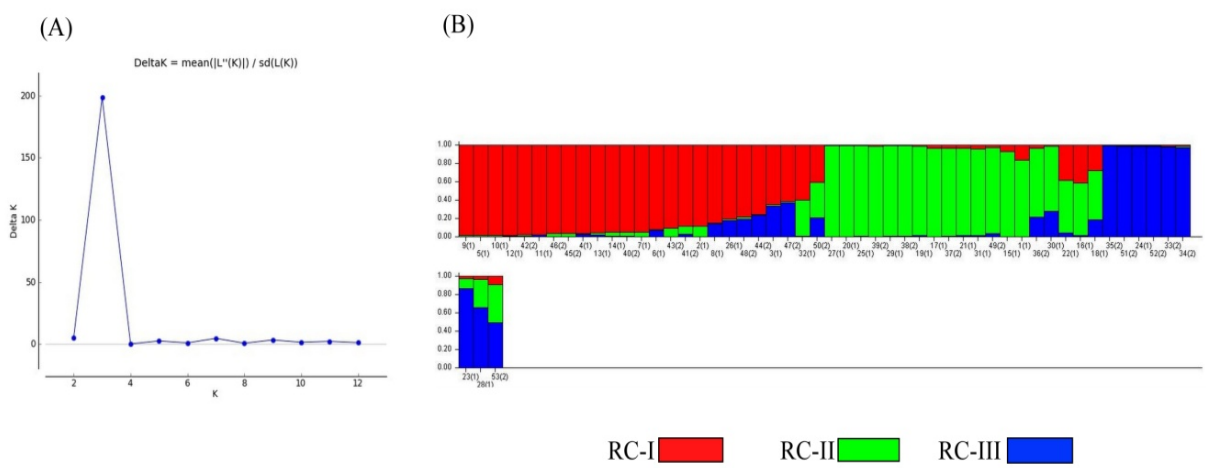


Figure 9. Model-based population structure analysis of 53 rice landraces (A) ΔK showing the presence of 3 hypothetical subpopulations (B) Population structure for 53 rice landraces of Sikkim sorted by kinship (Q) matrix at K = 3.

4.4.4. Marker-Trait Association

The marker-trait association (MTA) analysis for the two trait groups- biochemical and yield-related- was carried out using 42 polymorphic SSR markers across 53 rice accessions. Counting both GLM (62) and MLM (53), a total of 115 significant MTAs were observed ($P < 0.05$) for 21 traits which were contributed by 71% (30) of the SSR markers. In total, 25 MTAs for 17 traits were identical in both the models (Table 30), and the remaining 5 MTAs were detected in either of them.

In the case of GLM, a total of 62 significant MTAs ($P < 0.05$, $R^2 \geq 10\%$) were found, which ranged from a minimum of 1 to a maximum of 7. The minimum number of MTAs was detected for 1000-grain weight, kernel width after cooking, crude fat, and amylose content, while the maximum number of MTAs was reported for grain thickness. The correlation value (R^2) for GLM ranged from 10% to 26%. The highest R^2 value (26%) was between carbohydrate content and marker RM 528. In the case of MLM, 53 significant ($P < 0.05$, $R^2 \geq 10\%$) MTAs were detected. The minimum number of MTA (1) was detected for grain width, kernel width after cooking, kernel length after cooking, number of effective tillers, stem thickness, and amylose value. The maximum number of MTAs (4) were detected for 100-grain weight, number of panicles per plant, flag leaf width, carbohydrate content, and crude fiber content. The correlation value (R^2) for MLM ranged from a minimum of 10% in several MTAs to a maximum of 22%. The highest R^2 value (22%) was found between the kernel length and marker RM 489. In addition, the following seven markers were associated with two or more than two traits: RM 5 (associated with grain width and kernel width); RM 408 (associated with grain length and crude fiber); OSR 13 (associated with grain thickness and effective tillers per plant); RM 551 (associated with grain thickness and no. of panicle per plant); RM 10 (associated with grain thickness and kernel thickness); RM 489 (associated with 1000-grain weight and kernel length); RM 447 (associated with the number of panicles per plant and carbohydrate content). Further, the markers RM 447, RM 6, RM 408, and RM 118 were found to be associated with carbohydrate, crude protein, crude fiber, and crude fat contents.

Table 30. Marker-Trait Associations obtained from the General Linear Model (GLM) and the Mixed Linear Model (MLM).

Trait			GLM		MLM	
	Marker	**Chr. No.**	**p-value**	**R^2**	**p-Value**	**R^2**
Grain length	RM 5	1	0.001	0.19	0.015	0.12
Grain width	RM 408	8	0.014	0.11	0.012	0.14
Grain thickness	OSR 13	3	0.023	0.11	0.018	0.11
	RM 551	4	0.020	0.11	0.024	0.10
	RM 10	7	0.008	0.14	0.013	0.13
1000 grain weight	RM 489	3	0.006	0.15	0.029	0.11
Kernel breadth	RM 5	1	0.001	0.18	0.009	0.14
	RM 215	9	0.010	0.12	0.025	0.11
Kernal length	RM 489	3	0.009	0.14	0.004	0.22
	RM 44	8	0.005	0.20	0.016	0.16
Kernel thickness	RM 283	1	0.012	0.13	0.014	0.13
	RM 10	7	0.005	0.15	0.009	0.15
Kernel length after cooking	RM 452	2	0.001	0.17	0.017	0.12
Number of effective tillers	OSR 13	3	0.024	0.11	0.028	0.10
Number of panicles per plant	RM 551	4	0.015	0.11	0.021	0.11
	RM 447	8	0.002	0.17	0.024	0.10
	RM 515	8	0.001	0.18	0.022	0.11
Flag leaf length	RM 271	10	0.017	0.10	0.017	0.12
Flag leaf width	OSR 13	3	0.015	0.11	0.008	0.15
Plant height	RM 514	3	0.001	0.18	0.010	0.14
Protein	RM 6	2	0.010	0.12	0.006	0.16
Carbohydrate	RM 447	8	0.015	0.10	0.021	0.11
	RM 223	8	0.006	0.14	0.021	0.13
Crude fat	RM 118	7	0.020	0.10	0.021	0.11
Crude fiber	RM 408	8	0.009	0.13	0.008	0.16

Similarly, markers OSR13, RM 551, and RM 10 were associated with grain thickness, and RM 489, RM 215, and RM 44 were associated with kernel length only. Likewise, RM 551, RM 447, and RM 515 were associated with the number of panicles per plant.

