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RESEARCH ARTICLE

Endangered indigenous rice varieties as a source of B vitamins for the undernourished population

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Abstract

Background and Objectives: Rice is a staple food for half of the world's population and plays an important role to deliver several micronutrients including B vitamins to humans. The present investigation was carried out to detect some B vitamins and estimate their concentrations in 309 traditional indica rice landraces, compared with three modern rice varieties predominantly available in the Indian market.

Findings: Liquid chromatographic examination of the rice samples demonstrated that a large number of traditional rice landraces contained considerable amounts of different B vitamins. In the landraces examined, vitamin B1 (thiamine) was recorded to be present in the range of 0.01–10.55 mg/100 g, vitamin B2 (riboflavin) 0.01–2.63 mg/100 g, vitamin B3 (niacin) 0.20–4.52 mg/100 g, vitamin B5 (pantothenic acid) 0.01–18.55 mg/100 g, vitamin B6 (pyridoxine) 0.01–0.86 mg/100 g, and vitamin B7 (biotin) 0.01–5.90 mg/100 g in different rice landraces.

Conclusion: Compared with traditional rice, modern rice cultivars seem to have substantially lower B vitamin levels. It appears that these vitamin-rich traditional rice landraces if incorporated into daily diet, may serve to attain nutritional security of the poor.

Significance and Novelty: Our results show that many traditional rice landraces are nutritionally superior to any modern rice cultivar, even though traditional rice landraces are normally not in priority for agronomic research and development. This study shows how native rice landraces may be leveraged to constitute novel nutritious diet that could enhance human health.

KEYWORDS
B vitamins, HPLC, landrace, nutrition, traditional rice

1 | INTRODUCTION

Rice (*Oryza sativa* L.) is an important part of the human diet because it contains a large amount of carbohydrates, and small quantities of protein, fatty acids, dietary fibers, B vitamins, and minerals. B vitamins are critically important vitamin groups, as they help keep the nervous system (Calderón-Ospina & Nava-Mesa, 2020), skin and eyes (Shabbir et al., 2020), liver (Khan et al., 2009), brain function (Klenner, 2005), and gastrointestinal tract

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(Tappenden & Deutsch, 2007) healthy and functional. These vitamins concertedly work to promote metabolism, facilitate adequate oxygen supply to cells, detoxify organs, stabilize nervous system functions, prevent vision problems (Gonçalves & Portari, 2021), and also to treat debilitating conditions (Tice, 2010).

B-vitamin deficiencies have diverse aetiologies, including inadequate intake, increased needs at different life history stages, malabsorption, drug-nutrient interactions, and other factors like hereditary diseases or health conditions (Porter et al., 2016). In many developing countries, vitamin B inadequacies are very common, particularly in those with diets that are low in animal products, fruits, and vegetables, and where cereal grains are milled before consumption. The most vulnerable groups to vitamin B deficiency are newborns, adolescents as well as pregnant and lactating women (Ashley, 2016). Infantile beriberi, a potentially fatal condition brought on by thiamine deficiency, is widely believed to be a sickness of the past in regions of the world where milled white rice consumption is the norm. Recent case reports, however, have demonstrated that thiamine deficiency is still a contributing factor in infant mortality in South and Southeast Asia. In the United States and UK, riboflavin deficiency is uncommon, but prevalent in developing countries in Asia and Africa. Niacin deficiency in the diet is usually common during periods of the food crisis, and frequent in Africa and Asia's maize-eating regions. Pyridoxine deficiency mostly occurs when the body has low levels of other B vitamins, especially vitamin B12 and folic acid, which is common in South Asia (Harding et al., 2018).

Fortification of rice with different vitamins and minerals is considered to be an important step toward addressing endemic malnutrition in rice-growing countries. While rice endosperm is virtually devoid of micronutrients, brown rice is known to contain several vitamins and minerals (Deb et al., 2015; Mondal et al., 2021; Rezaei et al., 2022; Roy et al., 2021). Knowledge of vitamin contents of specific rice landraces is important for strategizing the nutritional security of populations through the public distribution of vitamin-rich rices, as a viable and cheaper alternative to rice fortification programs. However, there is very limited information on the presence of B vitamins in rice landraces in its brown, or polished form. Liquid chromatographic method has evolved as one of the best methods for the identification and quantitative determination of B vitamins in food matrixes (Nguyen et al., 2021; Rezaei et al., 2022). Thiamine, riboflavin, nicotinic acid, biotin, pantothenic acid, and so forth have been reported by liquid chromatographic methods in a small number of *indica* rice landraces (Cho et al., 2020; Deepa et al., 2008; Priya et al., 2019; Roy et al., 2021; Sumczynski, et al., 2018). Vitamin B12 is reported to be absent in the plant system (Watanabe, 2007), although vitamin B12 derived from microbial biosynthesis is rarely found in some processed plant foods (Jedut et al., 2021). On a larger scale, there is a research gap on the profiling of B vitamins and their concentrations in diverse rice (*O. sativa* ssp. indica) landraces. In this study, we report the results of a quantitative analysis of seven crucial B vitamins in 309 *indica* rice landraces, compared to three modern rice cultivars, and discuss the compositional diversity to address nutritional security.

