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Mining nutri-dense accessions from rice landraces of Assam, India

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ABSTRACT

The Indian subcontinent is the primary center of origin of rice where huge diversity is found in the Indian rice gene pool, including landraces. North Eastern States of India are home to thousands of rice landraces which are highly diverse and good sources of nutritional traits, but most of them remain nutritionally uncharacterized. Hence, nutritional profiling of 395 Assam landraces was done for total starch, amylose content (AC), total dietary fiber (TDF), total protein content (TPC), oil, phenol, and total phytic acid (TPA) using official AOAC and standard methods, where the mean content for the estimated traits were found to be 75.2 g/100g, 22.2 g/100g, 4.67 g/100g, 9.8 g/100g, 5.26%, 0.40 GAE g/100g, and 0.34 g/100g for respectively. The glycaemic index (GI) was estimated in 24 selected accessions, out of which 17 accessions were found to have low GI (<55). Among different traits, significant correlations were found that can facilitate the direct and indirect selection such as estimated glycemic index (EGI) and amylose content (−0.803). Multivariate analyses, including principal component analysis (PCA) and hierarchical clustering analysis (HCA), revealed the similarities/differences in the nutritional attributes. Four principal components (PC) i.e., PC1, PC2, PC3, and PC4 were identified through principal component analysis (PCA) which, contributed 81.6% of the variance, where maximum loadings were from protein, oil, starch, and phytic acid. Sixteen clusters were identified through hierarchical clustering analysis (HCA) from which the trait-specific and biochemically most distant accessions could be identified for use in cultivar development in breeding programs.

1. Introduction

Rice (*Oryza sativa* L.*)* is the primary staple food consumed by the world and a source of nutritional and food security. The Indian subcontinent harbors various rice landraces, but the Green Revolution brought along the cultivation of improved varieties rather than

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being considerate towards indigenous landraces [1]. Improved varieties are high yielding but prone to biotic and abiotic stresses, lacking local adaptability, whereas heterogeneous indigenous landraces contain various stress-resistant genes and are genetically diverse. Northeast (NE.) India is a secondary centre of origin of rice where, it is the primary crop for food and nutritional security occupying 4.8 mha [2], comprising about 10,000 rice landraces, with Assam being 9th among the top ten rice producers in India. Scientific evidences have revealed the renewed consumer interest in brown rice due to increasing health consciousness in recent years where such a shift in consumption patterns would assure nutritional security.

Brown rice landraces are bestowed with a significant quantity of nutritional traits, where nutritional attributes such as starch, amylose, total dietary fiber, protein, and oil contents determine the functionality of rice germplasm. Rice grain is majorly composed of carbohydrates (approx. 80 g/100g), moisture (8–10 g/100g), fiber (4–5 g/100g), and fat (3–4 g/100g) [3]. Starch is the major carbohydrate in rice (80 g/100g) which is highest amongst cereals and consists of amylose and amylopectin. Based on amylose content (AC), rice can be categorized as waxy (1–2 g/100g), very low AC (2–9 g/100g), low AC (9–20 g/100g), intermediate AC (20–25 g/100g) and high AC (25–33 g/100g) [4]. In contrast, total dietary fiber (TDF) correlates to the non-starch polysaccharides (lignin and resistant starch), which are resistant to the endogenous secretions of the human gut. Foods rich in insoluble dietary fiber are necessary for gastrointestinal function, and those rich in soluble dietary fiber are required for metabolic effects on glucose and lipid metabolism. The total protein content (PC) is another nutritionally relevant component in rice (approx. 9.5 g/100g) which is comparable to that of maize (9 g/100g), and wheat (12.5 g/100g). The PC is also related to the texture of cooked rice since it inhibits water absorption and swelling of starch upon cooking [5]. Brown rice grains are rich in oil content (5.5 g/100g) which is greater than maize (approx.4 g/100g) and similar to pearl millet (5.95 g/100g) [6]. Apart from the nutritional characteristics, rice grains possess some anti-nutritional components such as polyphenols and phytic acid, which act as free radical scavengers and chelators of pro-oxidant metals, preventing low-density lipoprotein oxidation [7]. Different types of polyphenolic compounds have been identified based on the pericarp color of rice grains [8], where rice grain with red and black pericarp has the highest concentration [9]. For light brown pericarp, phenolic content for rice bran and rice grain was reported between 0.190 and 5.03 g/100g GAE and 0.025–0.535 g/100g GAE, respectively [10]. The amount of phytic acid is abundant in rice specifically in the bran, representing 65–73% of the total P content [11] where variable amounts of phytic acid have been previously reported in brown rice grain from 3 Indica rice cultivars ZN7, ZN60 & ZN34 as 0.734 g/100g, 0.399 g/100g, and 0.679 g/100g, respectively [12].

Assam is bestowed with a wide diversity of rice landraces which are cultivated and preserved by farmers since a long time. Different types of landraces such as *Joha*, *Bora*, *Baodhan* and *Chokuwa* are commonly cultivated throughout Assam districts. These different landraces also exhibit compositional variations with respect to nutrition and are used in the preparation of various delicacies. The *joha* rice is aromatic and used for cooking of *kanji, pulao, payas* and other recipes. B*aodhan* is deep water rice and is a boon to flood prone areas, *bora* rice is glutinous which is used in the preparation of *ghila pitha* and served with meat dishes, *chokuwa* rice is semi glutinous soft rice and used as for making 'instant rice'. Rice has been the staple food crop of Assam and contributes to 95.51% of total food grain production of the state. It is also well known that the most commonly consumed rice varieties in Asian countries have a high glycaemic load which is the primary cause for type II diabetes mellitus [13]. Apart from the entrenched day to day and cultural preferences for rice consumption, Assam has a lower collective percentage of diabetic population (3.7%) compared to the other predominantly rice consuming states of India such as Odisha (4.9%), West Bengal (5.3%), Andhra Pradesh (6.9%), Kerala (8.1%) and Tamil Nadu (5.5%) [14]. It is believed that specific rice cultivars can elicit lower glycaemic responses which can be correlated to the low diabetes incidences in Assam. A number of studies have tried to innumerate the associations between grain composition and GI of rice [15–17]. Specifically, amylose and amylopectin ratio have been proved to be the major contributor in GI score where amylose being slowly digested would correlate negatively with high GI value. Similarly, rice with high fat, fibre, protein, antioxidants and low starch would also prove to be low in their glycaemic potency. It is presumed that these rice landraces will have wide variability in nutrient composition, particularly for amylose, starch, protein and oil content, which is also indicated through their cooking characteristics and uses for specific recipes.

