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Original Research Article

Nutritional composition patterns and application of multivariate analysis to evaluate indigenous Pearl millet ((*Pennisetum glaucum* (L.) R. Br.) germplasm

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ABSTRACT

The nutritional composition of 87 diverse Pearl millet (*Pennisetum glaucum* (L.) R. Br.) germplasm including landraces and commercial varieties was assessed through standard protocols. The results indicated a substantial variability in total carbohydrates [starch (50.37–63.25), amylose (19.26–27.90), sucrose (0.58–1.53), glucose (0.32–0.75), resistant starch (RS) (1.49–3.52), total soluble sugars (TSS) (1.53–3.22), expressed as g/100 g], protein (8.07–18.15 g/100 g), total dietary fibre (TDF) (7.68–16.18 g/100 g), lipids and fatty acids [total lipid (5.24–9.99), palmitic (20.30–32.49), linoleic (32.11–46.91), oleic (21.99–33.43) and stearic acid (3.28–7.91) expressed as g/100 g], antinutritional factors [phytic acid (0.54–1.43 g/100 g) and raffinose family oligosaccharides (RFOs) (0.27–2.08 mmol/100 g)], phenols (0.04–0.21 g/100 g), and minerals. Multivariate analysis using hierarchical clustering analysis (HCA), and principal component analysis (PCA) was used to decipher the diversity of these attributes across germplasm. Multivariate data analysis (MVDA) can be applied for deciphering the differences/ similarities between multiple nutritional attributes, sample types or for projecting the object in a two/ three-dimensional factor-plane, determined based on various distinct characteristics. HCA revealed that Cluster I, II and III showed higher content of amylose, starch, moisture, cluster III had higher lipid content. cluster I, II, III and IV showed higher RS. Cluster II and III had higher TSS, cluster III showed higher sucrose content. Cluster V and VII were indicated by higher glucose and protein content. Cluster II, III, IV and VI showed phytic acid content and cluster III showed higher mineral content. The germplasm displayed distinct regiospecific variations in their nutritional content. Those derived from Gujarat, Maharashtra and Uttarakhand showed higher protein content. Those derived from Haryana, Karnataka, New Delhi, Punjab, Tamil Nadu and Uttar Pradesh showed high carbohydrates. Those from New Delhi, Punjab, Tamil Nadu and Uttarakhand showed high Iron content and high copper was found in germplasm from Maharashtra, Punjab and Tamil Nadu. High zinc was found in germplasms from Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh. More calcium was found in germplasm from Maharashtra, Punjab, New Delhi, and Tamil Nadu. The analysis can form the basis for the commercialization and utilization of pearl millet using efficacious breeding strategies.

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1. Introduction

The inherent biodiversity, high productivity, need for minimal agricultural inputs and the hardy nature of Pearl millet (PM) makes it a crop of choice for several farmers in India (Krishnan and Meera, 2018). This crop can be used as a staple food and can successfully fulfil the crucial nutritional requirements for a large population in developing and underdeveloped nations particularly in Asia and Africa (Annor et al., 2017). Lack of gluten in this crop makes it highly suitable for people suffering from coeliac disease and its hypoglycemic property can be used to effectively manage type II diabetes (Dias-Martins et al., 2018). Global millet production was estimated at around 30 million tons in 2016, out of which India (primarily Karnataka, Madhya Pradesh, Gujarat, Maharashtra, Haryana, Uttar Pradesh, and Rajasthan contributed to about 10.28 million tons (FAO 2016; Yadav, 2011).

Since the late 1980s, the production of PM in India has increased consistently with the advent of high-yielding hybrid varieties and the mining of biochemically diverse germplasm. There has been a recent increase in demand for natural food diversification through food products derived from whole PM grains owing to their high content of bioactive compounds, micronutrients, and dietary fibre (Gong et al., 2018). Carbohydrates are the major components of PM, chiefly starch (56–65 %) which is similar to that of wheat (69 %) and lower than that of maize (78 %), rice (85 %). About 20–22 % of this starch is present as amylose (Krishnan and Meera, 2018). The adequate composition of starch and amylose determines the functional properties of PM starch like gelatinization (important for the food industry) and hypoglycemic properties (Annor et al., 2017). The optimum quantity of resistant starch (2.8–5.1 %) and dietary fibre or non-starch polysaccharides (11.9–13.3 %) serve as important modulators of starch digestibility, cholesterol level and bowel health. Proteins are the second most important biochemical component in PM grains amounting to 11.8 %, which is higher than that of rice (8.6 %), maize (9.2 %) and similar to that of sorghum (10.7 %). PM protein is rich in glutamic acid, a non-essential amino acid (Adebiyi et al., 2017) that functions as a neurotransmitter or a precursor for γ-aminobutyric acid (GABA). It also reduces menopausal symptoms by attenuating estrogen deficiency (Han et al., 2015). PM grains have a substantially higher lipid content, of about 6.4 %, which is twice the amount in maize (3.3 %) and sorghum (3.4 %). The major fatty acids present in lipids include 20–21 % palmitic acid (C16:0), 21–27 % oleic acid (C18:1) and 39–45 % linoleic acid (C18:2) (Amponsah et al., 2015). Although unsaturated fatty acid-rich lipids negatively influence flour stability, they positively modulate human health and metabolism. Phenolic content of 1478 μg/g, in PM grains, is substantially higher than that of oats (472 μg/g), maize (601 μg/g), sorghum (746 μg/g), wheat (1342 μg/g), barley (1346 μg/g), and rye (1366 μg/g) (Dykes and Rooney, 2007). These phenolic compounds display potent antiproliferative, anti-inflammatory and antioxidant effects (Tian et al., 2020b).

Phytic acid (*myo*-inositol 1,2,3,4,5,6 hexa*kis* phosphate) is an antinutritional factor that reduces the bioavailability of essential divalent metal ions like manganese, calcium, magnesium, iron, zinc by chelating them (Marathe et al., 2018). The phytic acid content of PM grains ranges from 0.58 to 1.38 % and is close to that of cereals like sorghum, maize and higher than that of wheat, rye, barley, rice, and oats (Gabaza et al., 2018). PM grains also have another group of antinutritional factors called the raffinose family oligosaccharides (RFOs) which are composed of raffinose, stachyose, and verbascose. These indigestible heat stable oligosaccharides are known to cause abdominal discomfort and flatulence (Kumar Dixit et al., 2011). The ash content of PM is about 1.8 %, which is lower than that of barley (2.2 %), oats (2.5 %), higher than rice (0.9 %), maize (1.3 %) and similar to that of wheat (1.9 %) (Dias-Martins et al., 2018). With respect to minerals, PM is a rich source of iron, copper, zinc, potassium, calcium, sodium, magnesium and phosphorus (Oshodi et al., 1999; Krishnan and Meera, 2018). These minerals modulate the enzyme activities by acting as cofactors, regulating the immunity, metabolic systems, cell signalling and other vital activities (Mohammad et al., 2017).

Although PM is widely consumed as local traditional foods in form of sweets, couscous, flatbreads, porridges, paruthi paal (Kumar, 2019; Dias-Martins et al., 2018), and their nutritional attributes are well-described in the previous literature, the biochemical information of PM which is available in the database is very scanty and dispersed, especially for the popularly grown Indian cultivars and accessions. Also, comprehensive information on the nutritional, antinutritional, bioactive and mineral composition of this grain is still lacking specifically with respect to Indian scenario.

Traditionally the concentrations are usually determined by univariate analysis, which involves isolating a single variable. Despite being simple and intuitive, this data analysis approach is wasteful and limited. For example, if a UV–vis spectrum is generated for a specific analyte with 500 data points, only a single point is used for determining the concentration (absorbance at a single wavelength), while discarding 99.8 % of the generated data. Additionally, univariate measurements are exceedingly sensitive to interferents (Keithley et al., 2009). It is almost unfeasible to differentiate an analyte-specific signal from an interferent when viewing a single data point. Recently, a higher development level is reflected and will even escalate into increased complexity of data and variables associated with nutritional analysis. In this, multivariate approaches are highly profitable, being able to effectively manage complex data (Granato et al., 2018). Multivariate statistics circumscribes the concurrent analysis of one or more dependent variables (outcome variable), against two or more independent variables (input variable) (Hidalgo and Goodman, 2013). Also in many circumstances, these multivariate statistical procedures can compare a large number of dependent variables (responses) against a plenitude of predictors (independent variables). Appropriate use of multivariate data analysis (MVDA) is pertinent to assess the association between the biochemical characteristics of a sample under investigation. In the real scenario, MVDA can be applied for deciphering the differences/ similarities between multiple attributes, sample types or for projecting the object in a two/ three-dimensional factor-plane, determined based on various distinct characteristics.