2 | MATERIALS AND METHODS

2.1 | Samples

Freshly harvested grains of 309 traditional rice landraces were collected in 2019 from the Centre for Interdisciplinary Study's conservation farm Basudha (http://www.cintdis.org/basudha), located in Rayagada district of Odisha (19° 42′ 32.0″N, 83° 28′ 8.4″E) where all the rice landraces are cultivated with zero external input. The farm is situated in the northern Eastern Ghat, characterized by hot subhumid eco-region with annual rainfall ranging from 1030.21 mm to 1569.50 mm. Samples of three modern varieties, namely, IR36, IR64, and BPT5204, were examined for comparison. Samples of the first two were collected from Chinsurah Rice Research Station, Hooghly, India, and BPT5204 was procured from Rajendranagar market, Hyderabad, India. All rice samples were decorticated manually in the laboratory by rubbing against a pumice stone, keeping the rice germ and bran layer intact, and ground to a fine powder using mortar and pestle, and stored at −20°C for vitamin analysis.

2.2 | Chemicals

Thiamine hydrochloride (B1), riboflavin (B2), nicotinic acid (B3), D-pantothenic acid calcium salt (B5), pyridoxine HCl (B6), and biotin (B7) and cyanocobalamin (B12) standards were purchased from Sigma-Aldrich. Methanol, chromatography-grade water and analytical-grade hydrochloric acid were obtained from Merck.

2.3 | Preparation of stock solution

Stock solutions of thiamine hydrochloride, pyridoxine HCl, cyanocobalamin, nicotinic acid, and D-pantothenic acid calcium salt were prepared by dissolving 10 mg of the respective compound in 10 mL of deionized water (1 mg/mL), and stock solutions of riboflavin, and biotin were prepared by dissolving 10 mg of the respective compound in 10 mL of 0.1 mol/L NaOH (1 mg/mL). Stock solutions were prepared afresh before each analysis.

2.4 | Extraction of B vitamins

Extraction was carried out following the methods described in Puwastien et al. (2011) with slight modification, and detailed in Roy et al. (2021).

2.5 | Chromatographic procedure

High-performance liquid chromatography (HPLC) was used for the analysis of all B vitamins from the extracted rice samples. The reverse-phase-HPLC (RP-HPLC) method, reported by Heudi et al. (2005) was adopted for standardization, with slight modification. A gradient elution method was employed to get the baseline separation of the B vitamins (Roy et al., 2021). Briefly, gradient of two mobile phases were: methanol (A) and water with 0.02% aqueous H_3PO_4 (B) were set at: 0% A + 100% B for 3 min; 10% A + 90% B for 10 min; 30% A + 70% B for 15 min and 30% A + 70% B for 35 min. The injection volume was 20 µl. The flow rate was kept at 1 mL/min and analytes were scanned at 210 nm wavelength. The peaks were identified by comparing the relative retention time with proper peak integration, co-chromatography with standard, and calibration against absorption spectra obtained from the analytical standards. Considering the large number of samples and quick completion of the analysis, we have carried out the experiments using three different liquid chromatographic machines. However, the method was identical for B complex vitamin analyses in all the instruments. The instrumental details are (i) Shimadzu Prominence Analytical HPLC System attached with Zorbax SB-C18 column (4.6 mm × 150 mm, 3.5 micron, Agilent) with Photo Diode Array Detector; (ii) Waters HPLC attached with Atlantis dC18 column (100 Å, 5 µm, 3.9 mm × 150 mm) and UV-Vis detector, and (iii) Shimadzu Prominence UFLC (Ultra-Fast Liquid Chromatography), attached with Zorbax SB-C18 column (4.6 mm × 150 mm, 3.5 µm, Agilent, USA) with dual-channel UV-Vis detector. In all these cases, the separated peaks were calculated by comparing the relative retention time with the right peak integration, standard co-chromatography, and absorption spectra calibration obtained from the authentic standard.