Several studies have been conducted on Assam rice landraces (*joha, baodhan, chakuwa*, pigmented, and non-pigmented) along with high-yielding cultivars for assessing their nutrient variability [18–26]. However, the information regarding major landraces of Assam on wider germplasm with an assessment of essential nutritional traits including phytic acid, polyphenols, and the glycaemic index remains limited. Therefore, the present study aims to evaluate the nutritional variability of rice landraces from Assam valley by estimating total starch, amylose, dietary fiber, protein, oil, phenolic content, and total phytic acid of 395 brown rice germplasm. Based on the nutritional attributes the glycaemic index determination of 24 selected accessions was carried out to identify low GI rice landraces. Multivariate analysis techniques such as principal component analysis (PCA), hierarchical cluster analysis (HCA) and correlation studies were used to understand data structures better and determine how nutritional attributes are distributed and related. The nutritionally superior landraces would provide a broader gene pool for higher productivity and distinctive quality traits of rice such as grain GI reduction, enrichment of micronutrients and antioxidants.

2. Materials and methods

2.1. Sample collection and preparation

395 accessions of brown rice landraces were collected from Assam valley, particularly from Jorhat (Alangmora and Dangdhora) district. The sample set included local landraces along with aromatic (*joha*), deep water (*bao*), sticky (*bora* and *ampe*), and non-sticky (*sali/shali*) rice. All the samples were oven-dried overnight at 60 °*C* to remove any moisture that would interrupt husk removal from the kernel. The samples were cleaned and dehulled by subjecting them to the frictional action of rubber rollers on laboratory rice mill

(model JGMJ8098). About 20g of dried seeds were ground and homogenized by passing through a 1 mm sieve on Foss Cyclotec™ Sample Mill (Denmark). The flour thus obtained was further used for nutritional analysis.

2.2. Estimation of biochemical traits

2.2.1. Estimation of starch

The total starch content was estimated as per AOAC Method 996.11 [27]. Thermostable α-amylase was used to hydrolyze starch into branched and soluble/insoluble maltodextrin, while amyloglucosidase was used to hydrolyze maltodextrin to D-glucose further. Glucose oxidase peroxidase (GOPOD) was used to hydrolyze D-glucose into D-gluconate. The hydrogen peroxide liberated was estimated colorimetrically at 520 nm after the development of pink color and the results were expressed as g/100g.

2.2.2. Estimation of amylose content (AC)

The AC in brown rice was determined using a modified iodometric method based on the amylose-iodine binding capacity [28]. The homogenized sample was extracted for AC with sodium hydroxide and absolute ethanol, and the mixture was incubated for 15 min over the water bath. Gelatinized sample was diluted with distilled water to 50 mL and shaken vigorously. Sample aliquot was taken from the dilutions in amber tubes, and iodine solution was added under acetic acid conditions. Final dilution was done using distilled water and left for incubation at room temperature for 20 min. The absorbance was read at 620 nm against reagent blank on Benchtop Lab Systems Spectrophotometer (Cat# BT-VS-E) after the development of a blue-colored complex. A standard calibration curve was also obtained using potato amylose (Sigma Aldrich) to validate the method, and the results were expressed as g/100g.

2.2.3. Estimation of total dietary fiber (TDF)

The total dietary fiber content was estimated by the gravimetric and enzymatic method based on **AOAC 985.29** [27]**.** Duplicate samples of the dried sample (fat<10%) were first digested with heat-stable α-amylase, protease, and amyloglucosidase, and soluble fiber was precipitated with ethanol and filtered. The residue was washed with alcohol and weighed after drying. One of the duplicates was estimated for ash using a muffle furnace at 450 °C, while the other was estimated for protein by the Kjeldahl method. The result was expressed as g/100g on a dry weight basis.

2.2.4. Estimation of protein

The total nitrogen content (%N) of rice was estimated by the Kjeldahl method **(AOAC 984.13)** [27]**.** Dried flour was pre-digested overnight with a cold digestion mixture (sulphuric acid, selenium, hydrogen peroxide & lithium sulfate). The partially digested samples were subjected to a temperature of 450 °C for about 3–4 h till the solution became colorless, marking the complete digestion of the sample. Foss Tecator 2300 Kjeltech Nitrogen Auto analyzer was calibrated with ammonium sulfate for 21% total nitrogen. Ammonium from the sample was steam distilled with 40% alkali (NaOH) to liberate ammonia. An indicator solution of 1% boric acid with bromocresol green/methyl red was used to trap ammonia gas, accounting for the amount of total nitrogen present in the sample. The obtained result was %Nitrogen, and the total protein percentage was thus calculated by multiplying %N with a Jones conversion factor of 5.95.

2.2.5. Estimation of oil

The total oil content was estimated by a non-destructive, accurate, rapid method using pulsed Nuclear Magnetic Resonance (NMR) spectroscopy [29]. The method is based on the relaxation of protons when kept in an external magnetic field. The seeds were oven dried at 60 °C overnight and kept in a desiccator before analysis. The completely moisture-free samples were used to estimate oil content using Newport NMR Analyser equipped with 40 mL coil assembly, obtained from Oxfords Analytical Instruments Ltd., UK. The instrument was calibrated thrice with reference rice bran oil to acquire a 100% oil content value before sample estimations for utmost accuracy. The NMR results (signal/mass ratio) were matched with that of reference oil, and the results were expressed directly as %.

2.2.6. Estimation of total phenolic content (TPC)

The total phenolic content of brown rice was estimated using the Folin Ciocalteau reagent (FCR) spectroscopic method [30]. The ground sample was extracted overnight for phenols with 80% ethanol, centrifuged at 13000 rpm for 10 min, and the supernatant was pooled. The sample aliquot from the supernatant was treated with FCR (equal amounts of FCR and water) under sodium bicarbonate conditions. After 1 h of incubation at room temperature, the absorbance of a dark blue-colored complex was measured at 650 nm using Benchtop Lab Systems Spectrophotometer. The FCR is a mixture of phosphomolybdate and phosphotungstate, which gets reduced by phenols to form molybdenum blue under basic conditions. The measurement was compared to a calibration curve of Gallic acid, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (GAE g/100g).

