

Basudha Data Paper No. 9

**An Evaluation of Biochemical Characteristics of Rice
(*Oryza sativa* L.) Landraces from Northeastern India**

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Kolkata

2025

Introduction

Rice (*Oryza sativa* L.) is a major staple for half of the population worldwide. Different morphological and agronomic characteristics enable an easy and quick discrimination between varietal phenotypes. These characteristics are heritable, can be easily seen by the eye and are equally expressed in all environments. Assessment of landrace populations based on the durability, uniformity and stability (DUS) of these traits is essential to distinguish different landraces. Evaluation of these characteristics can be carried out by any farmer or researcher without any formal institutional training and technical support. A compilation of these DUS characters would be of great value to the farmers to ascertain the genetic purity and stability of the cultivars. Deb's (2005) pioneering compilation of 36 morphological and agronomic characteristics of 417 indica rice landraces is an example.

Although a few of these biochemical characterization can be carried out by ordinary farmers, proper characterisation would require the use of standardised reagents such as phenol, KOH and thymol blue, and therefore are ideally to be performed in a laboratory. These biochemical descriptors are useful for the management and maintenance of germplasm collection and to all users of rice genetic resources. We present here the first compilation of 5 biochemical descriptors of 142 rice landraces from northeastern India.

Materials

We examined a total of 142 rice varieties from Basudha's accession from four states of north east India, namely, Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim and Tripura (Fig. 1 and Table 1).

Table 1: The number of Rice Landraces from Seven North East Indian States Selected for Chemical Tests.

| States | Number of varieties |
|-------------------|---------------------|
| Arunachal Pradesh | 15 |
| Assam | 66 |
| Manipur | 2 |
| Meghalaya | 12 |
| Nagaland | 42 |
| Sikkim | 1 |
| Tripura | 4 |
| Total | 142 |