CHAPTER 5

5. DISCUSSION

5.1. Cataloging and documentation of Indigenous traditional knowledge

Rice has great antiquity in the Sikkim-Himalayan region as evident from the etymological significance of its old name "Denzong", meaning- "*the valley of rice*". Rice farming has been an inspiring enterprise here as it has evolved in response to the dynamic needs of a multi-ethnic society of diverse food habits, limited land holding, and inhabiting difficult topographies of a harsh climate. Their enduring creative labour, for centuries, has led to the selection of an array of landraces alongside evolving treasured Indigenous Traditional knowledge (ITKs) on cultivation and storage (Rahman and Karuppaiyan 2011). However, of late, like other Himalayan states, rice production in Sikkim is witnessing a steady shift from the traditional to modern agroecosystem. Consequently, landraces and their ITKs are being slowly repalced by the high-yielding varieties and new technologies (FS&AD 2018-19). It has been cautioned in many earlier reports that in the absence of appropriate scientific intervention, indigenous rice landraces and their farming technologies in the region might become history with the time (Zuberi 1997; Kambewa et al.1997). In view of this, the present thesis work was undertaken to document ITKs and characterize the rice landraces of the Sikkim-Himalayan region, in addition to gaining preliminary insights into their use in marker-assisted selection.

5.1.1. Documentation and collection of indigenous rice landraces

During the field trips, we documented and collected 74 rice landraces from the four districts of Sikkim. This included 26 reported earlier by Kapoor et al. (2019). After initial screening, nine duplicate entries were deleted, and 65 were retained for further study. Besides, we documented 35 indigenous technical knowledge practised by the Sikkimese farmers for ages.

For our survey, we deliberately chose elderly farmers as respondents. The reason was that they were the ones who authentically used indigenous technologies. From our interactions, this conjecture was found valid, and young farmers showed a greater inclination towards improved technologies, if not downplayed, the older ones.

Our interaction with farmers threw several insights. For instance, many respondents confirmed that almost all the landraces grown in the region are late maturing, low yielding and prone to lodging during grain filling and maturity stage. It was found that Krishna bhog and Attey (both Sano and Thulo) are the local favourites due to their excellent cooking quality, taste, and palatability, in addition to disease resistance. These findings are consistent with the reports of Sharma et al. (2015). Krishna bhog is premium rice highly sought after for its pleasant aroma and is used in the preparation of several traditional dishes like kheer, pulao, and dhakaney (prepared with milk and roasted rice) - popular in the region- in addition to its use in the ceremonial offerings. Besides, Attey is another very popular landrace and occupies the largest cultivation area in the state. There are two variants of Attey known viz. Thulo attey and Kanchi (Sano) attey, of which the Thulo attey is widely used. Its taste and palatability are considered superior compared to Sano Attey. Regarding the agronomic performance, Attey varieties are known to perform better than High yielding varieties (HYV) even under the low nutrient application (Rahman and Karuppaiyan 2011). The farmer's preference for this variety is also due to the non-formation of chaffy grain and higher biomass (Kapoor et al. 2019).

Among the upland rice, Tukmorzho with pink starch has been cultivated for ages by the Lepcha community of Dzongu in North Sikkim. The germplasm has been conserved through generations and is the most preferred rice in all the rituals of this primitive indigenous community. Pink starch is believed to have nutraceutical value and is good for consumption by women during and after the pregnancy. It is supposed to heal anaemia and has good iron content. Kalo nunia, or small Basmati, is another distinct landrace and is the only aromatic rice among the short, bold grain types with black-coloured husk. It has a delicate aroma, taste, and texture. The cost of production is high because seeds pure lines are not available easily, and the farmers are, therefore, reluctant to spare. This variety typically grows in tropical areas having temperatures >20ºC**.** Birinful is another aromatic rice with highly conspicuous awns, which is famous for its unique aroma, good cooking qualities, and excellent palatability. During germplasm collection, we observed that farmers deliberately mix the seeds of more than one landrace. Understandably, this practice has helped to avoid monoculture and preserve biological diversity while also acting as a buffer against the effects of biotic and abiotic stress.

We also documented and characterized seven lesser-known landraces of upland rice (Kalo tukmar, Seto tukmar, Tukmorzho, Mumpupzho, Marbonzho, Chinizho, and Kalo dharmali), which were scarcely available for research untill now due to their limited access. Their cultivation practices and consumption have been largely restricted to selected indigenous communities. We were informed that these landraces could be grown on less fertile soil and water availability and are also known to show resistance against abiotic stresses, particularly cold. Despite being poor yielders, they appear to offer great diversity in their natural habitat. Such unique genetic resources are likely to play a significant role in future rice improvement programs.

Finally, in our structured interview, we tried to learn the reasons for the depletion of rice landraces in the region reported but not investigated in earlier studies (Kapoor et al. 2019). From the responses gathered, we could infer the following:

Typical of any hill agriculture, rice cultivation in Sikkim is a labour-intensive operation. Shortage of manpower is the principal cause of its decline in recent years. Agricultural labourers have diverted to better employment opportunities leaving large stretches of rice fields uncultivated. Lack of irrigation sources due to drying water streams (Jhoras) has added to the vows. This has led farmers to shift to improved varieties, gradually replacing landraces, resulting in the loss of valuable genetic resources. Many respondents shared that they are raising the traditional varieties only as a continuation of the ancestral practice.

Further, due to small landholdings, many marginal farmers have diverted their rice fields to cultivate remunerative cash crops, mainly vegetables (Sharma and Rai 2012) like beans, cole crops, and seasonal and local vegetables which fetch them premium prices in the market. Similar observations have been recorded earlier by Awasthe et al. (2017). This reality closely resembles the situation in other Asian rice-cultivating countries which are witnessing the drifting of their young generation from farming, especially traditional rice farming (Krishnankutty et al. 2021), either to modern agriculture or migrating to bigger cities for better employment. In the light of these findings, it is cautioned that many indigenous rice landraces and their farming technologies in Sikkim might become endangered/disappear with time as they are passed on through the tradition. Such concerns are also raised by the researchers of other northeast states and West Bengal (Zuberi 1997; Kambewa et al.1997; Anonymous 1989 and Anonymous 2000, Das et al. 2013; Deb 1995 and Das and Ahmed 1995), indicating the phenomenon is pervasive. Thus, our study affirms the need for continuing dialogue between the extension, research and policy systems to sensitize the next generations on preserving the biodiversity of this heritage crop of the region.

5.1.2. Documentation of the Indigenous Traditional Knowledge (ITKs)

With regards to ITKs, we documented 35 of them practised by the Sikkimese farmers. The maximum number of ITKs was recorded for plant protection against diseases (10 nos.), and two ITKs were noted in water management. They are discussed here with the possible rationale. Emphasis is given for the scientific explanation, either evinced from the subject matter specialists or derived from the relevant literature wherever possible.

Firstly, for sowing in the next season, the farmers collect seeds through the first two thrashings using the manual method. A similar practice is in use among the Boro indigenous people of the Kamrup district of Assam. Talukdar et al. (2012) opined that the rationale behind this could be to recover healthy and filled grains with high vigour after eliminating the diseased and partially filled grains.