Pattern recognition techniques like principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been used as primary statistical tools for augmenting our understanding of the data structure and decipher the distribution and association between nutritional traits. PCA is a useful tool and exclusively used for exploratory data analysis. It provides a comprehensive overview of the data under study while uncovering the existing complex interrelations among and between samples and specific variables (Giuliani, 2017). PCA algorithms explore the direction of the highest variance in a multidimensional data space, based on the hypothesis that high variability (indicated by a high variance value) corresponds to a high amount of information. For discerning the correctness of this direction, the data matrix must be at least column-wise mean-centred, ensuring that the axes rotate about the centroid of the data. The rotated axis, accounting for the maximum variance, depicts the lowest-order (first) principal component (PC1). The second axis lies in the direction keeping the highest variance orthogonal (non-correlated) among all directions with respect to PC1. Consequently, the second PC (PC2) describes the maximum information which is not explained by PC1. All the further PCs (PC3, PC4 and so on) can be defined as per the same scheme (Granato et al., 2018). Every sample can be projected in a space defined by new variables: the obtained coordinate values are defined as scores. PCs are described as a linear combination of the original variables, with each multiplied by a weight coefficient known as the loading. From a geometrical standpoint, loadings represent the cosine angle values between the original variables and PCs. These values can fall in a range between −1 and +1, suggesting their importance in defining a given PC. The larger the value of a cosine, the smaller is the angle of rotation between the variable and the PC, the closer are the two directions, the greater is the contribution of that

specific variable to the PC. The fundamental feature of PCA is its unique ability to represent a large amount of complex information present in the data through basic bidimensional or tridimensional plots [loading plots, score plots and biplots(superimposition of loading and score plots)].

HCA is a branch of exploratory analysis that can differentiate groupings among samples, or rarely among variables through density or similarity measurement. HCA, a clustering technique that explores the organization of samples into groups and among groups, delineating a hierarchy. The results of HCA are generally presented as a dendrogram, a plot showing sample organization and its association in form of a tree where the samples are connected through branches at corresponding similarity levels. Similarities in traits among two different samples are normally defined by an inverse function of their inter-distance. This is based on the presumption that the samples which are fair in the descriptor space are quite dissimilar. A number of metrics are used for evaluating the distance between two samples in multivariate space, the most commonly used ones are Mahalanobis distance and Euclidean distance. Ward's method, uses the optimal value of a specific function. Clustering techniques belong to two families: hierarchical (divisive or agglomerative) and nonhierarchical. In agglomerative clustering, each sample is originally considered as a cluster and subsequently, the cluster pairs are merged. In a divisive approach, the algorithm starts with a single cluster encompassing all the samples, recursive splits are performed.

The present study was undertaken to evaluate carbohydrates (starch, amylose, sucrose, glucose, resistant starch, total soluble sugars), protein, total dietary fibre, lipids (total lipid, palmitic acid, linoleic acid, oleic acid and stearic acid), antinutritional factors (phytic acid and RFOs), phenols, ash, moisture, and minerals (iron, copper, zinc, sodium, potassium, calcium, magnesium, manganese, nickel, phosphorous, molybdenum, selenium and cobalt) content of 87 diverse PM varieties, landraces and accessions grown all across the Indian subcontinent. Since PM-based foods can be ascertained as predominant constituents of human diet, the composition of nutritionally relevant biochemical traits of the commonly consumed and cultivated cultivars can usher our understanding to further aid in sustainably exploiting this crop for human nutrition and food diversification. Also, the present data were evaluated through MVDA techniques like PCA and HCA for augmenting our understanding of the data structure and decipher the distribution of these nutritional traits.

2. Materials and methods

2.1. Materials and sample preparation

Experiments were carried out on the grains of 87 PM genotypes, containing, landraces and accessions representing different parts of India. The regions include Gujrat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, New Delhi, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, and Uttarakhand (Supplementary Table S1). These grains were harvested at maturity and further cleaned through winnowing followed by hand cleaning and oven-drying to a grain moisture content of 8–10 % and stored at 4 °C. About 10 g of dried seed samples were ground, homogenized and sieved through 1 mm sieve on Foss Cyclotec™ 1093 Sample Mill (FOSS Analytical, Denmark), to a flour particle size range of 500–900 μm. The homogenized samples were further subjected to nutritional analysis.

2.2. Quantification of nutritional parameters

2.2.1. Total protein

The total protein content was estimated by the Kjeldahl method (Sáez-Plaza et al., 2013). About 0.1 g of the homogenized samples were weighed in triplicates with an accuracy of 0.1 mg and placed in the digestion tubes. About 10 mL of ice-cold digestion mixture was added to the digestion tubes and left overnight for pre-digestion. The digestion tubes were placed in a digestion block at 420 °C for a period of 20–30 min, till complete digestion. Foss Tecator 2300 Kjeltec nitrogen auto-analyser (FOSS Analytical, Denmark) was calibrated by 0.15 g ammonium sulphate. Ammonium from the sample was converted to ammonia by using an alkali containing NaOH (four times the volume of H_2SO_4 used in the digestion mixture). The solution containing boric acid (1%) with bromocresol green /methyl red was used for trapping ammonia and as an indicator solution. The results were obtained as nitrogen %, which were converted to protein by multiplying with a conversion factor of 5.95. The results were expressed as g/100 g DWB (dry weight basis).

2.2.2. Total dietary fibre (TDF)

Total dietary fibre was determined by using an assay kit (Megazyme, K-TDFR-100A, Wicklow, Ireland) by a gravimetric and enzymatic method based on AOAC Method 985.29 and AACC method 32−05.01 (AOAC - Association of Official Analytical Chemists., 2005; AACC - American Association of Cereal Chemists., 2010). About 1.0 g samples were weighed in triplicates to an accuracy of 1 mg in duplicates and placed at ~ 100 °C with heat-stable α-amylase for gelatinisation, hydrolysis and depolymerisation of starch molecules and further incubated at 60 °C with protease (protein depolymerization) and amyloglucosidase (starch hydrolysis to glucose). The samples are then treated with 95 % ethanol (280 mL) to eliminate the depolymerized glucose and proteins and allow the soluble fibres to completely precipitate. The precipitate is filtered and washed 95 % ethanol, dried and weighed. The TDF was calculated by subtracting the weight of ash and proteins from the weight of filtered and dried fractions. The results were expressed as g/100 g DWB.

2.2.3. Total lipid

Total lipid content in dried PM whole grains was analyzed by a non-destructive method using Newport NMR analyzer (Model-4000) from Oxford Analytical Instruments Ltd. U.K, having a 40 mL coil assembly (Shukla et al., 2018). The grains were samples in triplicates and dried at 108 °C for 16–18 h to a grain moisture content of 4–5%. The instrument was calibrated using pure PM seed oil which was extracted using a Soxhlet apparatus (Zhao and Zhang, 2014). The calibration was done at an incessant gain in the audio frequency of 400, radiofrequency current of 225 lA and 1.0 G gate width (Abraham et al., 2010). The NMR response in the form of (signal/mass) from seed samples was matched with the NMR response of pure oil to directly obtain the lipid percentage. The results were expressed as %.

2.2.4. Total phytic acid

The total phytic acid fibre was determined by using an assay kit (Megazyme, K-PHYT, Wicklow, Ireland) by an enzymatic method based on the spectrophotometric detection of inorganic phosphorus, after and before the enzymatic degradation of phytic acid (McKie and McCleary, 2016). Accurately weighed 1.0 g of homogenized sample in triplicates were digested overnight at room temperature in 50 mL Falcon tubes by 0.66 M hydrochloric acid (20 mL) with continuous agitation. To determine the total phosphorous, the sample was subjected to phytase which converts phytic acid (*myo*-inositol hexakisphosphate) into *myo*-inositol phosphate and inorganic phosphate (Pi) and alkaline phosphatase which further hydrolyses the *myo*-inositol phosphate into *myo*-inositol and Pi. Phosphorous was calorimetrically quantified as molybdenum blue by an increase in absorbance at 655 nm. The results were expressed as g/100 g DWB. Total phytic acid content was calculated by substracting free phosphorous in the sample from total phosphorous, entirely released after enzymatic digestion.