2.6 | Detection of B vitamins and chromatographic separation

Following the recent understanding (Sasaki et al., 2020) that all B vitamins can be spectrophotometrically detected at 210 nm. We used 210 nm wavelength to detect the presence of all seven B vitamins (Supplementary Figure S1) in our experiment. The chromatographic separation of seven B vitamins in the mixture of standard solution using the gradient elution method is shown in Supplementary Figure S2 (I). The elution order was vitamin B1 (thiamine), B3 (niacin), B6 (pyridoxin), B5 (pantothenic acid), B7 (biotin), B12 (cyanocobalamin), and B2 (riboflavin). Supplementary Figure S2 (II) shows the chromatographic separation of B vitamins from an extracted rice sample G37. All seven B vitamins were separated to the baseline and eluted as sharp peaks within 20 min. The reproducibility of the retention time was checked three times over, and only after getting an acceptable standard deviation value the method was adopted. By combining the stock solutions in the proper proportions and diluting them with mobile phase, the analytical solutions were used for assessing the linearity, range, LOD, and LOQ. Peak areas were plotted against five comparable concentrations (µg/mL) of each vitamin B molecule to develop calibration curves. Using the outcomes of these analyses, the linearity (with $R^2 > 0.998$), range, LOD, and LOQ were estimated (Table 1). The concentrations at which vitamin B compound peaks could be identified without being interfered with by baseline noise were used to determine LOD and LOQ. All vitamins were quantified by using the standard validation method and finally, the amounts of the analytes were expressed as mg/100 g. Results below the LOD were considered as not detected (ND) in case of all B vitamins.

2.7 | Statistical analysis

Data are presented as the mean of three replications. Pearson's correlation and principal component analysis (PCA) were performed using R statistical software (v4.2.2; R Core Team 2022). Considering replications ($df = 2$), the confidence limit was set at $p < .05$. Using the same data sets, bidirectional heatmap clustering was performed.

3 | RESULTS AND DISCUSSIONS

3.1 | Analysis of B vitamins in rice samples

The concentrations of different B vitamins in 309 rice landraces and three modern rice cultivars examined

TABLE 1 LOD and LOQ of B vitamins in chromatographic detection and variation of B vitamins across rice lines achieving recommended daily intake.

Name of B vitamins	LOQ (ppm)	LOD (ppm)	Variations B vitamins in rice landraces (mg/100 g)	Average recommended Daily Intake[a] for an adult (19-60 years)	A few recommended rice lines with high amount of B vitamins
Thiamine	0.04	0.05	0.01–10.55	1.2 (mg)	C09, DD16, G43, TT16, B24
Riboflavin	0.07	0.01	0.01–2.63	1.3 (mg)	B38, M04, M33, K71
Nicotinic acid	0.02	0.008	0.20–4.52	16 (mg)	SS04, M51, S54, G12
Pantothenic acid	0.03	0.005	0.01–18.55	5 (mg)	A10, DD18, S61, T01
Pyridoxine	0.02	0.01	0.01–0.86	1.3 (mg)	C12, B21, S54, G12
Biotin	0.03	0.008	0.01–5.90	30 (μg)	SS02, SH06, SH04, M33
Cyanocobalamin	0.01	0.006	N.D.	2.4 (μg)	---

Abbreviations: LOD, Limit of detection; LOQ, limit of quantitation; N.D., not detected.

[a]Taken from Kennedy (2016).

here are given in Supplementary Tables S1 and S2, respectively. Vitamin B1 (thiamine) was present in the range of 0.01–10.55 mg/100 g, vitamin B2 (riboflavin) 0.01–2.63 mg/100 g, vitamin B3 (nicotinic acid) 0.20–4.52 mg/100 g, vitamin B5 (D-pantothenic acid calcium salt) 0.01–18.55 mg/100 g, vitamin B6 (pyridoxine hydrochloride) 0.01–0.86 mg/100 g, and vitamin B7 (biotin) 0.01–5.90 mg/100 g in different rice landraces. In comparison with several traditional rice landraces, the modern rice cultivars IR36, IR64, and BPT5204 contained much lower quantities of B vitamins in their grains (Supplementary Table S2). In contrast, among the 309 landraces studied here, there were several landraces containing remarkably high levels of different B vitamins in their grains (Figure 1), implying that their consumption could meet the recommended daily intake (RDI) of these vitamins (Table 1). There was no strong correlation among the levels of different B vitamins in the rice landraces examined here (Supplementary Figure S3).