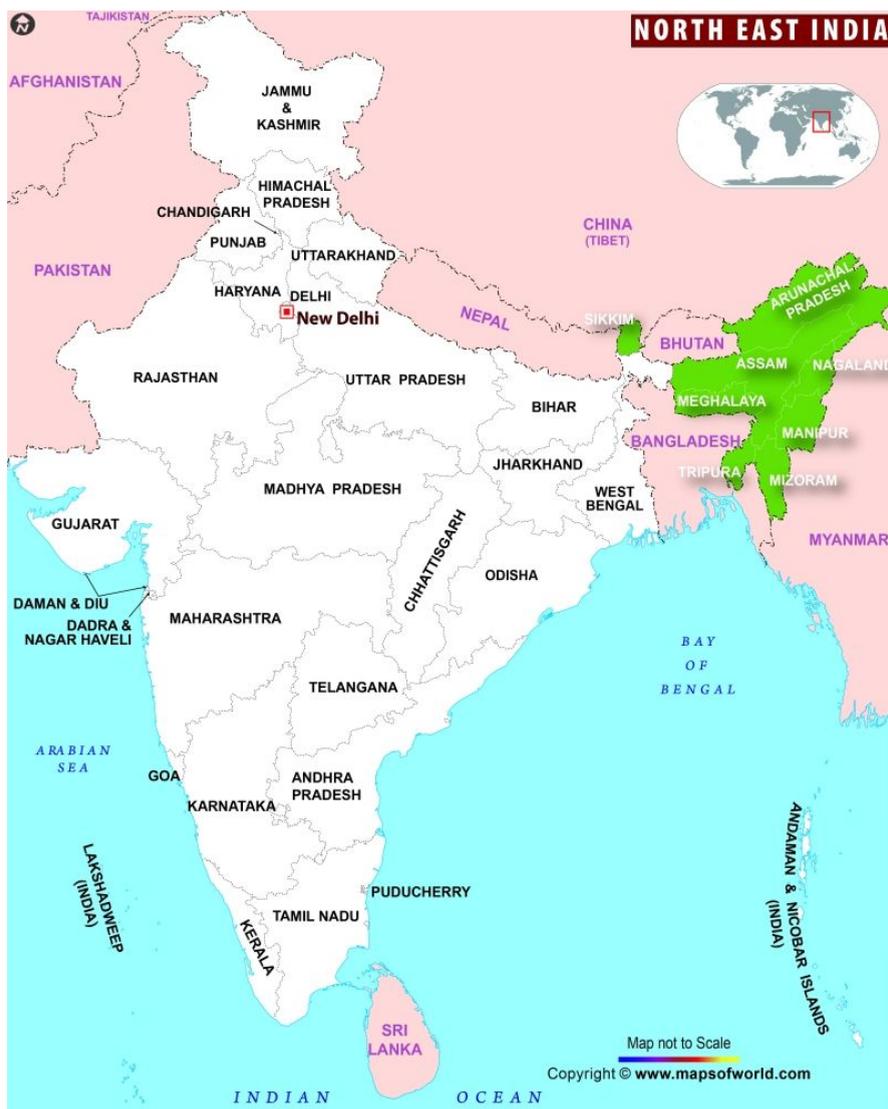


Fig. 1: Map Showing the States of Origin of the Rice Accessions from Northeastern India (in green).

Methods

For biochemical characterization of the selected rice landraces, we evaluated the following descriptors: endosperm type, based on amylose content; aroma, based on the presence of 2-acetyl 1-pyrroline (2AP); alkali digestion; gel consistency, based on the distance travelled by rice starch gel; and phenol reaction of the rice hull.

(a) *Endosperm type*

By visual observation, two types of endosperms of polished rice kernels are distinguishable. In glutinous rice, which does not have amylose, the endosperm appears a waxy white. In non-glutinous rice, which contains

amylose, the endosperm appears cloudy and translucent (Bioversity International, IRRI & WARDA 2007). The standard classification is given in **Table 2**.

Table 2: Visual Classification of Endosperm Type.

| Visual Description | Endosperm type |
|--------------------|----------------|
| Translucent | Non-glutinous |
| Cloudy | Intermediate |
| Waxy | Glutinous |

(b) *Aroma*

Basmati-like aroma in fragrant rice landraces is detected when the rice kernel contains 2-acetyl 1-pyrroline (2AP). As there is no transducer for chemical senses, the only reliable method of detection of aroma is by employing the human nose to smell the samples. For smelling, we relied on at least two trained persons to detect the aroma.

Alkali is known to enhance the release of 2AP, and acidification inhibits the release of 2AP. The characteristic smell of 2AP disappears after acid treatment and reappears upon re-alkalization. Accordingly, we used the method described by Brahmachary and Poddar-Sarkar (2015). We took 4 – 5 grains of unpolished rice kernels in a Petri dish, added 3 drops of 2% (w/v) KOH on the kernels, and left the sample under glass cover for 20 minutes. After 20 minutes the cover was removed for smelling the sample. Regardless of detection of any basmati-like aroma, we subsequently added 4 drops of 2% (w/v) HCl to acidify the sample, upon which the aroma disappeared. After this we added 5-6 drops of 2% (w/v) KOH to re-alkalize the kernels, and cover the Petri dish. After 20 minutes we smelled the sample again, and the presence or absence of Basmati-like aroma was recorded. The strength of the aroma was recorded according to the standard code (Table 3):

Table 3: Standard Code for the Strength of Aroma due to Presence of 2AP.

| Strength of Aroma | Code |
|-------------------|------|
| No aroma | 0 |
| Mild aroma | 1 |
| Strong aroma | 2 |

(c) *Alkali Digestion*

Alkali digestion was measured by using methods of Little et al. (1958) and Chemutai et al. (2016), with slight modification. We placed 3 - 4 unpolished kernels of each rice landrace in a petri-dish. 3 to 4 drops of 2% (w/v) KOH were added onto the kernels, submerging them in the solution. The petri dishes were then covered and placed at room temperature for 16 hours. After 16 hours the degree of disintegration of the kernels was rated visually according to standard evaluation system for rice by Bioversity International (**Table 4**).