2.2.7. Estimation of total phytic acid

The phytic acid content in brown rice was estimated by enzymatic method using a Megazyme phytate assay kit [31]. The method included acid extraction of inositol phosphates followed by treatment with phytase from IP6 (phytic acid) and lower phytate forms IP2, IP3, IP4, and IP5. Further treatment with alkaline phosphatase ensures the release of final phosphate from the IP1 form. The total phosphorus released was read colorimetrically at 655 nm, and results were expressed as g/100g phosphorus in the sample.

2.2.8. Estimation of glycaemic index

24 rice accessions were selected from 395 samples based on various combinations of high/low amylose with high/low starch, high oil, phytic acid and phenol contents. The Glycaemic Index of brown rice was estimated by the modified method of Frei et al., 2003 [32]. Briefly, 80 mg of a cooked rice sample and 10 mL of 0.2 M HCl–KCl buffer (pH: 1.9) were added and homogenized for about 2 min. Subsequently, 0.2 mL of pepsin solution was added and incubated in a shaking water bath at 40 °C for 1 h. This was followed by the addition of 10 mL of tris-maleate buffer and 5 mL of α-amylase and the mixture was incubated at 37 °C. Aliquots of 200 μL were collected from the incubated samples at the 30-min interval for a total duration of 3 h. After inactivating α-amylase by placing the test tubes in boiling water for 5 min, 50 μL of amyloglucosidase was added and incubated in the water bath at 50 °C for 45 min to hydrolyze starch to glucose. The glucose thus liberated was measured using GOPOD reagent by measuring the absorbance at 510 nm. GI was estimated from the hydrolysis index obtained by dividing the area under the starch hydrolysis curve of the samples with that of white bread used as a reference.

2.3. Quality control

All the estimations were carried out in duplicates to ensure the reproducibility of the results and suitable standards and reagent blanks were used to ensure accuracy. ASFRM-Rice-2 from PT -8 obtained from INMU, Thailand, was used for method validation and check recovery of protein and TDF. At the same time, Total starch control kit (K-TSCK) flours, viz. wheat starch; high amylose maize starch were used for method validation of starch. Rice reference materials (BCR-465, 466, and 467) obtained from Sigma-Aldrich were tested for method standardization and validation of amylose estimation. Oat flour control powder included in the Megazyme assay kit was used as a standard for the validation of the phytic acid estimation method. The total fat content estimation is validated for ISO10565:1998 and ISO10632:2000 standards for oilseed and their defatted residues. The instrument was calibrated thrice with reference rice bran oil before estimation to ensure the accuracy of the instrument [33].

2.4. Statistical analysis

The descriptive statistics of the data were calculated using IBM SPSS version 7.0 software, including mean, standard error, range, and standard deviation values. The histogram and box plots were developed by for each trait by Jamovi statistical package version 1.6.9 [34]. Hierarchical clustering with squared Euclidean distance method using IBM SPSS software version 7.0 was done to obtain nutritionally distinct clusters of rice. The nutrient values were used as input, and a dendrogram was obtained to form the clusters of the landraces. The linear correlation between starch, amylose, total dietary fiber, protein, oil, phenols, and phytate was done using a Pearson correlation matrix and plotted using the Jamovi statistical package. The data was also subjected to PCA using Jamovi to identify superior traits responsible for the variation among them [35].

3. Results and discussions

3.1. 1Nutritional profiling of brown rice

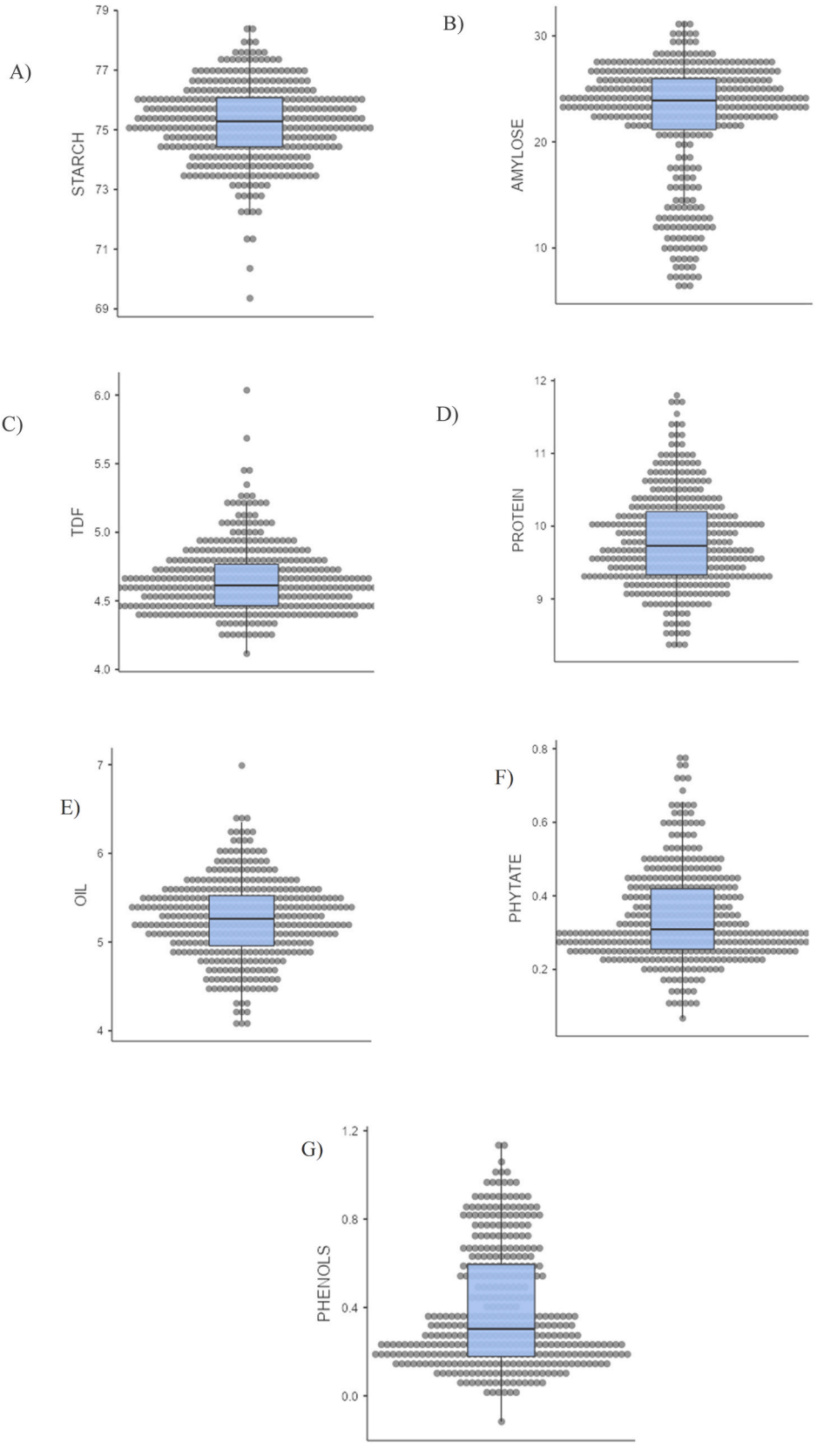
The descriptive statistics of the nutritional analysis of brown rice are summed up in Table 1. The variability of data based on box and whisker plots is presented in Fig. 1A–G. The stacked data points in the individual trait plots reveal the trait-wise data distribution. The plots show the distribution frequency in each nutritional attribute's entire range of variability. The total starch content ranged from 69.4 to 78.5 g/100g (75.2 ± 1.31), whereas AC ranged from 6.03 to 31.33 g/100g (22.3 ± 5.61). The TDF and protein contents (PC) of brown rice ranged from 4.12 to 6.04 g/100g and 8.34–11.80 g/100g with mean and standard deviation as 4.67 ± 0.26 and 9.78 ± 0.67 respectively. The total oil content ranged from 4.05 to 6.99% (5.27 ± 0.46). The total phenols and phytic acid values ranged from 0.02 to 1.34 GAE g/100g (0.40 ± 0.27) and 0.07–0.78 g/100g (0.34 ± 0.13) respectively.