In Sikkim, the planting of rice seedlings is strictly carried out alongside the festival of "*Aasar Pandra*" or "*Ropain*". This seems quite appropriate as the period depicts the optimal time for sowing of the rice seedlings. It has considerable bearing on the panicle initiation time during October, which requires a clear and sunny sky, thereby avoiding the cold and dry months of November or rainy months of mid-August to September (*Bhadau* as per the Hindu calendar). Otherwise, exposure to low temperatures and high humidity generally attracts moulds and cause heavy damage by insects and pests. Similar observations of selecting an optimal time for transplanting rice seedlings have been documented by Singh and Sureja (2011) on the rainfed agroecosystem of Madhya Pradesh.

Likewise, as in all other rain-dependent agriculture, rainfall pattern*s* or jharis as they are called here is another important feature in rice cultivation. A total of nine such jharis have been categorized (Sharma et al. 2016). They are Titey jhari, Naurethey jhari, Sisney jhari, Bhadaurey jhari, Sauney jahri, Makurey jhari, Bhangeri jhari, Kartikey jhari and Sohrasaradey jhari. We found that every jharis has its own significance. Particularly in rice cultivation, Naurethey and Bhadaurey jhari are of utmost importance. Naurathey jhari showers during September and October before the *Dusshera* festival. During this time, heading initiates in the paddy plant, requiring plenty of water. It is believed that this rain will determine the productivity for the year. Similarly, Bhadaurey jhari occurs during the month of *Bhadau* (15 July-15 August), immediately after the paddy is transplanted in the main field. During this time, the perennial and seasonal spring water sources need to swell up owing to the continuous rainfall to provide enough water to the rice field.

To protect their harvest, the ITKs used in Sikkim are based on locally available plants and plant materials such as leaves, twigs, seeds, rhizomes etc. The plants generally used for this purpose are– neem patti (*Azadirachta indica* A. Juss.), hardi [*Curcuma longa* (L.)], adhuwa (*Zingiber officinale* Roscoe), bhojo (*Acorus calamus* L.*)*, bakainu leaf (*Melia azedarach* L.), and kadi patta [*Murraya koenigii* (L.) Spreng.], sajana (*Moringa* *olifera*) and tulsi (*Ocimum* sp.). All these plants have been previously shown to have pest and insect repelling properties. It has been demonstrated that the drumsticks tree's leaves and barks prevent the stem borer's infestation due to pesticidal properties (Ojiako et al. 2013; Nathan 2006). Leaves of neem, bakainu, and curry leaf, are well known for their insecticidal properties and are used extensively for preventing damage by storage pests (Carpinella et al. 2007; Faraga et al. 2011). These ITKs are corroborated by other validation studies reported earlier (Gopi et al. 2016; Chhetry and Belbahri 2009; Talukdar et al. 2012).

Further, animals such as dead frogs/toads/crabs and other products like wood ash and fresh cow dung are used to control pests and diseases. This may be reasoned by the fact that pests and bugs are attracted and crowded around the dead matter, thus saving valuable soft grains. Similar analogies have been drawn by Deb and Chakraborty (1981), Gogoi and Majumdar (2001) in the case of traditional agricultural pest management practices followed in West Bengal, Arunachal Pradesh and Assam.

We also documented several traditional storage structures, viz. *Bhakari*, *Kotha/Kothe* and *Dhikutis* used by the farmers for the protection from storage pests, insects, rodents etc. They are usually plastered with cow dung and mud paste mixed with leaves of neem patti (*Azadirachta indica* A. Juss.), bhojo (*Acorus calamus* L.), bakainu leaf (*Melia azedarach* L.). It is implicit that the anti-microbial properties of these sealing agents will protect against invading microbes and prevent seeds from being damaged by the pest and insects. Lamichaney et al. (2019) have confirmed the scientific utilities of these indigenous grain storage structures in their study on the Lepcha and Limboo communities of Sikkim.

Thus from the foregoing discussion, it can be inferred that the indigenous traditional knowledge associated with rice cultivation and storage in Sikkim has a considerable scientific basis. They are undeniably less dangerous to the people and the environment and are affordable, biodegradable, environmentally friendly, and act through a variety of processes in a more targeted manner (Souto et al. 2021). Thus it is time to popularize them among farmers as a sustainable alternative to chemical-based insecticides and pesticides.

5.2. Phenotypic diversity based on grain and kernel traits

Physical parameters and cooking quality traits play a very important role in determining the commercial value of the rice. Consumers base their preference on the appearance, size and shape of the grain besides behavior upon cooking, taste, tenderness and flavour. However, these preferences vary among the consumer group and regions and even within the same region. For instance, in the Middle East, consumers prefer long-grain, well-milled rice with aroma, while the European community generally prefers long-grain rice with no scent (Traore 2005). Even within India, people in the Southern parts of the country prefer medium/short slender types of grains (Viratmath et al. 2010), while in northeastern states, including Sikkim, people prefer short, bold and medium slender grains (Kapoor et al. 2019). The constituents that vary among rice's key cooking and eating quality are amylose content, gelatinization temperature, and gel consistency (Traore 2005). Hence we have investigated these parameters in the rice landraces of Sikkim as part of this work.

5.2.1. Morphometric characterization

We observed four types of grain lengths in our study: one very long type (>10.5 mm), 15 long type (9-10.5 mm), 26 medium type (7.5-9 mm) and 23 short type (<7.5 mm). Their variability was highly significant ($p<0.001$). Among the 65 genotypes, Doodhkatey recorded the highest seed length (10.63 mm). The trait showed a positive correlation (>0.70) with the Grain L/B ratio, Kernel L/B ratio, Cooked rice length and Elongation index at 0.01 level. Similar observations in morphological as well as agronomical variability of grain characters have been recorded by Patra and Dhua (2003) in upland rice germplasm of the Jaypore tract of Orissa and some important aromatic rice varieties of India (Meena et al. 2010).

We also classified them based on their breadth as narrow (21), medium (26), broad (15) and very broad (3). Vanangamudi et al. (1987) and Belsnio (1980) had earlier used grain length and width to group them into usable categories. Rickman et al. (2006) reported that size and shape being the stable varietal properties, could be important parameters for identifying a variety. The variability observed for grain traits in our study is also consistent with the earlier observations in some aromatic Indian rice varieties (Meena et al. 2010) and Sikkim rice landraces (Kapoor et al. 2019)

Further, by using the ratio of length and breadth, grains of our landraces were categorized as bold (4), medium (26) and slender (35). Nascimento et al. (2011) used this categorization in ascribing agro-morphological characters of the upland rice varieties. Correlation between grain breadth and kernel breadth showed a significantly positive relationship (0.86**) at 0.01 level. This result conforms to the earlier studies on rice landraces of Meghalaya (Longvah and Prasad 2020). Good phenotypic diversity in our study recorded may be due to the diverse geographic location of the collection with varying climatic and edaphic conditions (Roy et al. 2010).