The total phytic acid was calculated by the formula

$$Total\ phytic\ acid\ \left(\frac{g}{100g}\right) = \frac{mean\ M \times 0.1112 \times \Delta A_{phosphorous}}{0.282} \quad (1)$$

Where

$\Delta A_{Phosphorous}$: Optical density of [Total Phosphorus (extract 2) – Free phosphorous(extract 1)]

mean M: phosphorus standard mean value μg/ $\Delta A_{phosphorous}$

2.2.5. Raffinose-family oligosaccharides, sucrose and D-glucose

The saccharides were quantified after extraction by the method outlined by Tahir et al. (2012), using an assay kit (Megazyme, K-RAFGL, Wicklow, Ireland). The analysis was done in triplicates. In this method, sucrose and RFOs were hydrolyzed by invertase and α-galactosidase into their constituent D-fructose, D-glucose and D-galactose units. D-glucose is then determined by GOPOD (glucose oxidase/peroxidase) reagent. Glucose oxidase catalyzes the oxidation of D-glucose to D-gluconate and hydrogen peroxide. 4-aminoantipyrine, p-hydroxybenzoic acid further react in presence of peroxidase to produce pink coloured quinoneimine which was quantitatively detected at 510 nm. RFOs, sucrose and D-glucose were determined by the following equations. The results were expressed as mmol/100 g DWB.

$$D-Glucose\left(\frac{mM}{100}g\right)=\Delta A\times F\times 50 \tag{2}$$

$$Sucrose\ (mM/100\ g)=(\Delta B-\Delta A)\times F\times 50 \tag{3}$$

$$RFOs\ (mM/100\ g)=(\Delta C-\Delta B)\times F\times 50 \tag{4}$$

Where

ΔA, ΔB and ΔC: GOPOD absorbance for solution A, B and C respectively

$$F=\frac{0.556\ (\ \mu M\ glucose)}{GOPOD\ absorbance\ for\ 0.556\ \mu M\ glucose} \tag{5}$$

2.2.6. Resistant starch

Resistant starch was quantified by using an assay kit (Megazyme, K-RSTAR, Wicklow, Ireland). The determination method was in agreement with the principles specified in official AACC Method 32–40.01 and AOAC Method 2002.02 (Goñi et al., 1996). The samples in triplicates were incubated with pancreatic α-amylase and amyloglucosidase in a shaking water bath at 37 °C for 16 h. During this period these two enzymes hydrolyzed the non-resistant starch to D-glucose units. An equal volume of ethanol was used to terminate the reaction and RS is recovered as a pellet after centrifugation at 16,000 *g* for 30 min. The pellet was then washed two times by ethanol (50 % v/v) followed by centrifugation. RS present in the pellet was dissolved in 2 M KOH, and the solution is further neutralised by acetate buffer. Amyloglucosidase was used to hydrolyze starch into glucose. This glucose was quantitatively detected at 510 nm as pink colour by GOPOD reagent. The resistant starch was calculated by the formula and the results were expressed as g/100 g DWB.

$$Resistant\ Starch\ (g/100g\ sample)=\Delta E\times\frac{F}{W}\times 9.27 \tag{6}$$

Where

ΔE: Sample absorbance

$$F=\frac{100\ \mu g\ glucose)}{GOPOD\ absorbance\ for\ 100\ \mu g\ glucose} \tag{7}$$

W: Sample dry weight

2.2.7. Preparation of extracts for starch (pellet), phenols and TSS (supernatant)

Briefly, 100 ± 5 mg of homogenized samples in triplicates were weighed into screw-capped falcon tubes and vortexed for 2 min after adding 5 mL of 80 % ethanol and kept in a water bath at 80 °C for 30 min. The tubes were further cooled to room temperature and mounted on a rotator for 60 min. the tubes were subsequently centrifuged at 16,000 *g* for 15 min and supernatant collected and extracted two more times following the same method. The supernatant was collected in fresh tubes and the final volume of the supernatant was made to 10 mL. The pellet was used for starch quantification while the supernatant was used for estimating phenols and TSS (both the pellet and the supernatant can be briefly stored at −20 °C). The complete extraction of sugars and phenols was tested in a pilot study using five diverse accessions where extraction was done up to five times and each supernatant was kept separate. We estimated sugar and phenols from each extract and only traces could be detected in the fourth extract, hence extraction of samples was done only up to 3 extracts.

2.2.8. Starch

Total starch was determined by using an assay kit (Megazyme, K-TSTA-100A, Wicklow, Ireland) based on AACC 76−13.01 and AOAC Method 996.11 with few modifications. About 200 μL 80 % ethanol was added to the pellet (Section 2.2.7) containing tubes and placed in a boiling water bath for 5 min. The sample is subjected to thermostable α-amylase which hydrolyze starch into branched, soluble and unbranched maltodextrins. Followed by amyloglucosidase, which hydrolyses the maltodextrins into D-glucose. The released glucose was quantitatively detected at 510 nm as pink colour by GOPOD reagent. The total starch content was calculated by the formula and the results were expressed as g/100 g DWB.

Starch % can be calculated by the formula

$$Starch\ \%=\Delta A\times F\times EV\times\frac{D}{W}\times 0.90 \tag{8}$$

Where

ΔA: Sample absorbance

$$F=\frac{100\ \mu g\ glucose)}{GOPOD\ absorbance\ for\ 100\ \mu g\ glucose} \tag{9}$$

EV: Sample extraction volume (100 mL)

D: Dilution factor (1), undiluted

W: Weight of the sample in mg

2.2.9. Phenols

Total phenols were determined according to a method by Tian et al., 2020a with slight modifications. The 500 μL of already prepared extract in triplicates (2.2.7) was taken in test tubes and completely evaporated over a water bath at 100 °C. Three mL of double distilled water was added to these test tubes and vigorously vortexed. Simultaneously blank was prepared by adding 3 mL double distilled water in separate tubes. For the preparation of standard, gallic acid (GA) (0.01, 0.02, 0.03, 0.04, and 0.05 mg) was added to a separate set of tubes and the volume was made to 3 mL. Subsequently, 500 μL of Folin-Ciocalteu reagent (FCR) (equal part of FCR and water) was added to each of the three sets of test tubes (sample, blank and standards). The test tubes were vortexed for 2 min, followed by the addition of 2 mL of 20 % w/v, Na_2CO_3. The tubes were allowed to incubate at room temperature for 1 h and the absorbance of each solution (sample, standard) was measured at 650 nm against a blank. FCR is a mixture of molybdates and tungstates and works by the oxidation-reduction reaction. The mixture of molybdates-heteropolyphosphotungsates is reduced by phenolic compounds forming a blue coloured chromogen under basic condition by Na_2CO_3. The total phenolic content was expressed as GAE g/100 g (g gallic acid equivalence per 100 g), quantified by the gallic acid standard curve.

2.2.10. Total soluble sugars

TSS were determined according to a method by (Hansen and Møller, 1975) with slight modifications. Already prepared 200 μL of extract in triplicates (2.2.7) was taken in a test tube and completely evaporated over a water bath at 100 °C. One mL of double distilled water was added

to these test tube and vigorously vortexed. Simultaneously blank was prepared by adding 1 mL double distilled water in separate tubes. For the preparation of standard, D-glucose, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg was added to a separate set of tubes and the volume was made to 1 mL. Subsequently, 4 mL of ice-cold anthrone reagent was added to each of the three sets of test tubes (sample, blank and standards). The tubes were incubated in a water bath at 80 °C for 8 min. Sulphuric acid in anthrone dehydrates the carbohydrates to generate furfural. Anthrone then reacts to the furfural to generate a green-blue colour which is spectrophotometrically quantified. The absorbance of each solution (sample, standard) was measured at 630 nm against a blank. The total soluble sugars were expressed as g/100 g DWB and quantified by D-glucose standard curve.