The association between the rice landraces was investigated using important B vitamins to determine suitable rice landraces for future studies. The outcomes of the heat-map analysis of the rice landraces are shown in Supplementary Figure S4. The efficacy of applied methodologies in identifying rice landraces based on phenotypic data is highlighted in the heatmap, which clearly classifies 309 landraces into five main clusters and 12 sub-clusters. Our PCA failed to detect any distinctive group among our rice samples Supplementary Figure S5. However, separation was visible in the first two main components, which collectively account for 54.7% of variation. The principal component 1 (Dim-1, 36.2%) score and loading indicated that the rice lines had greater concentrations of B1, B2, B3, B5, and B6 with high and positive relations among each other. On the other hand, the level of B7 was higher in the PC2 loading (Dim2, 18.5%) plot and score than those in PC1. These findings, however, do not indicate that the concentrations of any B vitamin had any influence on the presence of other B vitamins in the rice grains. It is likely that edaphoclimatic diversity of origin of the landraces and different agronomic factors (Choi et al., 2012)—in addition to the varietal genotype—may independently influence the B vitamin contents in these rice landraces.

3.1.1 | Vitamin B1 (thiamine)

Almost 96% of the rice landraces examined here contained much greater than 0.1 mg/100 g of thiamine in their grains (Figure 1). The higher contents of thiamine were found especially in DD16, C09, G43, and TT16 samples, in a range between 9.03 and 10.55 mg/100 g. As Supplementary Table S1 shows, traditional rice landraces are a rich source of thiamine. The major forms of this vitamin in cells are free thiamine, thiamine monophosphate, and thiamine pyrophosphate, which are one of the cofactors for the enzymes of basic metabolic pathways like glycolysis, pentose phosphate pathway, and tricarboxylic acid pathway, amino acid and acetyl Co-A biosynthesis pathways (Rapala-Kozik, 2011). Deficiency of thiamine leads to beriberi, which affects cardiovascular and nervous systems (Lonsdale, 2006). Our finding suggests that RDI may be attained by consuming whole grain (unpolished) rice in a daily diet (Table 1).