3.1.1. Starch content

Starch is the major carbohydrate constituent in rice comprising 60–80% of the total composition, and is also the main source of energy in the rice grain. The average starch content found in our study for brown rice landraces was 75 g/100g, where the lowest was observed for Malbhog (IC 206031) (69.4 g/100g) and highest for Guru Pukhi (IC 350725) (78.5 g/100g). Longvah & Prasad [36] reported average starch content of 70.65 g/100g in rice landraces from Arunachal Pradesh, whereas Pachau et al. [37] reported 83.5 g/100g mean starch content for glutinous rice from Mizoram. Ashraf et al. [38] reported a mean starch value of 75.5 g/100g in pigmented and scented rice from Kashmir, which is comparable to the present study. The variation in starch contents among different

Table 1
Descriptive statistics of 395 brown rice accessions from Assam.

	PROTEIN	STARCH	AMYLOSE	TDF	OIL	PHENOL	PHYTIC ACID
N	395	395	395	395	395	395	395
Mean (%)	9.80	75.2	22.2	4.67	5.26	0.40	0.34
Std. Deviation	0.65	1.29	5.73	0.24	0.446	0.27	0.13
Range (%)	7.85–11.8	69.4–78.5	6.03–31.3	4.12–6.04	4.05–6.99	0.02–1.34	0.067–0.777



(caption on next page)

Fig. 1. A–G: Box and whiskers plots showing the variability of nutritional composition across all the traits.
(Note: Starch, amylose, TDF, protein, phenols, and phytic acid are expressed as g/100g, and Oil content is expressed as %.)

Indian states may be explained by the fact that rice genotype is intricately affected by farmers' management practices and preferences, which highly influences the genetic diversity of rice germplasm. Rice starch offers various advantages to the food and processing industries in end-use quality over other starches since it is white, hypoallergenic, tastes bland, and has a smooth gel texture. Different food products require diverse starch properties such as biscuits or crackers require waxy and low amylose starches, whereas puffed rice, noodles, and bread require high amylose starch [39].

3.1.2. Amylose content (AC)

The amylose content in rice is a governing factor for its cooking characteristics and textural properties. It is directly proportional to cooked rice's firmness, grain expansion, and water absorption, whereas AC is inversely proportional to its glossiness, stickiness, and tenderness [40]**.** The rice landraces from Assam exhibited a wide variation in AC in the present study. Many accessions containing very low AC (glutinous) were observed such as Poita bora (IC464430) (6.03 g/100g), Borni (IC463883) (6.9 g/100g), Ronga Bora 1 (IC464351) (7.8 g/100g) and those with intermediate and high AC were also observed such as Maniki Madhuri 2 (IC463708) (16.1 g/100g), Malbhog (IC206031) (20.2 g/100g), Ronga Sali (IC459978×) (24.2 g/100g) and Moina Giri (IC463704) (31.3 g/100g). The obtained range is slightly wider than that reported by Sahu et al. [41] with an AC range of 11.8–33.2 g/100g for Chhatisgarh rice landraces. Govindraju et al. [42] also **r**eported the AC range of 7.5–28.6 g/100g for indigenous rice varieties of Assam. Rice with high AC tends to have harder cooking properties, while low amylose containing rice tends to have a softer texture. AC in rice can dramatically affect the consumer's preferences and hence has achieved importance from the breeder's point of view.