In terms of test weight, Panbhara and Manipuri recorded the highest values (29.70 g and 29.43 g), the lowest test weight was recorded in Ramjeera (15.73 g) and Tulashi (18.40 g). Landraces with long grain lengths showed higher thousand weight values; these were Champa, Dhood kalami, Japani, Lal bachi, Manipuri, Panbhara and Pountain. Test weight is one of the most variable traits among the rice landraces, as reported by Devi (2000). Values of thousand grains weighing 20 to 30 g are regarded as excellent, whereas values less than 20 g may indicate the presence of immature, damaged, or empty grains (Adu-Kwarteng et al. 2003). Low thousand-grain weight may also be due to the low moisture content, which increases the damage to grains during the milling (Rather et al. 2016). Mahadevappa and Nandisha (1987) opine that heavier seeds produce vigorous seedlings and result in high grain yields. Thus our results indicate promising variability for test weight in the landraces of Sikkim.

5.2.2. Organoleptic qualities of cooked rice (Cooking quality)

Cooking quality is a critical factor in determining the consumer's preference. Cooking quality is a complex trait influenced by several physico-chemical properties of grain (Juliano 1979; Tomar and Nanda 1982; Hussain et al. 1987). Additionally, amylose content, gelatinization temperature, and gel consistency also affect the texture of the cooked rice. These traits are known to vary between the varieties (Juliano 1971).

Linear elongation after cooking is one of the most important properties of quality rice. When cooked, grains of certain varieties expand more than others. Length-wise expansion without width increase is regarded as highly desirable (Sood and Sadiq 1979). Contrary to this, the breadth-wise increase is considered an undesirable trait (Danbana et al. 2011). The trait is determined by the L/B ratio as well as the amylose concentration (Singh et al. 2005; Danbana et al. 2011). In our study, "Doodh katey" produced the highest value for grain elongation after cooking and thus may be regarded as high-quality rice. The other landraces with a higher elongation ratio included Kalo nunia-I (1.99 mm), followed by Kalo nunia-II and Sano tulashi, with a similar value of 1.86 mm. That said, grain elongation in cooking also depends on genetic factors and the degree of milling.

5.2.3. Biochemical properties

Another major cooking quality trait, the gelatinization temperature, ranged from low - high in our study. Most of the landraces (49) exhibited low gelatinization temperatures, while 14 recorded intermediate alkali digestion and gelatinization temperatures. This trait is important as gelatinization temperature is directly linked to cooking time. According to Bhattacharya and Sowbhagya (1971), the higher the gelatinization temperature, the longer it takes to cook rice. Bhattacharya et al. (1980) also assessed the Indian rice landraces for quality traits, gelatinization temperature, grain type and protein content and reported moderately high gelatinization temperature for the Indian rice varieties (72-74^{0}C).

Alkali spreading value is also one of the important traits, and its score in the range of 4-5 indicates intermediate alkali digestion. Among the rice landraces we investigated, 75% showed high alkali scores, and 22% were found to have intermediate scores. Only 3%

showed low alkali scores, which were recorded in only two landraces, Phudungey and Tukmorzho. Therefore, these could be used as parental lines to improve the lower alkali content. Medium grain disintegration could be considered a highly desirable property for quality rice (Bansal et al. 2006; Bhonsle 2010). Our results showed an alkali spreading value from 3.0 to 7.0. A similar observation of alkali digestion within the range of intermediate to high alkali scores were recorded by Rathi et al. (2002) in 100 traditional upland/*ahu* rice cultivars of Assam.

The amylose content of the cooked rice determines its texture, and rice grains with >25% amylose content absorbs more water and has a fluffy texture after cooking (Frei and Becker 2003). Short and bold grains with intermediate amylose content, which become fluffy on cooking, are mostly preferred in Sikkim (Kapoor et al. 2019). In our study, Amylose content ranged from 13.85 to 28.25%. Almost 41% of the tested rice showed intermediate Amylose content (<20-25%), and about 53% showed low AC content (12-20%). Among the landraces, Phudungey and Mumpupzho showed the highest percentage of amylose, while Chewrey dhan and Jogi dhan recorded the least value. This means Phudungey and Mumpupzho can be recommended for diabetic patients since starchy foods with high amylose levels are associated with lower blood glucose and slower emptying of the human gastrointestinal tract compared to those with high levels of this macromolecule (Frei and Becker 2003). This finding corroborates with the earlier reports on amylose content in the landraces of Himachal Pradesh (Singh et al. 1998), medicinal rice cultivars in Manipur (Thongbam et al. 2010) and *sali* rice of Assam (Pathak et al. 2017).

Fragrance in rice is due to specific compounds that give the rice a refreshing pop-corn-like aroma. These are usually volatile compounds released during the cooking process. In our study, almost 70% of the collected germplasm was non-aromatic, while the remaining 18% was strong, and 12% was mildly aromatic. Strongly scented rice landraces are Krishna bhog, Birinful, Kalo nunia (I, II, & III), Kalo dhan, Kataka, and Bhagey tulashi.

Our study also recommends the reorganization in the list of scented rice reported from Sikkim. For instance, accessions like Kaltura, Kataka, Tulasi and Timburey, which were reported as non-aromatic types by the previous study (Kapoor et al. 2019), were determined to be aromatic in our analysis. Likewise, landraces like Lal bacchi, Taprey, Khamti and Charinangrey, which are reported as aromatic, have been determined to be non-aromatic.

Nonetheless, the scented rice genotypes identified in our study may form a new addition to the list of potential aromatic rice landraces that can be used in the breeding programs.

5.3. Proximate nutritional and mineral composition

The nutritional value of rice depends on the nature of de-husking, which affects the nutritional parameters except for the carbohydrate content. When rice is de-husked by milling, which is the standard practice for improving the commercial value, there is a substantial loss of most of the nutrients. This is because, during milling, the outer membranous layer called the pericarp is lost, which contains a major proportion of mineral, lipid and crude fiber (Tontisirin et al. 2002). Loss of pericarp results in the loss of protein (6% to 29.1%), lipid (84.6% to 86.5%), crude fiber (33.3% to 81.8%) and ash content (24% to 70.5%), while loss of carbohydrate is only marginal in the range of 4.7 to

6.5% (Grist 1975). In the case of manually de-husked grain, the pericarp remains almost intact, and so all the nutritional components remain intact. However, grain called "whole grain" is normally meant for domestic consumption by farmers. Therefore, for academic purposes, particularly for germplasm evaluation in terms of nutritional value, the values obtained for de-husked rice represent the actual characteristics of a particular cultivar or variety. Therefore, we have used the de-husked seeds in all our proximate analyses.

The protein content is considered an important index for determining the nutritional quality of the rice, and its content ≥ 10% is considered above average (Resurrection et al. 1979). In our study, the highest protein content was observed in Doodh katey (11.28%). Besides, as many as 11 other landraces showed protein content >10%. The result demonstrates the superiority of the rice germplasm of Sikkim for this trait vis-à-vis some of the landraces of Assam, where protein content was in the range of 7.20% to 10.86% with a mean of 9.12% (Dutta et al. 2018) and those reported from 33 Manipuri rice varieties (7.5%) (Longvah et al. 2021). However, Baruah et al. (2006), working with ten indigenous deep water paddy cultivars of Assam, all of which are coarse grain type, found a mean protein value of 11.78%, with three landraces showing >13% protein. Subsequent works by Loying (2010) involving 10 deep water paddy of Assam found protein in the range of 9.63% to 13.22 % with a mean of 11.06%. Our results are in conformity with these findings in addition to that of Prasad et al. (2021), who reported 6.95 to 12.46 % protein content in 60 locally cultivated rice landraces of Tamilnadu. Caution, however, is warranted here as the protein content of any paddy is variable up to 7% due to environmental effects (Juliano et al. 1964; Cagampang et al.1973).