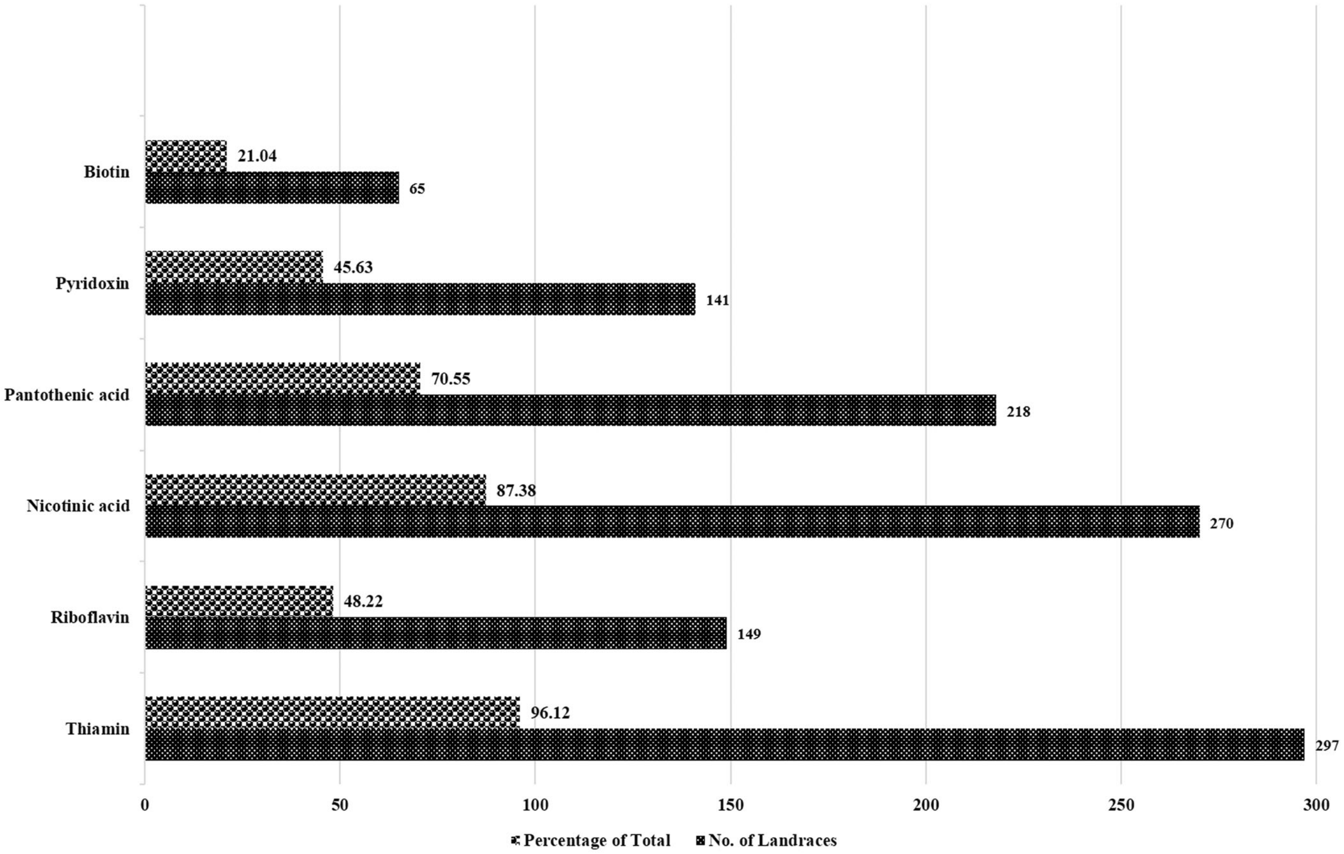

FIGURE 1 The number of landraces (dotted bars) and their proportions (bubble bars), Containing > 100 µg of different B complex vitamins in 100 g of sample. Figures beside the bubble bars indicate the % of $N = 309$ landraces examined.

3.1.2 | Vitamin B2 (riboflavin)

Considerably high riboflavin levels were found in M04 (2.63 mg/100 g) and B38 (1.05 mg/100 g) rice landraces. Riboflavin is a precursor of various redox-active coenzymes associated with different proteins, which act as cofactors in different metabolic enzymes (Giancaspero et al., 2013). Deficiency of vitamin B2 in the diet leads to anemia, neurological, and developmental disorders (Powers, 2003). Our study indicates that RDI may be achieved by consuming some traditional rice landraces, which were a good source of vitamin B2 and could be recommended to patients suffering from ariboflavinosis (Table 1).

3.1.3 | Vitamin B3 (nicotinic acid)

The higher contents of niacin were found in SS04 (4.52 mg/100 g), M51 (4.05 mg/100 g), S54 (3.86 mg/100 g), and so forth rice landraces. However, 87.38% of the rice landraces examined here contained more than 0.1 mg/100 g in their grains (Figure 1, Supplementary Table S1). Niacin is a precursor of pyridine alkaloids (Noctor, 2006) and helps to metabolize macronutrients, resulting in the healthy functioning of the nervous system. Severe deficiency of niacin in the diet causes pellagra, poor concentration, anxiety, depression, and so forth (Penberthy & Kirkland, 2020). Consuming the rice varieties examined here may not meet the need of RDI of vitamin B3 in diet (Table 1), but a moderate amount of niacin present in rice landraces could be helpful for people who are suffering from vitamin B3 deficiency.

3.1.4 | Vitamin B5 (D-pantothenic acid calcium salt)

The higher contents of pantothenic acid were found in 70.55% of rice landraces. Pantothenic acid (B5) is commonly available among other B vitamins and could be found in both animal and plant-based food items. It is the precursor of coenzyme A which is essential for fatty acid metabolism. It is also responsible for the secondary metabolite synthesis pathway (Coxon et al., 2005). Pantothenic acid deficiency in humans is extremely rare and has received less attention. The RDI for vitamin B5 could be easily achieved by consuming traditional rice landraces, which are an excellent source of pantothenic acid as our data suggest (Table 1).

3.1.5 | Vitamin B6 (pyridoxin hydrochloride)

The higher contents of pyridoxine (B6) were found in 45.63% of the total rice landraces (Figure 1). Vitamin B6 is a potent antioxidant that helps to metabolize sugars and fatty acids and acts as a cofactor in different enzymatic reactions (Drewke & Leistner, 2001). Deficiency of vitamin B6 leads to depression, lower immunity, kidney diseases, rheumatoid arthritis, and so forth (Sharifzadeh et al., 2018). Vitamin B6 may lower the risk of cancer (Mocellin et al., 2017), help with brain function by lowering levels of homocysteine (Rutjes et al., 2018), and prevent cardiovascular diseases. Our results suggest that a good number of traditional rice landraces contain this vitamin in moderate amounts.