3.1.3. Amylose:Amylopectin ratio (Amy:Amyp)

Starch is the major component of the rice grain and consists of two glucose polymers-linear amylose and the highly branched amylopectin. Therefore, amylopectin content can be calculated as total starch minus total amylose contents (Table 1 supplementary data). The amylose and amylopectin molecule behavior contributes to starch digestibility and therefore the digestive properties of rice significantly depend on amy:amyp ratio [43]. The average amy:amyp ratio of the landraces was 29/67, which corresponds to moderate amylose content (≤25%). The lowest amy:amyp ratio of 7/80 was observed for Poita bora (IC464430) followed by Lugum ampe (IC610256) (2/21) and Borni (IC463883) (5/51), which belong to the sticky rice category from Assam namely *bora* and *ampe.* The higher amy:amyp ratio was observed for Moina Giri (IC463704) (30/41), Buruli Bao 1 (IC464451) (7/10), and Amona Bao 1 (IC591483) (7/10). The *in vivo* and *in vitro* starch digestion rate is negatively correlated with amylose content and positively correlated with amylopectin content. This could be explained by the fact that amylose-rich starch is prone to retrogradation or re-crystallinity, which is a phase separation involving the alignment of linear starches forming a rigid gel with inhibited enzyme hydrolysis and reduction in starch digestion. Whereas, waxy starches (low amy:amyp ratio) lead to imperfect crystal formation during retrogradation, providing access to digestive enzymes and a lower reduction in *in vitro* starch hydrolysis [44]**.** Thus, the digestibility of starch accounts for the rapid release of glucose and influences the Glycaemic Index (GI). Therefore, low glycaemic rice is in demand to meet the needs of the fast-rising diabetic population for whom rice is a primary staple food.

3.1.4. Total dietary fibre (TDF)

The mean TDF content obtained for brown rice was 4.67 g/100g, and the results obtained in this study were comparable to that reported for popular Indian rice varieties with a range of 2.73–4.89 g/100g [45]. Total dietary fiber (TDF) is largely plant cell wall material that consists of non-starch polysaccharides (insoluble and soluble fibers), lignin, oligosaccharides, and resistant starch, where cellulose, arabinoxylans, and pectin substances are the major fiber constituents in rice. The physical properties of dietary fibers (soluble) significantly affect the digestion process by reducing lipid absorption and hindering the action of hydrolytic enzymes in the small intestine. Meanwhile, the insoluble fibers are fermentable in the gut, resulting in the production of short-chain fatty acids required for glucose modulation and lipid metabolism in the liver. Whole-meal cereals containing dietary fibers affect the metabolism by slowing down glucose absorption, inferring that higher the viscosity of food in terms of fiber, the lower is its GI value [46]. Accessions like Jeera Joha 2 (IC458454×) (6.04 g/100g), Kola Amona Bao 3 (IC332920) (5.35 g/100g), Kon Joha (25) (IC579766) (5.21 g/100g) could be consumed for their high TDF, moderate amylose and desirable PC. These accessions also contain higher oil content, which can be explained by the positive correlation between TDF and oil (Table 4). The study is evident of the significant amount of dietary fiber in brown rice; hence, regular consumption of brown rice can be useful in sufficient fiber uptake.

3.1.5. Protein content (PC)

Apart from starch, protein is an essential macronutrient of rice which is present in the form of enzymes, nucleic acids, and amino acids. A protein content of more than 10% in rice is classified as high protein, and various accessions were identified as unique with significantly high PC, such as Ghiw Bora (IC558248) (11.74 g/100g), Boga Joha 2 (IC512867) (11.71 g/100g) and Rajdhan (IC463183) (11.54 g/100g). **Longvah & Prasad** [36] reported a protein range of 6.2–10.2 g/100g for Arunachal Pradesh rice landraces, and **Rayala et al.** [47] reported a mean PC value of 8.1 g/100g for Indian rice landraces from Hyderabad. **Patil et al.** [48] reported comparable PC for Chattisgarh rice landraces, whereas a comparatively lower PC mean of 6.63 g/100g was reported for diverse Indian landraces by **Roy et al.** [49]**.** The maximum PC was observed for Pakhi bora (11.8 g/100g) amongst the landraces from Assam. Protein content enhancement through plant breeding programs can be considered if rice's nutritional value is concerned,

especially in countries where rice is a part of every meal. PC affects the texture of cooked rice as highly dispersed protein bodies associated with starch granules would restrain starch from swelling and limit the softening effect of cooking [50]. While protein-rich rice will be tougher and chewier than low-protein rice, reports available on rice suggest that the endogenous protein in foods reduces starch hydrolysis. These proteins have been reported to probably reduce the GI of Indica rice cultivars by coating the starch granule and preventing enzyme action [51].

3.1.6. Total oil content

The total oil content for brown rice ranged from 4.05 to 6.99% and the highest was observed for Malbhog (IC206031) (6.99%), followed by Gettu (IC362048) (6.44%), Betguti 1 (IC267330) (6.24%), whereas the lowest was observed for Ishaguni Ahu (IC449605) (4.05%). The obtained oil content in brown rice was significantly higher than that reported by **Tegegne et al.** [52] **(**0.61–2.35%) for Ethiopian rice varieties and 2.63% for hybrid rice varieties from Assam [26]**.** The obtained oil range for Assam rice was narrower than the lipid range reported by **Abubakar et al.** [53]**,** i.e., 2.90–8.90% for Malaysian rice. High-quality unsaturated fatty acids (MUFA) in starchy foods like rice provides enhanced palatability where high oil-containing accessions could be promoted for market purposes. Total fat in rice is required for the solubilizing and intestinal absorption of lipid-soluble vitamins such as A, D, E, and K [53]. Lipids have also been known to form amylose-lipid inclusion compounds, which have slow digestibility and thus reduce post-prandial serum glucose and insulin response [54]. Rice bran removed during milling rice is commercially exploited for extracting high food value rice bran oil. Since most of the fat is concentrated in the aleuronic (bran) layer of the rice kernel, the difference in the milling of accessions may result in a variation in rice fat content. Therefore, brown rice accessions from this study can be extracted for rice bran oil since it contains essential fatty acids like linoleic acid and no cholesterol, making it a suitable substitute for cooking oil [4]**.**