In terms of moisture content, all rice landraces revealed an acceptable level of moisture content (<12%) for long-term storage without damage from pests or affected by microbial activities (Nielsen 2002). The differences in moisture content among the rice landraces (7.86-12.24%) might be due to the variations caused due to factors after the harvesting (Asaduzzaman et al. 2013). The values are similar to many earlier reports on rice (Sompong et al. 2011; Oko et al. 2012).

It is reported that in rice, carbohydrate exhibits maximum variation in the range of 77.0% to 86.0% (Grist 1984). In the present study, we observed considerable variability in the total carbohydrate content, ranging from 70.25% to 82.97%, with a mean of 75.23%. All the genotypes exhibited a fairly higher amount of carbohydrates, which are somewhat similar and/or a little above the desired range of 80% (Juliano 1985). The results are in conformity with the earlier findings on Joha rice landraces of Assam (Roy et al. 2010), Chokuwa landraces (semi-glutinous) (Dutta et al. 2018), deep water paddy cultivars of Assam (Baruah et al. 2006; Loying 2010) and Manipuri rice landraces (Longvah et al. 2021).

Rice grain contains a low but uniform amount of lipids, mostly in the pericarp. Its dietary importance is well recognized. It is a source of essential fatty acids and phospholipids. In the present study, the lipid content varied within the range of 0.63% to 3.83%. The highest lipid content was found in Baghey tulashi (3.83%), and the lowest was recorded in Jhapaka with 0.63%. Bhagey tulashi is known for its delicious taste and texture. It is known that high lipid content contributes to good taste and delicacy (Gopalan et al. 1989).

Our findings for crude fat content are similar to that of rice cultivars of northeast India, having values ranging from 1.2 to 4.2%. On the other hand, a lower content of fat (0.27–0.30%) was recorded in different non-glutinous Thai rice varieties by Thumrongchote et al. (2012). Earlier works by Juliano et al. (1964) revealed that in *indica* type of rice, lipid content varied from 2.33% to 3.13%, which drastically reduced to 0.30% to 0.55% upon milling; the corresponding values for *japonica* rice were 3.05% to 3.58% for de-husked rice and 0.44 % to 0.54% for milled rice. Pathak et al. (2017), working with some indigenous pigmented hill rice of Assam, reported lipid content in the range of 4.37% to 5.27%. Baruah et al. (2006) reported a relatively higher lipid level in the range of 2.42% to 4.64% in deep water landraces of Assam. This was corroborated by Roy et al. (2010) for scented rice landraces of Assam, the range being 2.03% to 3.73% with a mean value of 2.49%. The findings from our study indicate that the landraces of Sikkim have medium-level lipids except for one or two landraces where the content is higher.

Crude fiber is not a nutritional component in the true sense of the term since it is not digested and assimilated by the body unlike protein, carbohydrate etc. However, its importance in human nutrition is well known (Gopalan, 1989). Indian Council of Medical Research (ICMR) recommends a daily intake of 25 - 40 g of crude fiber, which underlines its importance.

In the present study, the crude fiber content varied from 0.61% to 1.13% with a mean of 0.82%, which is higher than the values reported by Baruah et al. (2006) and Loying (2010) for deep water paddy, which ranged from 0.36% to 0.53% and 0.5 to 0.9% respectively. Rabha (2011), working with the indigenous coarse and fine grain landraces of Assam, reported that the mean value for the coarse type was 1.42%, while for fine grain, it was 1.26%. Dutta et al. (2010), working with Joha rice, found crude fiber in the range of 0.33% to 0.75%. However, another group of fine-grain paddy, Chokuwa, contained a relatively high amount of fiber in the range of 0.92% to 1.40%, with a mean of 1.17% (Dutta et al. 2018). Compared to these findings, the rice landraces of Sikkim contain a rich amount of fiber.

Ash content in grain, although typically low, is critical to metabolism due to the involvement of different minerals. Like other food components, a considerable variation exists between de-husked and milled rice. Juliano et al. (1965) reported that, in de-husked rice, ash content varied from 1.52 to 2.13%, which comes down to 0.52 to 0.74% for milled rice. In the present study, total minerals in the form of ash content varied from 0.67% to 4.29%, with a mean value of 2.61%. The value recorded is much higher than the values reported by Baruah et al. (2006) and Loying et al. (2010) for indigenous deep water paddy, which is in the range of 0.71 to 1.07% and 1.13% to 2.0 %. The value obtained in the current study is much higher than the Joha rice (Dutta et al. 2010) and Chokhuwa rice (Dutta et al. 2018), which were from 1.00 % to 1.66% and 0.90% to 1.43%. The higher ash content recorded in Sikkim rice compared to other northeast states may be attributed to the differences in the mineral content of the soil, fertilizer inputs and the water used for irrigation (Shayo et al. 2006). On the application side, the landraces like Bhotangey and Panbhara could be a valuable germplasm for breeding the mineral content in rice as they exhibit highest minerals in terms of ash content.

Since rice is the staple food for >90% of Sikkim's population, it is important to assess how much mineral it can provide. In our study, Fe content varied from 0.74 mg/100 g in Kalo nunia IV to 1.29 mg /100g in Tukmorzho, with a mean of 1.03 mg/100 g. The highest Fe content was recorded in Tukmorzho (1.03 mg/100g). Natives believe that Tukmorzho has high nutraceutical value and therefore is good for consumption by women during and after pregnancy to heal anaemia. However, the average Fe (1.03 mg/100g) content recorded in our landraces was lower than that recommended for the dietary intake of a healthy individual (1-1.5 mg/100g) (Mahender et al. 2016). This might be due to the poor soil properties, as farming in Sikkim is done with low input and without any external applications for soil amendments (Kapoor et al. 2019).

The highest manganese content was found in Phudungey (4.87 mg/100g), and the lowest was found in Dorakhey (1.73 mg/100g). These findings are similar to the earlier report of Anjum et al. (2007). Similarly, the highest copper content was found in Kalo dhan (0.92 mg/100g), and the lowest was found in Basmati-II (0.16 mg/100g). The average Zn (2.10 mg/100g) content was higher than the recommended threshold level of 1.2 mg/100g and could be a dietary source for people suffering from Zn deficiency (Institute of Medicine (US) Panel on Micronutrients 2001). However, the average Zn content (2.10 mg/100g) in the present study is slightly lower than the Zn content of 130 Indian rice landraces (2.25 mg/100g) reported by Deb et al. (2015), Meghalaya landraces 2.26 mg/100g and Arunachal Pradesh landraces 2.55 mg/100g reported by Longvah et al. (2020). This might be due to the variation in mineral, nutrient availability and pH of the soil, which are highly variable between the regions (Zimmermann and Hurrell 2002).