2.2.11. Amylose

Amylose content was determined by the iodometric method by (Perez and Juliano, 1978) with several improvements. Briefly, 50 mg homogenized flour was added to a test tube in triplicates followed by 500 μL ethanol. The tubes were vortexed and then placed in a boiling water bath for 20 min. The tubes were removed from the water bath and the contents were emptied in a 50 mL volumetric flask and the volume was made up by double distilled water. From the volumetric flask, 500 μL sample was drawn into amber falcon tubes and 500 μL double distilled water (for blank), in another fresh set of amber tubes. This was followed by the addition of 100 μL glacial acetic acid and 200 μL iodine solution (2 g iodine dissolved in potassium iodide, 20 g/L) in each tube. The volume was made up to 10 mL by double distilled water. The tubes were allowed to incubate at room temperature for 20 min and the absorbance of the sample was measured at 620 nm against a blank. Iodine integrates with the helical amylose coils, producing a blue coloured complex. The Amylose was expressed as g/100 g DWB and quantified using a standard potato amylose curve.

2.2.12. Moisture and ash content

Quartz crucibles were kept at 575 °C in a muffle furnace, for 16 h and weighed to constant weight. Homogenized samples (0.5 g) were weighed in triplicates in the pre-weighed quartz crucibles and heated to 105 °C for 16 h. The crucibles were weighed again, and the difference between the sample weights (initial weight–dry weight) was calculated. This difference was computed as a per cent of initial weight and was determined as the moisture % (g/100 g). The crucibles were subsequently heated for 18 h at 575 °C to constant weight. The weight of the remaining ash was evaluated as the percentage of the initial sample weight (g/100 g) (Sluiter et al., 2008).

2.2.13. Minerals

Minerals (iron, copper, zinc, sodium, potassium, calcium, magnesium, manganese, nickel, phosphorous, molybdenum, selenium and cobalt) were estimated using PM whole grains homogenized by mortar and pestle to prevent metal contamination from Foss CyclotecTM 1093 Sample Mill. The 100 mg ground samples in triplicates were digested using a di-acid mixture ($HClO_4$: HNO_3, 4:9). The volume of the digested extract was made to 50 mL by a volumetric flask and further filtered through a Whatman No. 42. The samples were run on an inductively coupled plasma optical emission spectrometer (ICP-OES; model 5110, Agilent Technologies, Santa Clara, CA, USA) which was calibrated using standard solutions. The mineral concentration was expressed as ppm.

2.2.14. Fatty acids

Fatty acids (palmitic acid, linoleic acid, oleic acid and stearic acid) were profiled using a simplified, cost-effective and rapid GC–MS protocol outlined by the Division of Genetics, ICAR-IARI, New Delhi, India. The fatty acids were evaluated by a GC–MS (ThermoFisher Scientific, USA, DSQ-II model) as methyl esters of fatty acids. the methyl esters were prepared according to the protocol by Vasudev et al. (2008) with few modifications as follows. Briefly, 100 ± 5 mg of homogenized sample in triplicates was weighed into test tubes. Each tube was added with 5 mL methanol followed by 100 μL of concentrated H_2SO_4. The test tubes were vortexed for 3 min and incubated in a water bath for 1 h at 65 °C. The tubes were cooled at room temperature, followed by the addition of 2 mL hexane. The tubes were vigorously vortexed and then allowed to stand still, till the hexane layer containing methyl ester was separated. About 1.0–1.5 mL of the hexane layer was carefully removed and transferred to fresh 2 mL glass vials for further analysis. GC–MS analysis was performed using an HP-5 MS capillary column (0.25 mm, 30 m long i.e., a film thickness of 0.25 μm). The carrier gas used was helium with a flow rate of 1 mL min^{-1}. The program of the oven temperature was as follows: Initial temperature at 100 °C was linearly increased to 240 °C at the rate of 3 °C min^{-1}, and the final temperature was maintained for 10 min. Temperatures of both the detector and injector were maintained at 230 °C. The sample volume of injection was 2 μL and was injected at a split flow of 10 mL min^{-1}. The mass spectrometer of DSQ II series was operated in the electron impact mode at 70 eV and in the scan range of 50–450 *m/z*. The fatty acid composition was expressed as the total fatty acid percentage.

2.3. Statistics

All the analysis was performed as triplicates unless stated otherwise. Statistical significance was set at a 95 % confidence level. Hierarchical cluster analysis (HCA) was done by Ward clustering algorithm using squared Euclidean distance using a trial version of IBM SPSS. The correlation between carbohydrates, protein, TDF, lipids, antinutritional factors, phenols, ash, moisture, and minerals was evaluated by computing their linear relationships by Pearson's correlation coefficient. The correlation study was performed using Jamovi version 1.2.27 at a 5% level of significance. The differences between various nutritional parameters were statistically assessed by one-way analysis of variance (ANOVA). The differences among means were evaluated by posthoc test (Duncan's multiple range test) at ($p < 0.05$) level for each of the evaluated nutritional parameters using a trial version of IBM SPSS. The data were subjected to principal component analysis (PCA) to assess the main nutritional traits that determine the observed variation and envision the probable relationships among these attributes using Jamovi version 1.2.27. Bartlett's test for sphericity and Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy (MSA) were used as assumption checks before subjecting the data to PCA and assessed by Jamovi version 1.2.27. For maximizing the parameter loading in a component axis and for better pattern interpretation for a specific parameter, an orthogonal varimax rotation was applied for PCA. Heatmap for relative composition and Pearson's correlation coefficient was made using a web interface (MetaboAnalyst) (Chong et al., 2019).

3. Results and discussion

3.1. Multivariate data analysis (MVDA)

To develop an enhanced understanding of the data structure and its distribution, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to envision the distribution of the nutritional traits across 87 germplasm.

3.1.1. Hierarchical clustering analysis (HCA)

HCA was used in the present study to assess the multivariate relationship between the biochemical attributes. The evaluated germplasm was organized into seven clusters depicting their hierarchy and presented in form of a dendrogram. The germplasm was agglomeratively grouped where each sample was initially contemplated as an independent cluster, and these clusters were subsequently merged. Euclidean distance was used as a suitable sample distance metric and linkage benchmark among clusters. Germplasm grouped in seven clusters along with the mean value of their nutritional attributes is indicated through a

heat map in Fig. 1, Table 1. Mean values of 87 PM germplasm falling in each of the seven clusters are represented by Box-and-whisker plots in Supplementary Figure S1 (i to xvii), Figure S2 (i to xiii).

Cluster I is characterized by the germplasm having higher content of cobalt (0.37 ± 0.06 ppm) and low values of other constituents. Cluster II is characterized by germplasms having higher content of TSS (2.50 ± 0.30 g/100 g), RS (2.38 ± 0.37 g/100 g), linoleic acid (40.61 ± 3.00 %), selenium (0.22 ± 0.09 ppm) and lower values for stearic acid (4.87 ± 0.46 %), calcium (234.62 ± 28.10 ppm), magnesium (1635.23 ± 130.63 ppm), sodium (29.99 ± 5.80 ppm), zinc (28.71 ± 12.15 ppm) and copper (3.06 ± 0.72 ppm). Cluster III is characterized by germplasm with higher content of starch (60.33 ± 2.48 g/100 g), amylose (25.03 ± 1.03 g/100 g), lipid (9.99 ± 0.41 g/100 g), phenols (0.16 ± 0.001 GAE g/100 g), moisture (11.26 ± 0.46 %), sucrose (1.22 ± 0.04 g/100 g), RFO (1.76 ± 0.07 mmol/100 g), oleic acid (31.07 ± 1.28 %), stearic acid (5.87 ± 0.24 %), calcium (405.59 ± 16.68 ppm), iron (84.22 ± 3.46 ppm), sodium (42.81 ± 1.76 ppm), zinc (58.64 ± 2.41 ppm), nickel (1.91 ± 0.079 ppm), copper (5.8 ± 0.24 ppm) and lower content of phytic acid (0.76 ± 0.03 g/100 g), protein (12.20 ± 0.50 g/100 g), ash (0.88 ± 0.04 %), TDF (8.85 ± 0.36 g/100 g), glucose (0.38 ± 0.016 g/100 g), palmitic acid (25.09 ± 1.03 %), linoleic acid (37.97 ± 1.56 %), phosphorous (215.44 ± 8.86 ppm), potassium (2495.57 ± 102.63 ppm), selenium (0.008 ± 0.00 ppm), molybdenum (0.66 ± 0.027 ppm), manganese (8.21 ± 0.34 ppm). Cluster IV is characterized by germplasm with low content of iron (65.33 ± 12.13 ppm). Cluster V is characterized by germplasms having high values of ash (1.16 ± 0.23 g/100 g), molybdenum (0.78 ± 0.07 ppm) and low values of sucrose (1.07 ± 0.18 g/100 g) and nickel (0.60 ± 0.12 ppm). Cluster VI is characterized by germplasms having high content of manganese (12.57 ± 2.10 ppm). Cluster VII is characterized by germplasms having high content of phytic acid (1.10 ± 0.20 g/100 g), proteins (14.25 ± 3.17 g/100 g), TDF (13.77 ± 2.12 g/100 g), glucose (0.60 ± 0.10 g/100 g), palmitic acid (28.61 ± 2.21 %), phosphorous (310.96 ± 59.07 ppm), potassium (4994.72 ± 253.97 ppm), magnesium (2386.71 ± 197.41 ppm), manganese (12.57 ± 2.21 ppm) and low content of starch (55.54 ± 3.16 g/100 g), amylose (22.18 ± 2.66 g/100 g), lipid (7.07 ± 0.52 g/100 g), TSS (2.07 ± 0.14 g/100 g), phenols (0.12 ± 0.02 GAE g/100 g), RS (1.90 ± 0.30 g/100 g), moisture (10.36 ± 0.53 %), RFO (1.16 ± 0.42 mmol/100 g), oleic acid (26.15 ± 0.85 %) and cobalt (0.33 ± 0.04 ppm).