3.1.7. Total phenolic content (TPC)

The TPC of brown rice ranged from 0.016 GAE g/100g to 1.34 GAE g/100g. The higher phenol content was observed in Phul Pakri (IC466567) (1.15 GAE g/100g), Shiyal Sali (IC380636) (1.007 GAE g/100g), and Bam Kokowa Bao (IC134763) (0.987 GAE g/100g) as compared to other cultivars of rice. Whereas Pokhar Kala Bora (IC332950) (0.051 GAE g/100g) with Kalamdani (IC465307) (0.063 GAE g/100g) and Katenewli (IC37003) (0.09 GAE g/100g) were observed towards lower phenol values. Various polyphenol compounds are found in food products derived from plants comprising of dietary-rich antioxidants which are protective against oxidative damage [55]. Several studies have reported higher values of phenolic content in brown and pigmented rice, which is also associated with anti-oxidative and metal-chelating activities. In the present study, it was observed that deep water (*bao*) rice from Assam generally contained higher polyphenolic content. The *bao* rice accessions with red kernel, such as Negheri bao (IC332991) (0.834 GAE g/100g), Jhul Bao (IC516684) (0.860 GAE g/100g), and Bam Kokowa bao (IC34763) (0.987 GAE g/100g) contained higher TPC values. Not strictly enumerated, it is reported that Assam contains more than 70 *bao* landraces, and they are rich in dietary polyphenols such as anthocyanins, which have sufficient *in vitro* anti-oxidative activities [25]. Thithipramote et al. [56] reported similar polyphenol content of 0.069–1.018 GAE g/100g for red rice from Thailand. Polyphenolics have high anti-oxidative properties attributed to their chemical structure and high affinity towards reactive oxygen species (ROS). Moreover, antioxidative compounds are known to reduce *in vitro* starch digestibility [57]. And based on the TPC results, brown and pigmented rice landraces have high nutraceutical values, which are more health-beneficial food for people with diabetes than milled rice.

3.1.8. Total phytic acid (PA.)

Phytic acid has long been known as a chelating agent for critical cations like Ca2+, Fe2+, and Zn2+, which results in reduced absorption of minerals in the human body. Low-grain PA is a genetically governed factor and thus, identifying source accessions containing lesser PA amounts is essential for plant breeding programs. The total phytic acid content in our study ranged from 0.067 to 0.777 g/100g. Longvah & Prasad [36] reported a phytic acid range of 0.331–0.407 g/100g for rice landraces from Arunachal Pradesh, while Gyani et al. [58] reported a total phytic acid range of 0.117–0.334 g/100g in traditional Indian rice landraces. The present study consisted of many accessions with very low phytic acid contents, with the least in Kopak Ampe (IC458488×) (0.067 g/100g) and Borni (IC463883) (0.098 g/100g) followed by Kola Bora (IC464463) (0.104 g/100g). The accessions with low PA from the study could be used as promising donors for rice biofortification programs.

3.1.9. Estimated glycaemic index (EGI)

The glucose-raising ability of food in the blood categorises it as high (>70), medium (56–69), and low GI (<55) food. The glycaemic index score of 24 selected rice accessions ranged from 43.9 to 68.5. As anticipated, 17 of the 24 selected accessions with high amylose, phytic acid, and phenol in our study were found to be in the low GI category (Table 5) explained by the negative correlation between AC, PC, and TPC with GI. Moreover, ten landraces, including Lota Sali (IC466974), Edolia Bao (IC554930), Amona Bao (IC591483), were found to have GI < 50, which could be beneficial for the diabetic population and thus promoted for commercialization.

3.2. Statistical analysis

3.2.1. Principal component analysis

Principal component analysis has been traditionally used to decipher the information regarding the association of biochemical traits amongst each other. PCA extracts the relevant information from the dataset and presents it as new orthogonal variables, called principal components, along with the patterns of similarities of observations and variables in the form of scree plots.

395 accessions of brown rice were subjected to factor analysis, i.e., PCA for the worked biochemical traits, which is extensively used

for data reconstruction and dimension reduction. Loadings were studied based on sample grouping/differentiation and variance explained to understand and identify the relationship between data structure. Each principal component has a vector called the eigenvector, and the variance along the vector is called the eigenvalue, which was used to indicate the amount of variance contributed by the components. Four components have been identified (Table 2), where large positive factor loading values were indicated by protein (FL1: 0.925), phenols (FL4: 0.890), oil (FL2: 0.862), phytic acid (FL3: 0.803), TDF (FL2: 0.796) and amylose (FL3: 0.711). Negative factor loading values were indicated by starch (FL1: −0.886) and phytic acid (FL4: −0.340). In our study, the first four principal components accounted for 81.3% variability (Table 3), i.e., PC1, PC2, PC3, and PC4 (eigenvalue>1), where the rest of the principal components accounted for less variability. In PC1, protein (0.925) contributes to the maximum variability, followed by starch (−0.886) in the negative axis, accounting for 27.8% of the total variation. The second principal component, PC2, contributed 22.85% of the total variation, with maximum variability contributed by oil (0.862) followed by TDF (0.716). In PC3, phytic acid (0.803) contributed the maximum to the variability, followed by amylose (0.711), accounting for 16.05% of the total variation. The fourth principal component (PC4) accounted for 14.96% of the total variation, where phenols (0.890) contributed the most, followed by amylose (0.463), whereas phytic acid (−0.340) contributed in the negative axis.

3.2.2. Hierarchical clustering analysis

The hierarchical clustering analysis groups accessions based on their similarities and delineates a hierarchy between groups and subgroups. The HCA results are illustrated in a dendrogram, a tree-like plot depicting sample organization at different similarity levels.

The accession diversity is shown in terms of nutrition via a dendrogram obtained from hierarchical clustering. The dendrogram of rice landraces formed 16 major clusters, grouping the accessions with similar characteristics. Each sample was initially treated as an individual cluster, and then the clusters were subsequently merged based on similar characteristics. Euclidean square distance of 5 was used as a distance linkage metric between groups. The dendrogram of these landraces depicts their heterogeneity in terms of nutrition (Supplementary Fig. 1). The means of individual clusters and the number of cases in each cluster obtained from HCA are given in Table 4.