5.4. Molecular characterization and Association analysis

Information related to diversity and population structure plays an important role in determining the pre-breeding strategies for any crop. In addition, identifying favourable markers/alleles for yield, tolerance to high soil acidity, and lodging resistance via. Association mapping can provide fast and efficient means to strategize marker-assisted breeding programs. Hence, one of the objectives of this study was to determine the genetic diversity and relationship among the rice landraces of Sikkim. In addition, we have explored the prospects of association mapping through an extended experiment.

In our genetic diversity estimation, the number of alleles ranged from 2 to 11, with a mean value of 5.27 per locus. This suggests superior allelic diversity harboured in our germplasms compared to aromatic rice varieties of Assam (3.7; Talukdar et al. 2017), rice varieties released in India during 1940-2013 (3.11; Singh et al. 2016), as well as elite rice varieties of Bangladesh (4.18; Rahman et al. 2012) and Malaysia (4.09; Aljumaili et al. 2018); but lower as compared with eastern Himalayan landraces (Choudhury et al. 2013, 7.9- Das et al. 2013 and 8.49 - Roy et al. 2016). The variability in the number of alleles/locus observed in pan-Himalayan studies, including ours, reflects the diverse nature of the rice germplasm of this region. Besides, the number of effective alleles per locus ranged from 1.21 to 6.84, with a mean of 3.34 - a value close to (3.77) as reported by Chen et al. (2017), indicating a good number of alleles contributed to the genetic diversity. The major allele frequency across all the 42 SSR primers ranged from 0.46 (RM 277) to 0.93 (RM 322) with an average of 0.67 (Table 27), which is higher than the previous studies on Indian (0.53; Upadhyay et al. 2012) and Korean landraces (0.5; Li et al. 2014). The greater number of alleles indicates the usefulness of the selected SSR markers for genetic studies.

The PIC value is an excellent indicator of the marker's relevance for the linkage analysis. (Elston 2005). Often, PIC values reflect allelic diversity among the varieties (Meti et al. 2013). Our study showed a moderate PIC value with a mean of 0.32 (range: 0.121 to 0.375) for 53 accessions. A similar PIC value (0.37) was reported earlier by Choudhury et al. (2013) in rice landraces of northeast states of India. Saha et al. (2013) and Pachauri et al. (2013) also found mean PIC values of 0.37 and 0.38 in their study on different Indian rice varieties. However, higher PIC values up to 0.65 and 0.74 have also been reported in other studies from the eastern Himalayan region (Das et al. 2013 and Roy et al. 2016). This may be due to specific variability held by these landraces. Further, as PIC and inbreeding coefficient (FIS) are the functions of how heterozygosity is partitioned within and among the accessions based on differences in allele frequencies (Mulualem et al. 2018), it can be inferred that a large majority of the loci targeted by our markers are homozygous.

Our landraces revealed a gene diversity estimate of 0.217, which is much lower than 0.51 reported by Umakanth et al. (2017) for rice landraces of northeast India. The mean observed heterozygosity was 0.217, which is analogous to other studies on Indian rice varieties (Choudhury et al. 2014; Nachimuthu et al. 2015). Shannon's information index (I) ranged from 0.343 to 0.365, with an average of 0.354. This is lower than the findings of Aljumaili et al. (2018) and Suvi et al. (2019), who reported an index of 0.88 and 0.82, respectively. The moderate value of Shannon's information index is another indication of the moderate genetic diversity among the on-hand germplasm. Further, the inbreeding coefficient (FIS) represents the average deviation of the population's genotypic proportions from the Hardy-Weinberg equilibrium for a locus. The FIS values in our study revealed that only three of the 42 markers (RM 215, OSR13, and RM 514) showed higher heterozygotes (-0.081, -0.085, and -0.27).

In our UPGMA analysis, landraces from the same geographic locations or sharing similar traits such as aroma did not correspond to specific groups indicating the nominal influence of these attributes on the genetic relationship. Similar results have been observed for 67 hill rice accessions of northeast India (Roy et al. 2016) as well as 48 traditional aromatic rice landraces from eastern India (Meti et al. 2013). According to Mulualem et al. (2018), this may be due to their common descent. Conversely, high gene flow aided by cross-pollination might have also caused this (Musyoki 2015). However, the accretion of a significant number of aromatic accessions in a particular sub-group of UPGMA calls for further investigation into the role of aroma in shaping the phylogenetic relationships in rice landraces.

Population structure analysis using STRUCTURE revealed the highest ΔK value at $K=3$, indicating 53 rice accessions represented three sub-populations (Figure 9). They revealed 39 pure and 14 admixtures. The AMOVA showed significant genetic differences ($p \leq 0.001$) among and within individuals and lower variation among the population groups. Of the total genetic variation, 70% was due to variation among the individuals. A similar trend was reported even in earlier studies (Anandan et al. 2016; Roy et al. 2016; Islam et al. 2018). Such variability generates wide crosses and creates the desirable heterotic group in the base breeding population (Alam et al. 2015). On the other hand, the contribution of variation among the population groups was 1% indicating only a small collection within a given source captured the genetic diversity present in the test accessions. Further, the low mean value of Fst (0.011) and Gst (0.028) signify poor genetic differentiation (Wright 1978), and the allele frequencies between population groups are likely to be similar. This was further supported by a very high estimate of gene flow (Nm: 3.96). Put together, these results point to a close evolutionary lineage of the rice accessions of the Sikkim Himalayas or extensive out-crossing between the accessions (Nuijten et al. 2009). It is plausible that the exchange of rice accessions between the farmers may have also contributed to gene flow across different landraces.

In our association analysis, majority of the associations detected in the MLM were also supported by the GLM approach, with 25 MTAs for 17 traits being common in both (Table 30). In earlier works on the association analysis, Zhou et al. (2012) reported a total of 16 marker-trait associations ($P<0.01$) in the *japonica* rice varieties collected from China. Similarly, Anandan et al. (2016) reported 16 marker-trait associations in 629 rice accessions using 39 SSR markers for the identification of genes associated with early seedling vigour. Furthermore, Patil et al. (2014) found 13 significant SSR markers associated with the 18 agronomic traits in 58 rice accessions of Chhattisgarh, India.

Among the MTAs revealed, markers RM 489 and RM 408 were significantly associated with QTL controlling the grain length (GL) and grain width (GW). This is consistent with the findings of Huggins et al. (2019), who reported a strong association of RM 489 (chromosome 3) with grain length in brown rice and grain width in rough rice of USDA National small grains collections. Similarly, RM 6, RM 215, and RM 44, located on chromosomes 2, 9, and 8, were significantly associated with the protein content, kernel breadth (KB), and kernel length (KL). A related earlier study by Septiningsih et al.