The HCA revealed that germplasm in cluster I, II and III showed higher content of amylose, starch, moisture and higher lipid content in cluster III and its lower content in cluster I and II. These traits determine optimum techno-functional properties and thus these clusters could be suitably used by food processing industries and designing food products like cookies, bread and bakery products. A higher RS content was observed in cluster I, II, III and IV could enable the use of the germplasm

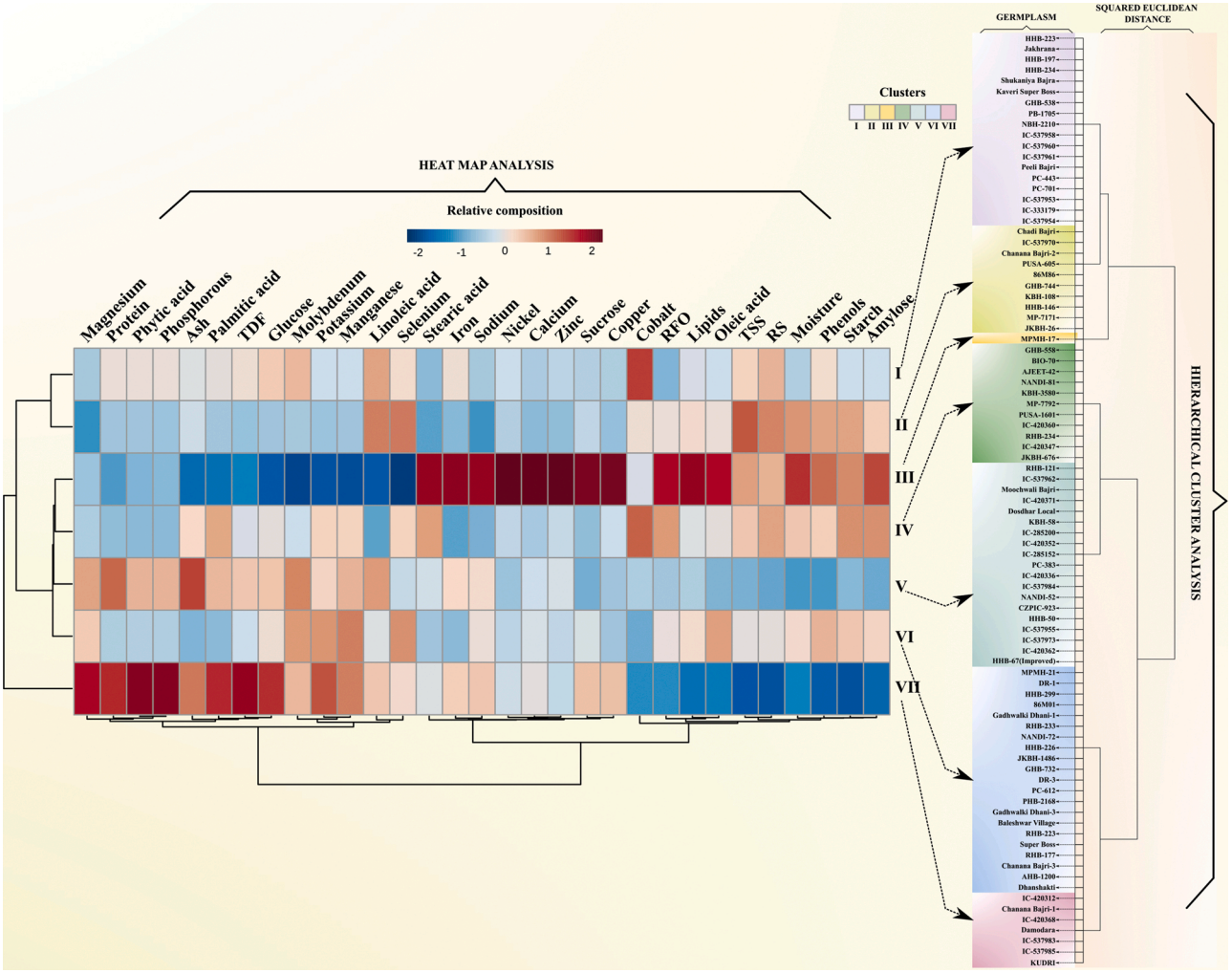

Fig. 1. The evaluated germplasm was organized into seven clusters depicting their hierarchy and presented as a dendrogram. The germplasm was agglomeratively grouped where each sample was initially contemplated as an independent cluster, and these clusters were subsequently merged. Euclidean distance was used as a suitable sample distance metric and linkage benchmark among clusters. The mean values of the relative nutritional attributes of these clusters are graphically represented as individual values contained in the matrix and represented as colours. The data is scaled between +2 and -2. Here TDF: Total dietary fibre; RFO: Raffinose family oligosaccharides; TSS: total soluble sugars; RS: Resistant starch.

Table 1
Mean values of 87 pearl millet germplasm falling in each of the seven clusters.