Cluster I is characterized by accessions with moderate amylose (22.2 g/100g) and high protein content (10.4 g/100g). Cluster II comprises accessions with very low AC (7.74 g/100g). Cluster III is characterized by low AC (10.5 g/100g) with high protein accessions (10.4 g/100g). Cluster IV contains only one accession Malbhog and is characterized by high oil (6.99%), high fiber (5.26 g/100g), low starch (69.3 g/100g), and moderate AC (20.2 g/100g). Cluster V contains accessions with high AC (25 g/100g) along with high PC (10.3 g/100g). Cluster VI comprises accessions with low AC (13 g/100g) and high PC (10.9 g/100g). Cluster VII contains very high amylose-containing accessions (29.6 g/100g) along with low oil (4.81%). Clusters VIII and IX are characterized by low amylose (16 g/100g) and moderate starch (approx. 75 g/100g) containing accessions. Cluster X is characterized by moderate AC (24 g/100g), and cluster XI showed high AC (27.2 g/100g) and phytic acid (0.46 g/100g). Cluster XIII is characterized by low amylose-containing germplasm (13.2 g/100g). Cluster XIV contains accessions with low AC (11.2 g/100g) and high starch content (77 g/100g). Cluster XV consists of high protein (10.9 g/100g), low amylose (18 g/100g), and moderate starch (74.3 g/100g) containing accessions. Cluster XVI is characterized by high oil (6%), low amylose (10.6 g/100g), low starch (71.3 g/100g), and high fiber (5.28 g/100g) contents.

Accessions such as Poita bora, Thupi bora, and Ronga bora are grouped in Cluster II, which have mean AC <10 g/100g. The AC of rice accounts for its cooking qualities, such as grain puffiness, stickiness, and firmness. Consumers in South Asia and the Middle East prefer dry and flaky rice, whereas Korea, Egypt, Taiwan, and Japan prefer sticky rice; therefore, cluster I can be used to prepare regional delicacies like sushi and bibimbap, while some sticky rice is also used in the preparation of rice beers due to its sweet taste [59]. Accessions falling in clusters I, V, X, and XII are high-quality cooking rice and hence can be used to prepare dishes like *biryani* which need firm and elongated rice. Clusters III, VI, and XV contain a high amount of protein and low amylose rice, which can be promoted towards protein enrichment programs. Clusters VII and XI accessions would be highly resistant to swelling and retain their integrity at elevated temperatures due to high amylose content. Cluster IV consists of a single accession Malbhog with the highest oil, lowest starch, low phytate, moderate amylose, and high fiber contents. Malbhog can not only be extracted for rice bran oil, but it can also be promoted for individuals with bowel disorders and cardiovascular ailments.

Moreover, Malbhog can be promoted for trait enhancement programs owing to its low starch content. Cluster XVI is marked by high oil, low starch, and low amylose rice. Besides being used as low glycaemic rice, they can also be extracted for rice bran oil which is commercially exploited due to its MUFA content.

Table 2
Component loadings for PCA with varimax rotation.

	Component				
	1	2	3	4	Uniqueness
PROTEIN	0.925				0.0997
STARCH	−0.886				0.1642
OIL		0.862			0.1519
TDF	0.387	0.796			0.2121
PHYTIC ACID			0.803	−0.340	0.2260
AMYLOSE			0.711	0.463	0.2265
PHENOLS				0.890	0.2010

Table 3
Principal component analysis and the contribution of each biochemical trait in data variance.

Component	Eigenvalue	% Of variance	Cumulative %
1	1.948	27.83	27.834
2	1.599	22.85	50.684
3	1.124	16.05	66.735
4	1.047	14.95	81.694
5	0.667	9.534	91.228
6	0.415	5.933	97.161
7	0.199	2.839	100.000

Table 4
Means of individual clusters (16) obtained from hierarchical cluster analysis.

CLUSTER	NO. OF CASES IN EACH CLUSTER	PROTEIN (g/100g)	TDF (g/100g)	STARCH (g/100g)	AMYLOSE (g/100g)	OIL (%)	Phenol (g/100g)	Phytic acid (g/100g)
I	31	10.40	4.65	74.00	22.32	5.16	0.684	0.251
II	17	9.86	4.65	75.74	7.74	5.52	0.087	0.345
III	10	10.43	4.70	74.61	10.52	5.41	0.710	0.275
IV	1	9.60	5.26	69.36	20.23	6.99	0.060	0.129
V	54	10.27	4.78	74.24	24.18	5.19	0.767	0.222
VI	9	10.95	4.78	73.78	13.07	5.13	0.104	0.249
VII	18	9.57	4.55	74.99	29.62	4.81	0.457	0.106
VIII	11	9.54	4.57	76.06	16.00	5.46	0.189	0.470
IX	48	10.05	4.67	74.35	26.21	5.22	0.183	0.418
X	61	9.44	4.59	76.10	24.14	5.26	0.106	0.394
XI	57	9.42	4.54	75.83	27.21	5.13	0.385	0.359
XII	39	9.24	4.61	76.69	21.70	5.43	0.768	0.098
XIII	15	9.68	4.59	75.52	13.23	5.42	0.168	0.394
XIV	10	9.18	4.58	77.04	11.23	5.67	0.195	0.278
XV	11	10.8	4.72	74.06	18.07	5.11	0.674	0.278
XVI	3	9.56	5.28	71.35	10.65	6.00	0.106	0.249

Table 5
Estimated Glycaemic Index (EGI) of 24 selected brown rice landraces.

S.No.	Sample ID	EGI	S.No.	Sample ID	EGI
1	Migum Sali	48.38	13	Kola Bora	68.53
2	Lota Sali	43.97	14	Edolia Bao 2	47.99
3	Kerker Shali	48.69	15	Shiyal Sali 2	48.97
4	Manipuri Joha	49.88	16	Damaguri Bao	46.23
5	Jengoni Ampe 1	59.61	17	Banki Sali 1	44.17
6	Dakdung Bao 1	51.08	18	Gela Jahingia	51.55
7	Getho	53.7	19	Poita Bora	59.04
8	Mishiri Sali	54.24	20	Buruli Bao 1	52.5
9	Ronga Chakowa Dhan	45.73	21	Bao Dhan 1	56.94
10	Jhul Bao	54.84	22	Kola Joha L	51.87
11	Kon Joha 2	51.92	23	Lugum Ampe	61.42
12	Negheri Bao 4	58.71	24	Amona Bao	46.5