(2003) revealed RM 6 and RM 215 to have associated with the grains per plant (GPP) and panicle length (PL). These parallels between our results and similar studies in rice from across the world suggest the reliability of our MTAs for further testing in the relevant breeding programs. For instance, markers RM 271 and OSR 13 mapped on chromosomes 10 and 3 are significantly associated with flag leaf length (FLL) and flag leaf width (FLW) in our study. An earlier work reporting QTLs for leaf width (Anandan et al. 2016) also established their involvement in leaf-related traits. Thus, it is worthwhile examining whether they could be used to improve leaf traits in Thulo attey, a popular rice landrace of Sikkim. Similarly, the other marker-trait associations could be explored as potential leads for testing in the relevant breeding programs.

CHAPTER 6

6. SUMMARY & CONCLUSION

Rice is a staple food for about 800 million people in India. It has played a significant role in the country's diet, economy, employment, culture and history. Rice accessions from India's northeastern states are thought to be a treasure trove of agronomically important genes. An estimated 10,000 such cultivars/landraces of ecological and cultural significance are conserved here.

The mountainous state of Sikkim, predominantly dominated by the indigenous communities, is known for a wide variety of rice landraces, with almost all wet arable lands uniquely filled with them. This study thus aimed to close the knowledge gap on Sikkim rice landraces by documenting the existing landraces, their ITKs and carrying out detailed nutritional and molecular characterization.

The outcome is summarized below:

6.1. Cataloging and documentation of rice landraces and Indigenous Traditional Knowledge

Our study found that Sikkim has a rich stock of rice landraces which needs to be characterized for novel genetic resources and genes for breeding programs. However, urbanization, unplanned developmental projects, and the introduction of improved varieties are rendering their rapid extinction. Landraces such as Botangey, Dharmali, Tukmar, Chinizho, Mumpupzho, Marbonzho, Taichung, Kalo dhan, Tukmorzho, Japani, Marshi, Sirkey, and Birinful are rarely found now and are cultivated at single locations, only by a few farmers, and are facing acute genetic drift.

Further, ITKs on agriculture practices have strong roots in Sikkim. This study documented 35 ITKs related to paddy cultivation. With further work on their utility and value addition, these technologies may serve as potential alternatives to modern agricultural tools. If the policy makers initiate steps to promote them among the farming community, it will help in bridging the gap between the two knowledge systems and help in restoring the economy and already fragile ecology of this beautiful Himalayan state.

6.2. Phenotypic diversity based on grain and kernel traits

In rice, consumers base their preference on grain appearance, size and shape, cooking qualities, taste, tenderness and flavour of the cooked rice. Phenotyping of seed traits from 65 rice cultivars revealed considerable variation in seed traits, cooking qualities and biochemical characteristics. Almost all the seed-based and cooking quality traits were positively correlated ($p < 0.05$), suggesting their utility in the breeding programs. The cultivars like Doodhkatey and Panbhara recorded the highest seed length (10.63 mm) and test weight (29.70 g), offering excellent materials for improvement. Almost 45% of the tested rice was found to possess intermediate Amylose content (20-25%), which indicates that a significant portion of the tested cultivars are of good quality and useful for diabetic patients. Among the cultivars studied, 55% showed intermediate alkali digestion and intermediate gelatinization temperature and 40% were found to have high GT, and only 5% had low GT. The cultivars with medium disintegration of grains could be highly desirable for quality rice. Almost 70% of the cultivars were non-aromatic, while the remaining 18% were strong, and 12% were mildly aromatic. Strongly scented cultivars were Krishna bhog, Birinful, Kalo nunia (I, II, & III), Kalo dhan, Kataka, Bhagey tulashi etc. These scented genotypes are suggested for use in breeding aromatic rice varieties.

Overall the excellent phenotypic diversity recorded may be due to the diverse geographic location of the collection with varying climatic and edaphic conditions. Therefore, these genetic resources could be used as parental lines in breeding programs.

6.3. Proximate nutritional and mineral composition

The results from our study revealed a good nutrient profile for the majority of the 65 rice cultivars of Sikkim. No significant difference was observed in the composition of aromatic and non-aromatic genotypes with respect to major nutrients. All the cultivars were found to possess moisture content within the acceptable limit for long-term storage. The highest lipid content of Baghey tulashi (3.83%) reasons well with its delicious taste. The highest protein content was found in Doodhkatey (11.28%), which is more than the desired range (7%-8%). During the study, 11 landraces showed protein content above 10%. The total carbohydrate content of all the landraces was higher than the desired values and ranged from 70.25% to 82.97%. The crude fiber content varied from 0.61% to 1.13%, with a mean of 0.82%. In comparison to the findings from other regions, the rice landraces of Sikkim revealed rich fiber content.

The average Zn (2.10 mg/100 g) recorded in this study was higher than the recommended threshold level of 1.2 mg/100 g and could be a dietary source for people suffering from Zn deficiency. The average Fe (1.03 mg/100 g) recorded was lower than the recommended dietary intake for humans (1-1.5 mg/100 g). The therapeutic properties of Tukmorzho in healing pregnant women before and after childbirth may be related to its high iron concentration.

Overall, the comprehensive nutrient profiling of the rice landraces of Sikkim, presented here, offers significant scope for their insertion among the promising rice genetic resources for the breeding program.

6.4. Molecular characterization and Association analysis

Genetic diversity analysis revealed moderate diversity, low divergence, and high gene flow among the rice landraces of Sikkim Himalaya. The deployed SSR markers, which produced a moderate PIC value (PIC between 0.121 to 0.375), showed considerable reliability for utility in the genetic studies. The high estimate of gene flow (Nm: 3.96) across different cultivars indicated close evolutionary lineage or extensive out-crossing among the rice accessions of the Sikkim Himalayas.

Further, the UPGMA tree, PCoA plot, and Bayesian-based STRUCTURE revealed that the landraces from the same geographical area or aromatic/non-aromatic properties did not correspond to the specific grouping, implying that these characteristics have only a minimal impact on the genetic link. However, the accretion of a significant number of aromatic accessions in a particular sub-group of UPGMA calls for further investigation into the role of aroma in shaping the phylogenetic relationships in rice landraces.

In terms of Association mapping, moderate diversity, low divergence, and high gene flow among the landraces indicated significant prospects for their empanelment. We could establish that the majority of the associations detected in the MLM were also supported by the GLM, with 25 MTAs for 17 traits being common. Some of the associated markers were located in previously discovered Qualitative Trait Loci in rice, lending credibility to our findings. Thus, the leads from our AM studies offer significant scope for its extension involving a large number of genotypes from the region.

CHAPTER 7

7. FUTURE PROSPECTS

Improving the rice productivity through the introduction of HYV in place of existing popular landraces/cultivars is proving counter productive in the sensitive mountain ecosystems of Sikkim. Alternatively, upgrading the popular local varieties by Marker Assisted Backcross Breeding (MAB) offers a parallel strategy for boosting the productivity. This, alongside promoting and popularizing the time-tested ITKs, can prove synergetic and extremely rewarding.