Traits	Cluster I N = 18	Cluster II N = 10	Cluster III N = 1	Cluster IV N = 11	Cluster V N = 19	Cluster VI N = 21	Cluster VII N = 7
Protein	13.04 ± 1.99^{a}	12.57 ± 1.41^{a}	12.20 ± 0.50^{a}	12.47 ± 1.50^{a}	13.91 ± 1.99^{a}	12.64 ± 1.35^{a}	14.25 ± 3.17^{a}
Starch	58.27 ± 2.71^{b,c}	59.98 ± 1.93^{b,c}	60.33 ± 2.48^{c}	60.25 ± 2.55^{c}	57.37 ± 2.51^{a,b}	59.41 ± 1.23^{b,c}	55.54 ± 3.16^{a}
Amylose	23.44 ± 1.94^{a,b,c}	24.00 ± 2.06^{a,b,c}	25.03 ± 1.03^{c}	24.51 ± 1.43^{b,c}	22.81 ± 1.92^{a,b}	23.91 ± 1.33^{a,b,c}	22.18 ± 2.66^{a}
Lipid	8.23 ± 0.82^{b}	8.44 ± 0.48^{b}	9.99 ± 0.41^{c}	8.26 ± 0.78^{b}	7.93 ± 1.02^{b}	8.45 ± 0.76^{b}	7.07 ± 0.52^{a}
TSS	2.36 ± 0.38^{a,b}	2.50 ± 0.30^{b}	2.43 ± 0.1^{a,b}	2.38 ± 0.29^{a,b}	2.21 ± 0.29^{a,b}	2.32 ± 0.40^{a,b}	2.07 ± 0.14^{a}
Phenols	0.15 ± 0.02^{b,c}	0.16 ± 0.02^{b,c}	0.16 ± 0.001^{c}	0.15 ± 0.02^{b,c}	0.12 ± 0.03^{a,b}	0.16 ± 0.02^{b,c}	0.12 ± 0.02^{a}
RS	2.31 ± 0.45^{a,b}	2.38 ± 0.37^{b}	2.31 ± 0.00^{a,b}	2.34 ± 0.35^{a,b}	2.04 ± 0.41^{a,b}	2.21 ± 0.31^{a,b}	1.90 ± 0.30^{a}
Ash	1.02 ± 0.19^{a,b}	1.00 ± 0.21^{a,b}	0.88 ± 0.04^{a}	1.04 ± 0.25^{a,b}	1.16 ± 0.23^{b}	0.95 ± 0.16^{a,b}	1.12 ± 0.16^{a,b}
TDF	10.95 ± 1.43^{b}	9.88 ± 1.26^{a,b}	8.85 ± 0.36^{a}	10.64 ± 1.55^{a,b}	11.43 ± 1.76^{b}	10.46 ± 1.51^{a,b}	13.77 ± 2.12^{c}
Moisture	10.62 ± 0.54^{ab}	11.02 ± 0.51^{bc}	11.26 ± 0.46^{c}	10.89 ± 0.53^{abc}	10.42 ± 0.59^{ab}	10.80 ± 0.50^{abc}	10.36 ± 0.53^{a}
Glucose	0.52 ± 0.1^{bc}	0.46 ± 0.05^{ab}	0.38 ± 0.016^{a}	0.49 ± 0.07^{b}	0.52 ± 0.08^{bc}	0.50 ± 0.10^{bc}	0.60 ± 0.10^{c}
Sucrose	1.13 ± 0.13^{a}	1.14 ± 0.07^{a}	1.22 ± 0.04^{b}	1.09 ± 0.22^{a}	1.07 ± 0.18^{a}	1.17 ± 0.18^{a}	1.25 ± 0.13^{a}
RFO	1.25 ± 0.33^{a}	1.41 ± 0.16^{ab}	1.76 ± 0.07^{b}	1.55 ± 0.28^{ab}	1.30 ± 0.55^{a}	1.40 ± 0.33$_{ab}$	1.16 ± 0.42^{a}
Phytic acid	0.86 ± 0.20^{a}	0.78 ± 0.13^{a}	0.76 ± 0.03^{a}	0.76 ± 0.12^{a}	0.92 ± 0.20^{a}	0.80 ± 0.11^{a}	1.10 ± 0.20^{b}
Palmitic acid	26.74 ± 1.87^{a,b}	26.10 ± 2.22^{a,b}	25.09 ± 1.03^{a}	27.54 ± 3.41^{a,b}	27.36 ± 2.09^{a,b}	25.92 ± 2.24^{a,b}	28.61 ± 2.21^{b}
Linoleic acid	40.35 ± 2.10^{a}	40.61 ± 3.00^{a}	37.97 ± 1.56^{a}	38.69 ± 1.99^{a}	40.42 ± 2.00^{a}	39.58 ± 3.05^{a}	40.04 ± 1.96^{a}
Oleic acid	27.92 ± 1.43^{a,b}	28.39 ± 2.85^{a,b}	31.07 ± 1.28^{c}	28.28 ± 1.24^{a,b}	27.06 ± 2.43^{a,b}	29.47 ± 2.52^{b,c}	26.15 ± 0.85^{a}
Stearic acid	4.97 ± 0.76^{a,b}	4.87 ± 0.46^{a}	5.87 ± 0.24^{b}	5.47 ± 1.00^{a,b}	5.15 ± 0.72^{a,b}	5.02 ± 0.79^{a,b}	5.18 ± 0.70^{a,b}
Phosphorous	241.74 ± 56.87^{a}	220.57 ± 36.34^{a}	215.44 ± 8.86^{a}	216.19 ± 33.56^{a}	260.14 ± 56.66^{a}	226.30 ± 32.33^{a}	310.96 ± 59.07^{b}
Calcium	247.07 ± 34.40^{a}	234.62 ± 28.10^{a}	405.59 ± 16.68^{b}	245.75 ± 38.26^{a}	262.29 ± 29.31^{a}	266.43 ± 37.59^{a}	273.53 ± 51.34^{a}
Iron	72.24 ± 20.88^{a}	66.85 ± 14.98^{a}	84.22 ± 3.46^{a}	65.33 ± 12.13^{a}	72.98 ± 15.27^{a}	67.06 ± 16.04^{a}	73.70 ± 23.11^{a}
Potassium	3727.51 ± 111.18^{c}	3442.19 ± 140.06^{b}	2495.57 ± 102.63^{a}	4162.66 ± 137.10^{d}	4125.09 ± 156.06^{d}	4589.58 ± 126.87^{e}	4994.72 ± 253.97^{f}
Magnesium	1790.15 ± 156.75^{b}	1635.23 ± 130.63^{a}	1773.05 ± 72.92^{b}	1805.07 ± 90.06^{b}	2105.10 ± 117.93^{c}	2010.37 ± 100.86^{c}	2386.71 ± 197.41^{d}
Sodium	32.46 ± 4.07^{a}	29.99 ± 5.80^{a}	42.81 ± 1.76^{b}	31.19 ± 3.81^{a}	35.34 ± 6.40^{a}	34.89 ± 6.20^{a}	36.29 ± 6.16^{a}
Zinc	31.80 ± 6.88^{a}	28.71 ± 12.15^{a}	58.64 ± 2.41^{a}	32.63 ± 9.13^{a}	34.07 ± 10.87^{a}	32.01 ± 7.00^{a}	32.25 ± 13.60^{a}
Molybdenum	0.76 ± 0.07^{b}	0.72 ± 0.05^{a,b}	0.66 ± 0.027^{a}	0.73 ± 0.07^{a,b}	0.78 ± 0.07^{b}	0.77 ± 0.08^{b}	0.76 ± 0.05^{b}
Manganese	10.64 ± 1.17^{b,c}	10.14 ± 1.04^{b}	8.21 ± 0.34^{a}	11.45 ± 1.76^{b,c}	11.91 ± 1.48^{b,c}	12.57 ± 2.10^{c}	12.57 ± 2.21^{c}
Copper	3.25 ± 0.70^{a}	3.06 ± 0.72^{a}	5.8 ± 0.24^{b}	3.46 ± 1.61^{a}	3.16 ± 0.97^{a}	3.14 ± 0.82^{a}	4.16 ± 1.39^{a}
Cobalt	0.37 ± 0.06^{a}	0.35 ± 0.06^{a}	0.35 ± 0.014^{a}	0.36 ± 0.08^{a}	0.34 ± 0.07^{a}	0.34 ± 0.07^{a}	0.33 ± 0.04^{a}
Nickel	0.76 ± 0.18^{a}	0.70 ± 0.15^{a}	1.91 ± 0.079^{b}	0.72 ± 0.33^{a}	0.60 ± 0.12^{a}	0.71 ± 0.17^{a}	0.78 ± 0.21^{a}
Selenium	0.16 ± 0.15^{b}	0.22 ± 0.09^{b}	0.008 ± 0.00^{a}	0.17 ± 0.13^{b}	0.13 ± 0.13^{a,b}	0.21 ± 0.11^{b}	0.16 ± 0.03^{b}

Note: Data are expressed as Mean ± Standard Deviation; Means with different superscript within the same row for each cluster are significantly different at $p \leq 0.05$; N= Number of germplasm. All the results of protein, starch, amylose, TSS (total soluble sugars), phenols, RS (resistant starch), ash, TDF (total dietary fibre), moisture, glucose, sucrose and phytic acid were expressed as g/100g DWB (dry weight basis); lipids, palmitic acid, linoleic acid, oleic acid, and stearic acid were expressed as % and all minerals were expressed as ppm (parts per million).

in these clusters for designing low glycemic index diet foods for diabetics. Higher TSS in cluster II and III, higher sucrose content in cluster III, greater glucose and protein content in cluster V and VII could make these germplasm useful for designing foods for underdeveloped and poor countries, for addressing protein-energy malnutrition. Besides being used as low glycemic foods, higher TDF content in cluster IV, V and VII could also be used for individuals with cardiovascular diseases, hypercholesteremia and bowel disorders. Clusters I, V and VII could be used for designing foods for individuals sensitive to flatulence and other gastric disorders. The lower phytic acid content in cluster II, III, IV and VI and higher mineral content in cluster III (calcium, iron, zinc, copper) could accredit their use as functional food ingredients and food diversification.

3.1.2. PCA

The nutritional parameters for 87 PM germplasm were scaled and subjected to factor analysis through PCA to discern the nutritional parameters participating in the observed variability and envision the existing feasible associations between these parameters. PCA creates a statistical mechanics framework for modelling the biological systems without a need for prior theoretical assumptions. The principal components (PCs) embody the same information as the original parameters but display the added benefit of being reciprocally uncorrelated, avoiding any redundant information between these parameters. PCs are orthogonal to each other and iteratively calculated to encompass the maximum possible variation from the evaluated data, in a manner that PC1 delineates more variation than PC2, and PC2 explains more variation than PC3 and so on (Granato et al., 2018). Every PC accounting for a portion of the variation in the data set is identified with an Eigenvalue. Each Eigenvalue corresponds to an Eigenvector which determines the variance within the associated principal components. Only the factors having Eigenvalues greater than 1.0 were considered in agreement with Kaiser's criteria (Kaiser, 1960). The break in the scree plot was used to determine the key nutritional parameters.