3.3. Correlation

The correlation between total starch, amylose, dietary fiber, protein, oil, phenols, phytic acid, and GI was carried out, and the correlation matrix indicates a relationship among these traits of brown rice (Table 6). At $p < 0.05$, Pearson's correlation constant $r > 0$ is perfect positive, while $r < 0$ is a perfect negative correlation. Multiple factors like growing conditions, climate, and soil type can cause variations in the phytochemical composition of the rice grain. As evident from the 'r' values, a highly negative correlation was observed between starch and protein ($r = -0.69$, $p < 0.001$). Possibly for higher protein content, starch accumulation occurs, resulting in decreased activity of starch synthases due to thermal denaturation [60]. TDF with starch had a significant negative correlation ($r = -0.38$) since non-starch carbohydrates like fiber can complex with starch and inhibit starch digestion or antagonistically affect digestive enzymes [40]. A significant negative correlation was seen for amylose and protein ($r = -0.14$, $p < 0.01$) and amylose with TDF ($r = -0.12$, $p < 0.05$) as amylose comprising of α-1,4 glucose units constitute <30% of rice starch. The correlation of oil with protein was significantly negative ($r = -0.29$, $p < 0.001$) because the synthesis of storage proteins in protein-rich seeds would consume excess carbon skeletons lacking their availability for lipid biosynthesis [61]. Amylose and oil correlated negatively ($r = -0.3$, $p < 0.001$), which can be explained by the fact that in the case of high amylose, crystallization of starch is favored resulting in the rigidness

Table 6
Pearson Correlation data of biochemical traits for 395 brown rice accessions along with EGI of 24 accessions.

		STARCH	AMYLOSE	PROTEIN	OIL	TDF	PHENOLS	PHYTIC ACID	EGI
STARCH	Pearson's r								
	P value								
	N	395							
AMYLOSE	Pearson's r	0.035							
	P value	0.483							
	N	395							
PROTEIN	Pearson's r	−0.691***	−0.136**						
	P value	<.001	0.007						
	N	395							
OIL	Pearson's r	0.041	−0.299***	−0.293***					
	P value	0.416	<.001	<.001					
	N	395							
TDF	Pearson's r	−0.376***	−0.116*	0.227***	0.408***				
	P value	<.001	0.021	<.001	<.001				
	N	395							
PHENOLS	Pearson's r	−0.023	−0.167***	−0.098	−0.096	−0.076			
	P value	0.648	<.001	0.051	0.057	0.133			
	N	395							
PHYTIC ACID	Pearson's r	−0.101*	0.175***	0.326*	−0.052	0.057	0.152**		
	P value	0.044	<.001	0.012	0.304	0.261	0.002		
	N	395							
EGI	Pearson's r	0.22	−.803**	0.052	0.274	0.006	−.597**	−.521**	
	P value	0.917	0.00	0.808	0.195	0.980	0.002	0.009	
	N	24							

(*Correlation is significant at the 0.05 confidence level (2-tailed)), (**Correlation is significant at the 0.01 confidence level (2-tailed)), (***Correlation is significant at the 0.001 confidence level (2-tailed)).

of cooked rice. Whereas in the case of high-fat amylose-fat complex forms correspond to the increased springiness of the cooked grain [62].

There is a highly significant positive correlation between TDF and oil content (0.407, $p < 0.001$) as both fiber and fat decrease starch digestibility (as evidenced by the negative correlation of starch with TDF and oil) and thus would have a positive correlation with each other. A highly significant negative correlation was found between GI and amylose ($r = -0.803$, $p < 0.01$), this could be due to the formation of complexes between amylose and lipids upon heating, thus making them less accessible to enzymatic digestion, resulting in a slower rate of digestion [63]. Along with amylose, GI was significantly correlated negatively with anti-nutritional factors; phenols ($r = -0.597$, $p < 0.01$) as TPC is involved in direct inhibition of α-glycosidase enzymes and/or formation of complexes with starch [62] and phytic acid ($r = -0.521$, $p < 0.01$) due to the high chelation effect of phytic acid which interrupts the action of α-amylase activity [64]. Phytic acid is known to chelate divalent cations and reduce mineral bioavailability, and probably the utilization of protein for the formation of phytate-mineral chelate [65] explains the significant positive correlation of phytate with protein ($r = 0.326$, $p < 0.05$). Phytic acid is also capable of binding with starch or amylose through phosphate linkage resulting in decreased starch digestibility. Despite anti-oxidative properties, phenolic compounds are also considered to be Fe-binding inhibiting Fe absorption, which explains the positive correlation between phytic acid and phenols (0.152, $p < 0.01$). Amylose and phenols correlated negatively ($r = -0.167$, $p < 0.01$) since the presence of amylose in any solution could bond with water molecules reducing water availability for phenol extraction [66].

4. Conclusion

The present study may be considered the first report on nutritive profiling of diverse rice landraces from Assam. Wide genetic variability was found for most of the nutritional traits, indicating the potential of using these accessions as parents for quality improvement programs or being released directly as cultivars. Accessions like Malbhog (IC206031), Rajdhan (IC463183), Poita Bora (IC464430), Kokowa Bao (IC134763) were found superior in terms of nutritional aspect. Four principal components were revealed by PCA, where the maximum contributor to the variability observed was protein, followed by phenols, starch, and oil. HCA revealed 16 different clusters where many Nutri-dense accessions have more than one trait with nutritionally high values. The study would not only provide the most sought-after nutritive value of rice by consumers but would also help to orient the work of investigators toward varietal selection. Only a few components of the plant genetic resources of rice, particularly landraces are being used in breeding programs which may lead to the narrowing of the genetic base in improved varieties. The distinct groups identified in this study, if treated with agro-morphological and genetic traits, could be promoted for developing novel cultivars through breeding programs. Furthermore, these can be probable candidates for commercialization to provide nutrition to the population where rice is a staple food. The study included nutritional profiling of landraces from the Jorhat district of Assam, whereas, in the future, landraces from varied districts could be considered for quality evaluation which could be of significance in improved nutritive value while being more adapted to agro-ecological conditions in the state of Assam.

Author contribution statement

Racheal John: Performed the experiments; Wrote the paper. Haritha Bollinedi, Christine Jeyaseelan, Rakesh Singh, Sudhir Pal Ahlawat: Analyzed and interpreted the data. Siddhant Ranjan Padhi, Neha Sajwan: Performed the experiments. Dhrubjyoti Nath, Jai Chand Rana: Contributed reagents, materials, analysis tools or data. Rakesh Bhardwaj: Conceived and designed the experiments.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17524.

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