Table 4: Standards of the Degree of Alkali Digestion.

| Degree of Alkali Digestion | Classification |
|--------------------------------------|-----------------------|
| Grain not affected but chalky | Low |
| Grain swollen | Low |
| Grain swollen with collar incomplete | Intermediate |
| Grain collar complete and wide | Intermediate |
| Grain split with wide collar | Intermediate |
| Grain dispersed merging with collar | High |
| Grain fully dispersed | High |

(d) *Gel Consistency*

Gel consistency in rice genotypes was determined by using the methods given in Cagampang et al. (1973), Chemutai et al. (2016) and Sattari et al. (2015) with slight modification. For each landrace, 100 mg ground kernels were weighed and placed in a 125 mm × 13 mm test tube, to which 0.2 ml of 95% ethanol containing 0.025% thymol blue was added. 2 ml of 0.2 N KOH was added and stirred gently for 30 seconds on an electrical vortex. Subsequently, the test tubes with samples were placed in a boiling water bath at 102 °C for 10 minutes. The test tubes were then kept at room temperature for 5 minutes and then kept in ice bath for 30 minutes. Finally, the tubes were taken out and placed horizontally on a graph paper on a flat surface. After 1 hour, the distance travelled by the gel was measured. From the distance travelled by the gel, the consistency was inferred from the standard classification (**Fig. 2, Table 5**).

Table 5: Gel Consistency Standards.

| Gel Distance (mm) | Gel Consistency |
|--------------------------|------------------------|
| >60 mm | Soft |
| 40 – 60 mm | Intermediate |
| < 40 mm | Hard |

(c) *Phenol Reaction*

Phenol reaction was assessed by using the method described in Chang and Bardenas (1965), with slight modification. We took a clean Petri dish (90 mm diameter) and marked 4 zones for 4 samples in the Petri dish by writing the sample codes in their respective zones in the dish. We placed 3 rice grains (with husk) of each landrace on its demarcated position, onto which we added 3 drops of 2% phenol solution and kept each Petri dish for 48 hours in a dark place at room temperature. After 48 hours of soaking in phenol, each Petri dishes were taken out one by one and the colour change on the grains of each sample was recorded, following the standard classification (**Table 6**). Most of the *indica* varieties and wild *Oryza* species take on a dark brown or black coloration as a result of polyphenol oxidase activity whereas *japonica* varieties show no colour change.

Table 6: Standard Codes of Colour Change in Reaction to Phenol.

| Phenol Reaction | Code |
|------------------------|-------------|
| No color change | 0 |
| Mild color change | 1 |
| Purple or black color | 2 |

Results

The results of our assessment are given in **Table 7**. Our data presented here are the first quantitative assessment of biochemical characteristics of 142 rice landraces from north east India, and reveal that 16 landraces (11.34%) are aromatic. Gel consistency for 7 (4.96%) landraces was found to be considerably hard. A total of 30 landraces (21.27%) are glutinous, and 25 landraces (17.73%) are non-reactive to phenol, plausibly indicating the introgression of these traits from japonica ancestral lines.

Table 7: Evaluation of Biochemical Characteristics of 142 Landraces from North East India.