Bartlett's test checks whether the variables in the data set are correlated to the extent that they can be possibly outlined with a smaller factor set. This test checks whether the correlation matrix significantly deviates from a null correlation matrix. Thus if Bartlett's test is significant with ($p < 0.5$), the correlation matrix is substantially divergent from the null and thus can be suitably used in PCA. KMO is a statistic that determines the per cent variance in the data that could result from underlying factors. The MSA values between 0.5 and 0.7 are adequate, those between 0.7 and 0.9 are considered good while those between 0.9 and 1.0 are considered excellent for conducting PCA. If the values are below 0.5, the result for PCA won't provide any useful interpretation. The results of PCA were interpreted based on the relation between different components. Carbohydrates, protein, total dietary fibre, lipids, antinutritional factors, phenols, ash and moisture were subjected to PCA (PCA1) while the minerals were subjected to separate PCA (PCA2). The traits were subjected to different PCA based on KMO and Bartlett's test values. The values of Bartlett's test for PCA1, 2 were $p < 0.001$ [Supplementary Table S2 (A.1, A.2)] while the KMO values for PCA1 ranged from 0.583 to 0.887 [Supplementary Table S2 (B.1)] while for PCA2 ranged from 0.538 to 0.783 [Supplementary Table S2 (B.2)] indicating the adequacy of our data for PCA analysis.

After the establishment of representative PCs based on sample grouping/differentiation and variance explained, loadings were analyzed to decipher the underlying association between in the data structure. The loading is envisaged as a regression vector, which is a vector of correlation coefficients between the initial variables with each

PC score. Factor loadings (FL) with positive values suggest that the specific factor will be higher on the positive axis of that specific PC. In the present data analyzed under PCA1, amylose (FL1: 0.885), moisture (FL1: 0.832), starch (FL1: 0.789), phenols (FL1: 0.443; FL2: 0.596; FL3: 0.364), lipids (FL1: 0.317; FL2: 0.441; FL3: 0.547), TSS (FL2: 0.736), sucrose (FL2: 0.679), RFO (FL3: 0.589) and RS (FL2: 0.495) showed positive factor loadings [Fig. 2B, E, Supplementary Table S3 (A.1, A.2)]. In PCA2 nickel (FL1: 0.853), copper (FL1: 0.851), zinc (FL1: 0.567; FL3: 0.315), potassium (FL2: 0.882), manganese (FL1: 0.322; FL2: 0.759), magnesium (FL2: 0.706), cobalt (FL2: 0.712), molybdenum (FL2: 0.308; FL3: 0.704), selenium (FL3: 0.696), calcium (FL1: 0.472), and iron (FL1: 0.337) showed positive factor loading values. Correspondingly, a negative value of factor loadings determines that the specific factor will be higher on the negative axis of that specific PC. Correspondingly in PCA1, protein (FL: –0.892), phytic acid (FL1: –0.649; FL3: –0.310), ash (FL1: –0.519; FL2: –0.402) and TDF (FL1: –0.408; FL2: –0.529; FL3: –0.545) showed negative factor loading values.

In the case of PCA1, the first two components explained 85.2 % variability in terms of carbohydrates, protein, total dietary fibre, lipids, antinutritional factors, phenols, ash and moisture. PC1 described 71.4 % of the total variation, in which the dominant parameters include protein, amylose, moisture, starch, phytic acid and ash. PC2 described 13.8 % of the total variation in which the dominant parameters include total soluble sugars, sucrose and phenols. PC3 explained 4.7 % of the total variation in which the dominant parameters include glucose, RFO, lipid, TDF. In the case of PCA2, the first two components explained 99 % variability in terms of mineral content. PC1 described 89.5 % of the total variation, in which the dominant parameters include nickel, copper and zinc. PC2 described 9.5 % of the total variation in which the dominant parameters include potassium, manganese and magnesium. PC3 explained 0.6 % of the total variation in which the dominant parameters include cobalt, molybdenum and selenium.

These dominant factors displayed high component loading and low uniqueness values. Loading describes the contribution of each variable to PC and uniqueness is the portion of variation that is unique to a specific variable and cannot be described by PCs. Thus lower the uniqueness value, the greater is the contribution of a specific variable to PCA. Two dimensional PCA score plots for PCA1, 2 indicate the distribution of nutritional traits in 87 samples, statistically clustered by Ward clustering algorithm based on their calculated component loading values (Fig. 2A, B). The green lines in the PCA biplot determine the magnitude of nutritional parameters with respect to the germplasm. When two vectors represented by green lines form a small angle represents a positive correlation between the traits. When the vectors form a 90° angle, they are less likely to be correlated while if they diverge to form ~180° angle, shows a negative correlation (Supplementary Figure S3).

3.2. Nutritional composition

Starch is a macro carbohydrate constituent in PM and varies between 62.8–70.5 g/100 g, depending on the individual cultivar (Punia et al., 2021). It constitutes a large proportion of dietary calorific intake and provides most of the metabolic energy needed for brain functioning and muscle tissues. The starch content in the present study ranged from 50.37 ± 1.75 g/100 g in IC-537,983 to 63.25 ± 1.96 g/100 g in KBH-3580. Our results corroborate with that of El Hag et al. (2002) who reported the content of 67.7 g/100 g for non-Indian cultivars and 59.4 ± 0.36 g/100 g by Sharma et al. (2015a) for Indian cultivars. The results indicate that PM is a rich source of starch, which can have diverse applications for food industries.

Amylose, a linear polysaccharide made of α(1→4) linked α-D-glucose units, primarily determines the glycemic response (the inverse relationship between amylose and glycemic index) and governs the physicochemical properties of starch like viscosity, swelling power, gelatinization capacity, retrogradation and starch crystallinity (Cornejo-Ramírez et al., 2018). Amylose rich starch is naturally resistant to digestion and susceptible to retrogradation. The amylose content in our study ranged from 19.26 ± 0.72 g/100 g in IC-537,953 to 27.9 ± 0.76 g/100 g in Kudri. A similar range of 15.64–19.46 g/100 g was reported by Bhupender et al. (2013), 21.9–28.8 g/100 g by Jambunathan and Subramanian (1988), 10.5 and 15.4 g/100 g by Siroha et al. (2019), 13.6–18.1 g/100 g by Sandhu and Siroha (2017), 21.9–28.8 g/100 g by Jambunathan and Subramanian (1988) and 28.8–31.9 g/100 by Hoover et al. (1996) for Indian cultivars.

RS, also considered as a TDF component is the proportion of starch that cannot be enzymatically hydrolyzed in the small intestine, and passes unhydrolyzed to the large intestine (Singh et al., 2010). High amylose content in RS forms long and stable helices which are highly resistant to enzyme mediated hydrolysis (Lehmann and Robin, 2007).