3.1.6 | Vitamin B7 (biotin)

An adequate amount of biotin was found in 21% of the rice landraces examined here (Figure 1), which are capable to meet the RDI (Table 1). Plants, most bacteria, and some fungi can synthesize biotin, whereas animals and some other fungi must obtain biotin from their diet because they are unable to synthesize it. Biotin operates as a cofactor for enzymes that are required in many biological functions (Knowles, 1989). Deficiency of vitamin B7 leads to brittle and thin fingernails, conjunctivitis, red rash on the face, and neurological symptoms such as depression, lethargy, hallucination, and so forth (Penberthy & Kirkland, 2020).

There are some recent reports of a few traditional rice landraces containing B vitamins. Specifically, Njavara from Kerala and Jyothi from Karnataka are reported to contain thiamine (0.35–0.52 mg/100 g), riboflavin (0.053–0.071 mg/100 g), niacin (7.15–7.32 mg/1100 g), and folic acid (0.05 mg/100 g) (Deepa et al., 2008). Similarly, 32 folk rice varieties from Meghalaya are reported to contain thiamine (0.17–0.38 mg/100 g), riboflavin (0.03–0.09 mg/100 g), nicotinic acid (1.43–3.87 mg/100 g), pantothenic acid (0.36–3.10 mg/100 g), and pyridoxine (0.03–0.17 mg/100 g) (Longvah et al., 2020). Roy et al. (2021) also reported different B vitamins, namely, thiamine (0.11–1.38 mg/100 g), riboflavin (0.01–0.56 mg/100 g), niacin (0.17–0.80 mg/100 g), pantothenic acid (0.81–2.9 mg/100 g), pyridoxine (0.1–0.2 mg/100 g), and biotin (0.01–0.24 mg/100 g) in a few selected rice landraces, also included in our present study of 309 rice landraces. In this study, we detected an outstandingly high concentration of thiamine in some landraces, to the extent of more than 9 mg/100 g. In addition to thiamine, the concentrations of pantothenic acid, pyridoxine, niacin, biotin in these folk rice grains are much greater than in the three modern high-yielding rice cultivars (Supplementary Tables S1 and S2). In fact, most of the B vitamins are not yet reported in any modern high-yielding rice cultivars (Deepa et al., 2008; Roy et al., 2021). In this study, the richness of B vitamins in the grains of some traditional rice landraces implies a robust solution to the problem of vitamin B deficiency, especially in rice-eating cultures. We suggest that the recommended daily dietary requirement of many B vitamins may adequately be fulfilled by a large number of traditional rice landraces (Table 1), most of which are not yet adequately examined.

This research indicates how indigenous rice landraces might be utilized into a nutritious rice-based product that is high in vitamins or used as a component in novel functional foods that could improve human health (Itagi et al., 2023). While folk rice landraces are typically not high on the priority list for agronomic research and development, our findings indicate that a large number of rice landraces are nutritionally superior to any modern rice cultivar.

4 | CONCLUSION

The present work is the first quantitative study of several B vitamins in fresh decorticated grains of a large number (309) of *indica* rice landraces, compared to 3 modern rice cultivars. Most of the landraces examined here have almost disappeared from rice farms as a result of preferences for modern HYVs. The extinction of these landraces from rice farms indicates a substantial loss of a wealth of indigenous rice genetic diversity, with their great potential to ensure nutritional security for the poor. One apparent policy recommendation is to conserve and promote the cultivation and consumption of traditional rice landraces. Widespread cultivation and consumption would be a viable means to assuring nutritional security for the poor and marginal sections of the country's population.

AUTHOR CONTRIBUTIONS

Debal Deb and Priyabrata Roy conceived the presented idea. Debal Deb designed the analyses and selected the rice cultivars, Tanima Saha and Thalappil Pradeep verified the analytical methods. Debal Deb, Brindaban Roy, Tanima Saha, and Thalappil Pradeep enlisted

Priyabrata Roy and Arunan Suganya to investigate all the analyses, statistical computations, provided the access and instrumental facilities for all analyses and supervised the entire work. Priyabrata Roy and Tanima Saha prepared the first draft of manuscript. All authors contributed to the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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