| LANDRACE | Endosperm Type | Aroma | Alkali Digestion | Gel: Mean Distance (mm) | Gel Consistency | Phenol Reaction |
|--------------------------------|----------------|-------|------------------|-------------------------|-----------------|-----------------|
| STATE: ARUNACAL PRADESH | | | | | | |
| ADAM CHHILPA | Intermediate | 0 | Intermediate | 60 | Intermediate | 0 |
| AREYA HATH PUNKO | Intermediate | 0 | Low | 108 | Soft | 0 |
| BAHADO | Intermediate | 0 | Intermediate | 100 | Soft | 1 |
| BAHARI | Intermediate | 0 | Intermediate | 100 | Soft | 1 |
| BHAKTI | Intermediate | 0 | Low | 56 | Intermediate | 1 |
| BORA | Glutinous | 0 | Intermediate | 113 | Soft | 0 |
| EKAHARI | Intermediate | 0 | Intermediate | 35 | Hard | 1 |
| EKDALIA | Intermediate | 0 | Intermediate | 110 | Soft | 0 |
| GARO AIZAWR | Intermediate | 0 | Low | 58 | Intermediate | 2 |
| GENG GENG BINNI | Glutinous | 0 | Intermediate | 91 | Soft | 1 |
| KHAUN | Intermediate | 0 | Intermediate | 55 | Intermediate | 1 |
| NAMTA | Non-glutinous | 0 | Intermediate | 5 | Hard | 2 |
| PAJAB LAHI | Non-glutinous | 0 | Intermediate | 112 | Soft | 1 |
| PYAPING | Intermediate | 0 | Intermediate | 30 | Hard | 2 |
| PYATU MYPIYA | Non-glutinous | 0 | Intermediate | 100 | Soft | 1 |
| STATE: ASSAM | | | | | | |
| ABOR XALI | Non-glutinous | 0 | Intermediate | 50 | Intermediate | 2 |
| AMPAKHI BORA | Glutinous | 0 | Intermediate | 75 | Soft | 2 |
| AURAR | Non-glutinous | 0 | Intermediate | 105 | Soft | 1 |
| BAISHMUTHI | Non-glutinous | 0 | Intermediate | 102 | Soft | 2 |
| BETGUTIXALI | Non-glutinous | 2 | Intermediate | 108 | Soft | 0 |
| BEZI BHAN | Glutinous | 0 | Intermediate | 80 | Soft | 2 |
| BHABOLI JOHA | Non-glutinous | 2 | Intermediate | 95 | Soft | 0 |
| BHOG PRASAD | Non-glutinous | 2 | Intermediate | 85 | Soft | 0 |
| BOGA BONI | Non-glutinous | 0 | Intermediate | 110 | Soft | 1 |
| BOGI XALI | Non-glutinous | 0 | Low | 120 | Soft | 1 |
| BOKUI BORA | Glutinous | 0 | Intermediate | 55 | Intermediate | 2 |
| BOR JAHINGIA | Non-glutinous | 0 | Intermediate | 112 | Soft | 1 |