In this background, we have attempted to list important ITKs associated with rice cultivation from the region, in addition to providing an early insight into their nutritional and molecular attributes, including the scope for association analysis.

Based on the leads obtained, future work must focus on the following:

- Validation, technology intervention, promotion and popularization of the documented ITKs.
- Trait-specific grouping of germplasm for use in breeding programs.
- Trait-specific characterization for identifying rare genes and alleles for the genetic improvement programs.
- Analyze the stability and utility of the marker-trait associations under additional environments through multi-location trials.
- Developing MTAs for other important traits relevant to the region, particularly tolerance to cold and low phosphorous conditions.

CHAPTER 8

8. BIBLIOGRAPHY

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CHAPTER 8

9. PUBLICATIONS

Plant Genetic Resources: Characterization and Utilization

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Research Article

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Genetic diversity in rice (*Oryza sativa* L.) landraces of Sikkim-Himalaya and early insight into their use in genome-wide association analysis

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Abstract

The Sikkim-Himalaya represents one of the unique reservoirs of rice genetic resources in India owing to the presence of a large number of landraces adapted to extreme climatic and edaphic conditions. This valuable gene pool is now under threat due to the introduction of high-yielding varieties, wanting suitable genetic intervention for enhancing their productivity, and thus economic viability. Development of lodging resistant, high-yielding varieties tolerant to soil acidity through association mapping aided marker-assisted breeding programmes can help achieve this in a fast and efficient manner. But this requires information on genetic diversity, population structure, etc., of the germplasm collection, which is strikingly lacking. We, therefore, characterized a set of 53 rice landraces from Sikkim to address the above issues. The results revealed moderate diversity, poor divergence and high gene flow in our germplasm collection attesting its utility as an association mapping panel. Further, a total of 115 putative marker-trait associations ($P < 0.05$, $R^2 \geq 10\%$) were obtained using the general linear model and mixed linear models of which 25 were identical in both. Some of the associated markers were positioned in those regions where Qualitative Trait Loci have been previously identified in rice, providing credence to our results. The resources generated from this study will benefit the rice breeders from this region and elsewhere for targeting the yield and related traits, in addition to conservation efforts by the interested researchers.

Introduction

India is home to a large number of rice landraces (Pachauri *et al.*, 2013) with all the major rice-growing states having their preferred assemblage (Singh and Singh, 2003). Adaptation to wide-ranging edaphic and climatic factors has shaped >120,000 of them with extensive phenotypic variations (Khus, 1997). Rice accessions from northeastern (NE) states of India are regarded as the storehouse of agronomically important genes (Choudhury *et al.*, 2013; Das *et al.*, 2013) as they have endured extreme conditions of this mountainous terrain. It is estimated that about 10,000 such cultivars/landraces of ecological and cultural significance are preserved here (Hore, 2005; Das *et al.*, 2013) wanting in-depth evaluation on their offerings.

Sikkim has considerable antiquity in rice cultivation (Rahman and Karuppaiyan, 2011). Rice is grown here at an elevation ranging from foothills to 1700 m above sea level, and from sub-tropical to temperate climate. It is the second-largest crop after maize occupying 15% (10,327 ha) of the area under agriculture (Portel, 2015). As the state receives plenty of rainfall (3250 mm/annum), well distributed over 6 months from May to October, rice farming has emerged as the major source of livelihood for the majority of the indigenous communities inhabiting this pristine land. Landraces, which are grown in a traditional way in the hill slopes with and without terracing, dominate the rice production here with a 55–60% share in acreage (Rahman and Karuppaiyan, 2011). There are 70 plus such landraces reported so far (Sharma *et al.*, 2016) which await detailed characterization. However, barring a few scattered works (Rahman and Karuppaiyan, 2011; Choudhury *et al.*, 2013; Das *et al.*, 2013; Sharma *et al.*, 2016), not much has been done in this direction.

The majority of these landraces suffer from poor yield, lodging and late maturity although they boast other superior traits such as hardiness, pest tolerance/resistance, low input requirements and weed coexistence (Sharma *et al.*, 2015). In addition, the soils in Sikkim are highly acidic, leached and generally poor in fertility and water holding capacity (Das and Avasthe, 2016). Even though high-yielding varieties (HYV) are available (https://icar-nrri.in/released-varieties), their introduction in place of existing landraces/cultivars is perceived as detrimental

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Traditional rice landraces and Indigenous knowledge associated with the rice cultivation in Sikkim Himalaya: An analysis based on local farming practices.

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ABSTRACT

This paper reports collection of rice landraces and documentation of traditional knowledge associated with rice cultivation in Sikkim Himalayan region. The methodology employed was- field visits accompanied with survey using purposive-cum-random sampling method, particularly of the elderly farmers. A total of thirty seven farmers from twelve selected villages of the four districts of Sikkim state were interviewed randomly as respondents. As many as seventy four different rice landraces were collected as part of this research. The maximum number of Indigenous Traditional Knowledges ITK was documented under the plant protection against various diseases (10 nos.) and only two ITK was recorded in water management. The rationale on the use of indigenous knowledge, presented here, is derived purely based on the free opinion of the respondents. The results suggest that the indigenous knowledge in rice farming in Sikkim Himalayas is built upon the natural features of the region. The innovation/techniques are based on locally available resources and are rooted in their historical experiences which are passed on through generations. The information on ITK presented here could provide vital clues on the suitable alternatives to expensive modern farming system.

1. Introduction

Sikkim has a glorious history of agriculture that has been practiced from generations where people and nature lived in perfect harmony. As is the case elsewhere, the system graduated from hunting and gathering to shifting cultivation to settled agriculture (Subba, 2009). In Sikkim, the cropping pattern over the years has been transferred from cereal dominated subsistence agriculture to high value, cash crop dominated commercial agriculture. Since the state is a completely hilly area, the agriculture is mostly rain-fed and rice is transplanted (Singh, 2011). The system of farming here was time tested, deeply interwoven with ecological system and climatic conditions, and founded on sustainable agricultural practices. However, with the emergence of modern agricultural methods, the indigenous farming practices used for centuries have been sidelined. Traditional varieties have been replaced with high yielding ones. These crops and varieties have attracted new pests and diseases. Soil health is on deterioration and, microorganisms have declined. With an idea of transforming Sikkim into an organic state, the Government of Sikkim made a historic declaration to transform Sikkim into a totally organic State in the year 2013. The decision of the Government of Sikkim to go organic was based on the fact that farming in this hilly state was traditionally organic.

Rice is the most important staple cereal crop of Sikkim grown at an elevation ranging from foothills to 1700 meters above sea level (m asl). Paddy constitutes the second-largest area of about 15 percent (10,327 ha) of the total cultivated area of the state. Its average annual production is around 10,271 tonnes. The average yield per hectare is 694 kg, which is low compared to all India average of 1073 kg (Portel, 2015). The principal rice-growing districts in the order of importance are East (33.07%), West (14.31%), South (10.13%) and North (4.42%) (State Profile of Sikkim, 2011). The share of rice as the main food item in the total food production has been

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