| LANDRACE | Endosperm Type | Aroma | Alkali Digestion | Gel: Mean Distance (mm) | Gel Consistency | Phenol Reaction |
|--------------|----------------|-------|------------------|-------------------------|-----------------|-----------------|
| CHUBOK | Non-glutinous | 0 | Intermediate | 98 | Soft | 2 |
| DEWRI BAW | Non-glutinous | 0 | Intermediate | 112 | Soft | 2 |
| DIMRU | Non-glutinous | 0 | Intermediate | 112 | Soft | 0 |
| ERI | Non-glutinous | 0 | Intermediate | 100 | Soft | 1 |
| GARO JOHA | Non-glutinous | 1 | Intermediate | 5 | Hard | 2 |
| GAZEP XALI | Non-glutinous | 2 | Intermediate | 30 | Hard | 1 |
| HAGMUNI | Non-glutinous | 0 | Intermediate | 115 | Soft | 0 |
| HARFONI | Non-glutinous | 0 | Intermediate | 112 | Soft | 1 |
| HEERAJ JOHA | Glutinous | 2 | Intermediate | 82 | Soft | 2 |
| JABRA | Intermediate | 0 | Low | 58 | Intermediate | 2 |
| JAHINGIA | Non-glutinous | 0 | Intermediate | 95 | Soft | 2 |
| JANGANI | Non-glutinous | 0 | Intermediate | 100 | Soft | 1 |
| JANGANI BORA | Non-glutinous | 0 | Intermediate | 113 | Soft | 2 |
| JOHA BORA | Glutinous | 1 | Intermediate | 85 | Soft | 2 |
| JUHA | Non-glutinous | 0 | Intermediate | 92 | Soft | 1 |
| KALA LAHI | Non-glutinous | 2 | Intermediate | 10 | Hard | 2 |
| KALA NONI | Non-glutinous | 2 | Intermediate | 75 | Soft | 2 |
| KALO BONNI | Glutinous | 0 | Intermediate | 92 | Soft | 2 |
| KALO JOHA | Non-glutinous | 2 | Intermediate | 115 | Soft | 1 |
| KARSOLI | Non-glutinous | 0 | Intermediate | 95 | Soft | 0 |
| KASI XALI | Glutinous | 0 | Intermediate | 105 | Soft | 2 |
| KBA LYNGKOT | Intermediate | 0 | Intermediate | 80 | Soft | 2 |
| KHAMTI LAHI | Non-glutinous | 0 | Intermediate | 92 | Soft | 1 |
| KOKUA BAW | Non-glutinous | 0 | Intermediate | 55 | Intermediate | 1 |
| KOLA JOHA | Non-glutinous | 0 | Intermediate | 50 | Intermediate | 2 |
| KOLA XALI | Intermediate | 2 | Intermediate | 113 | Soft | 2 |
| KOMAL | Non-glutinous | 0 | Intermediate | 100 | Soft | 1 |
| KOMOL | Non-glutinous | 0 | Intermediate | 95 | Soft | 1 |
| KON JOHA | Non-glutinous | 2 | Intermediate | 55 | Intermediate | 2 |
| KONA MUSORI | Non-glutinous | 0 | Intermediate | 105 | Soft | 0 |
| KUNIJHUM | Non-glutinous | 0 | Intermediate | 92 | Soft | 1 |
| KUTKUTI XALI | Intermediate | 0 | Intermediate | 45 | Intermediate | 2 |

| LANDRACE | Endosperm Type | Aroma | Alkali Digestion | Gel: Mean Distance (mm) | Gel Consistency | Phenol Reaction |
|-------------------------|----------------|-------|------------------|-------------------------|-----------------|-----------------|
| MAGURI BAW | Non-glutinous | 0 | Low | 91 | Soft | 1 |
| MAIBILAI | Non-glutinous | 0 | Intermediate | 70 | Soft | 2 |
| MIREN KELOK | Non-glutinous | 2 | Intermediate | 115 | Soft | 1 |
| MIRET | Non-glutinous | 0 | Intermediate | 95 | Soft | 2 |
| MOTHONGA | Glutinous | 0 | Intermediate | 82 | Soft | 2 |
| NAGA BONNI | Intermediate | 0 | Intermediate | 40 | Hard | 2 |
| NAL BONNI | Glutinous | 0 | Intermediate | 113 | Soft | 1 |
| NEKERA | Non-glutinous | 0 | Intermediate | 98 | Soft | 1 |
| PURA BONNI | Non-glutinous | 0 | Intermediate | 92 | Soft | 2 |
| RANGA BORA | Glutinous | 0 | Low | 110 | Soft | 2 |
| RIBOR JHUM | Non-glutinous | 0 | Intermediate | 100 | Soft | 2 |
| RONGA | Non-glutinous | 0 | Intermediate | 112 | Soft | 1 |
| SADA JOHA | Non-glutinous | 0 | Low | 115 | Soft | 2 |
| SHILA PATHAR | Glutinous | 0 | Intermediate | 100 | Soft | 2 |
| SIRI LANGKA | Intermediate | 0 | Low | 98 | Soft | 1 |
| SOK DIMRO | Glutinous | 0 | Intermediate | 98 | Soft | 1 |
| SUAGMONI | Non-glutinous | 0 | Intermediate | 109 | Soft | 2 |
| TALMARI | Non-glutinous | 0 | Intermediate | 103 | Soft | 2 |
| THUPA BORA | Glutinous | 0 | Low | 90 | Soft | 1 |
| THUPI-SAL | Non-glutinous | 0 | Intermediate | 103 | Soft | 2 |
| TIKI | Glutinous | 0 | Intermediate | 70 | Soft | 2 |
| TULSI XALI | Non-glutinous | 2 | Intermediate | 90 | Soft | 0 |
| STATE: MANIPUR | | | | | | |
| CHAK HAO POIREITHON | Intermediate | 0 | Intermediate | 82 | Soft | 2 |
| MANIPURA BHATTA | Glutinous | 0 | Intermediate | 50 | Intermediate | 1 |
| STATE: MEGHALAYA | | | | | | |
| ADOLIA BAW | Intermediate | 0 | Intermediate | 95 | Soft | 2 |
| BURMA | Non-glutinous | 0 | Intermediate | 80 | Soft | 2 |
| KAJA JOHA | Intermediate | 0 | Intermediate | 75 | Soft | 0 |
| KBA SNEM | Non-glutinous | 0 | Intermediate | 92 | Soft | 2 |
| KOLA BORA | Glutinous | 0 | Intermediate | 85 | Soft | 2 |

| LANDRACE | Endosperm Type | Aroma | Alkali Digestion | Gel: Mean Distance (mm) | Gel Consistency | Phenol Reaction |
|------------------------|----------------|-------|------------------|-------------------------|-----------------|-----------------|
| LAI PAWA | Non-glutinous | 0 | Intermediate | 95 | Soft | 0 |
| LANGKA | Non-glutinous | 0 | Intermediate | 45 | Intermediate | 2 |
| MAW THLEN | Non-glutinous | 0 | Low | 90 | Soft | 2 |
| PMAH SAW | Non-glutinous | 0 | Low | 107 | Soft | 2 |
| PNAH LAMARE | Glutinous | 0 | Intermediate | 95 | Soft | 1 |
| RANGALI BAW | Non-glutinous | 0 | Low | 107 | Soft | 1 |
| SALAER | Non-glutinous | 0 | Intermediate | 92 | Soft | 2 |
| STATE: NAGALAND | | | | | | |
| AGAR ALI | Intermediate | 0 | Intermediate | 90 | Soft | 2 |
| AIJOM | Non-glutinous | 0 | Intermediate | 102 | Soft | 2 |
| CHANG BEM | Glutinous | 0 | Intermediate | 102 | Soft | 0 |
| CHANG PAL NGAT | Intermediate | 0 | Intermediate | 80 | Soft | 2 |
| CHANG VOM | Glutinous | 0 | Intermediate | 105 | Soft | 2 |
| CHANG VOM NGAT | Glutinous | 0 | Intermediate | 110 | Soft | 2 |
| CHANGKO MEI | Intermediate | 0 | Intermediate | 113 | Soft | 2 |
| CHIKIA MNIE | Non-glutinous | 0 | Intermediate | 112 | Soft | 2 |
| CHONG LOI MAN | Intermediate | 0 | Low | 105 | Soft | 1 |
| DACHA | Non-glutinous | 0 | Intermediate | 110 | Soft | 1 |
| DUNGLWA MEI | Non-glutinous | 0 | Intermediate | 103 | Soft | 2 |
| HENING KUNGLWA MNIE | Glutinous | 0 | Intermediate | 105 | Soft | 1 |
| HENING MNIE | Glutinous | 0 | Intermediate | 98 | Soft | 0 |
| HERAPUI MEI | Non-glutinous | 0 | Intermediate | 90 | Soft | 1 |
| HETTALU MNIE | Glutinous | 0 | Intermediate | 80 | Soft | 2 |
| HEZI MEI | Non-glutinous | 0 | Intermediate | 115 | Soft | 1 |
| KASIA MEI | Non-glutinous | 0 | Intermediate | 115 | Soft | 2 |
| KEMENIE | Intermediate | 0 | Intermediate | 90 | Soft | 1 |
| KETSARU | Intermediate | 0 | Intermediate | 87 | Soft | 2 |
| KEZI MEI | Intermediate | 0 | Intermediate | 115 | Soft | 2 |
| KONG HING | Non-glutinous | 0 | Intermediate | 97 | Soft | 1 |
| MEIGIE | Intermediate | 0 | High | 103 | Soft | 0 |
| MELHITTE KENYE LHA | Non-glutinous | 0 | Intermediate | 96 | Soft | 2 |

| LANDRACE | Endosperm Type | Aroma | Alkali Digestion | Gel: Mean Distance (mm) | Gel Consistency | Phenol Reaction |
|-----------------------|----------------|-------|------------------|-------------------------|-----------------|-----------------|
| METIAK KEGIE | Intermediate | 0 | Intermediate | 82 | Soft | 1 |
| MEZIA MNIE | Non-glutinous | 0 | Low | 73 | Soft | 1 |
| MEZIA REI | Non-glutinous | 0 | Intermediate | 115 | Soft | 2 |
| MOLVOM CHANG | Non-glutinous | 0 | Intermediate | 85 | Soft | 1 |
| NGOBA | Non-glutinous | 0 | Intermediate | 98 | Soft | 1 |
| PETCHIM MEI | Intermediate | 0 | Intermediate | 82 | Soft | 2 |
| PHEUIMEI JHUM | Non-glutinous | 0 | Intermediate | 55 | Intermediate | 2 |
| PIYA | Non-glutinous | 2 | Intermediate | 110 | Soft | 0 |
| SAMZIURAM MEI | Glutinous | 0 | Intermediate | 50 | Intermediate | 1 |
| SANA CHANG | Non-glutinous | 0 | Intermediate | 112 | Soft | 0 |
| SILKOT | Intermediate | 0 | Intermediate | 102 | Soft | 0 |
| SIRGUTI HABE | Glutinous | 0 | Intermediate | 80 | Soft | 0 |
| SIRGUTI TIKBE | Intermediate | 0 | Low | 68 | Soft | 1 |
| TANYE | Non-glutinous | 0 | Intermediate | 100 | Soft | 1 |
| TENENG HABE | Glutinous | 0 | Intermediate | 94 | Soft | 0 |
| TENENG TIKBE | Glutinous | 0 | Intermediate | 92 | Soft | 2 |
| TEPTHE LHA | Intermediate | 0 | Intermediate | 100 | Soft | 2 |
| THEVURU LHA | Non-glutinous | 2 | Intermediate | 104 | Soft | 2 |
| TSORUNYU | Non-glutinous | 0 | Intermediate | 75 | Soft | 0 |
| STATE: SIKKIM | | | | | | |
| TUKMOR | Intermediate | 0 | Intermediate | 60 | Intermediate | 0 |
| STATE: TRIPURA | | | | | | |
| GABRANG | Intermediate | 0 | Intermediate | 80 | Soft | 2 |
| GARO MALTI | Intermediate | 0 | Low | 82 | Soft | 2 |
| MAMI GURIA | Glutinous | 0 | Intermediate | 52 | Intermediate | 1 |
| MANIK-SAL | Non-glutinous | 0 | Intermediate | 98 | Soft | 0 |

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Acknowledgments

We are indebted to Mr. Avik Saha for providing full infrastructural and logistic support to our research. We are grateful to Dr Payel Das, Ms Sangita Dey and Ms Priya Mandal for standardization of all methods adopted in this study and for a preliminary assessment of some of the landraces examined here. This work received no institutional funding.

Conflict of Interest

Authors declare no conflict of interest.