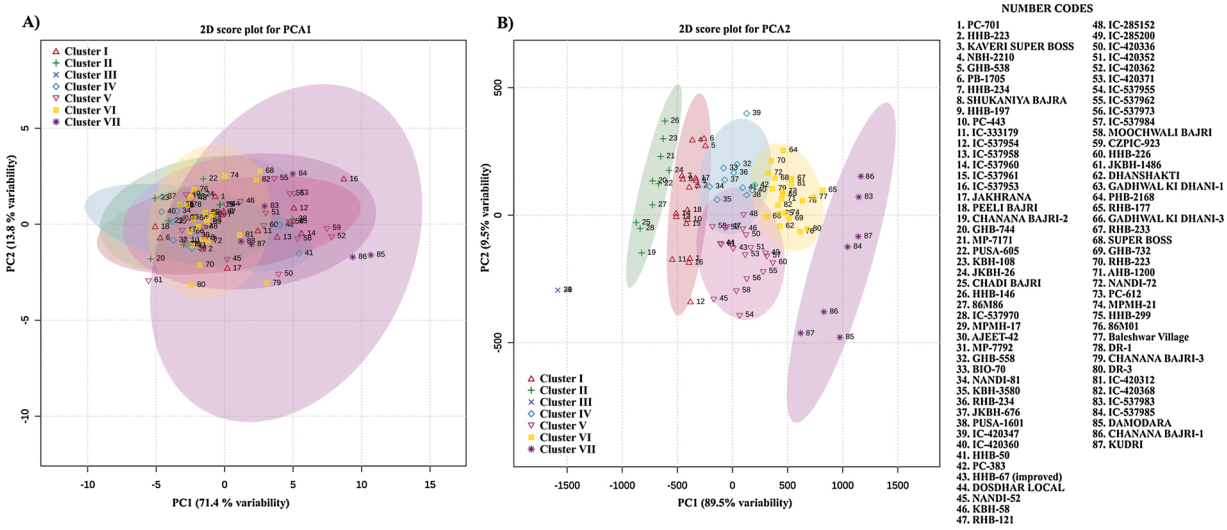

Fig. 2. A: Two dimensional and three PCA score plots for PCA1 indicate the distribution of nutritional traits in 87 samples, statistically clustered by Ward clustering algorithm based on their calculated component loading values for carbohydrates, protein, total dietary fibre, lipids, antinutritional factors, phenols, ash and moisture. **B:** Two dimensional and three PCA score plots for PCA2 indicate the distribution of nutritional traits in 87 samples, statistically clustered by Ward clustering algorithm based on their calculated component loading values for nickel, copper, zinc, potassium, manganese, magnesium, cobalt, molybdenum, selenium, sodium, calcium, phosphorous and iron.

The RS content in this study ranged from 1.49 ± 0.04 g/100 g in IC-420, 368 to 3.52 ± 0.1 g/100 g in PC-443. RS content of 2.41 ± 0.016 g/100 g was also reported by Sharma et al. (2015b) and 1.96 ± 0.01 g/100 g by Ragaee et al. (2006) for Indian cultivars.

Sugars are the prime determinants of glycemic index in foods. The low sugar content in PM contributes to their hypoglycemic property. The TSS content in PM was found in the range of 1.53 ± 0.04 g/100 g in Jakhrana to 3.22 ± 0.1 g/100 g in Nandi-72. A similar range of 2.16–2.78 g/100 g was reported by Subramanian et al. (1981), while 1.4–2.6 g/100 g was reported by Jambunathan and Subramanian (1988) for Indian cultivars and 1.4 and 2.78 g/100 g was stated by McDonough et al. (2000) for non-Indian cultivars. The sucrose content in our study ranged from 1.11 ± 0.03 g/100 g in IC-420,347 to 1.38 ± 0.05 g/100 g in 86M01 and the glucose content ranged from 0.32 ± 0.01 g/100 g in AHB-1200 to 0.75 ± 0.022 g/100 g in DR-3. Our results corroborate with the findings of Subramanian et al. (1981), who reported a range of 1.32–1.82 g/100 g sucrose and 0.09 to 0.16 g/100 g glucose for Indian cultivars. Sucrose content of 0.32–1.20 g/100 g and glucose content of 0.36–3.65 g/100 g was also reported by Hadimani et al. (2001) for non-Indian cultivars. Sucrose content of 1.51 g/100 g and glucose content of 0.063 g/100 g was reported by Mouquet-Rivier et al. (2008) for non-Indian cultivars.

Protein is an essential macronutrient and a fundamental constituent of tissues in humans and animals. They constitute the enzymes for metabolic pathways, maintenance and growth, acts as hormone and signalling molecule, maintains physiological pH, maintains immunity, stores and transports molecules etc (Kumar et al., 2021). PM is a rich source of dietary proteins, rich in essential amino acids (Dias-Martins et al., 2018). Protein content in the present study ranged from 8.07 ± 0.22 g/100 in Kudri to 18.15 ± 0.68 g/100 in IC-537953. The protein content of 15.25 ± 0.21 g/100 was reported by Osman (2011) and 8.38 ± 0.33 g/100 by Obadina et al. (2016) for non-Indian cultivars. The results indicate that higher protein content in these grains can be used for ameliorating protein malnutrition in a cost-effective manner. The protein isolates from these grains can also be used for designing functional protein-rich foods and food additives.

Dietary fibre is the portion of plant material that includes cellulose, noncellulosic polysaccharides like mucilages, gums, pectic substances, hemicellulose, and non-carbohydrate components like lignin, which are highly resistant to enzymatic digestion (Dhingra et al., 2012). PM is a rich source of dietary fibres and has a number of health benefits like controlling glycemic response, lowering cholesterol and the risk of cardiovascular disorders, alleviating constipation and colorectal cancers (Dhingra et al., 2012). TDF was found in the range of 7.68 ± 0.23 g/100 in KBH-108 to 16.18 ± 0.48 g/100 IC-537985. TDF content of 14.95 g/100 g was reported by Ragaee et al. (2006), 20.4 g/100 g was indicated by Kamath and Belavady (1980), 12–20 g/100 g was reported by Hadimani et al. (1995), for non-Indian cultivars and 15–20 g/100 was found by Chethan and Malleshi (2007) for Indian cultivars.

Lipids from PM are an indispensable source for vitamin E, provitamin A, vitamins and essential fatty acids, transporters for fat-soluble vitamins like A, D, E and K (Slama et al., 2020). In the present study, lipid content ranging from 5.24 ± 0.14 % in IC-420,336 to 9.99 ± 0.41 % in MPMH-17 was observed. Similar content of 5.06 % was reported by Slama et al. (2020), 2.7 ± 0.10–7.1 ± 0.25 was reported by Abdalla et al. (1998), 1.5–5% was indicated by Hadimani et al. (1995), 2.4–5% by Nambiar et al. (2011), 7.6 ± 0.2 % by Oshodi et al. (1999), 6.3 % by Sawaya et al. (1984), 7.69 % by Owheruo et al. (2019), 4.86 % by Amadou et al. (2013) for non-Indian cultivars, 5.5 ± 0.15 by Sharma et al. (2015a) for Indian cultivars.

PM lipids are rich in saturated fatty acids (SFA ~25 %), monounsaturated fatty acids (MUFA ~25 %) and polyunsaturated fatty acids (PUFA ~50 %). Linoleic acid constitutes ~45 % PUFA while oleic acid comprises ~25 % MUFA. SFAs like stearic acid and palmitic acid and PUFAs like linoleic acid are the predominant fatty acids constituting the major portion of PM lipids (Jukanti et al., 2016). Palmitic acid (C16:0) was found to range from 20.3 ± 0.62 % in RHB-223 to 32.49 ± 1.19 % in Chanana Bajri-1. Similar content of 16.7–25.0% was reported by Jellum and Powell (1971), 20.7–21.6 by Lai and Varriano-Marston (1980), 16.79 % by Slama et al. (2020) for non-Indian cultivars. Stearic acid (C18:0) was found in the range of 3.28 ± 0.14 % in RHB-233 and 7.91 ± 0.22 in PUSA-1601. A similar trend was observed by Slama et al. (2020) who found a stearic acid content of 5.02 % for non-Indian cultivars. Also, 1.8–8.0 % was observed by Jellum and Powell (1971), 6.1–10.1 by Lai and Varriano-Marston (1980) for non-Indian cultivars. Linoleic acid (C18:2) was found in the range of 32.11 ± 1.15 % in DR-1 to 46.91 ± 0.92 % in Chadi Bajri. A similar range of 40.3–51.7% was observed by Jellum and Powell (1971), 47.50 % by Slama et al. (2020) for non-Indian cultivars. Oleic acid (C18:1) was observed in a range of 21.99 ± 0.68 % in Dosdhar Local to 33.43 ± 1.4 % in PHB-2168. Our findings corroborated with that of Slama et al. (2020), who observed a content of 27.07 %. Also, 20.2–30.6 % was found by Jellum and Powell (1971), 27.2–28.2 by Lai and Varriano-Marston (1980) for non-Indian cultivars, 20.80–22.25 % by Yadav et al. (2012) for Indian cultivars.

Phytic acid exhibits a unique characteristic of chelating important cations like potassium, zinc, copper, iron, magnesium and calcium, thereby significantly reducing their bioaccessibility and bioavailability by animals and humans (Marathe et al., 2018). The phytic acid content in the present study was found in the range from 0.54 ± 0.02 g/100 g in Kaveri Super Boss to 1.43 ± 0.04 g/100 g in Kudri. Our results corroborated with that of Pushparaj and Urooj (2014) who reported a range of 0.26 to 0.99 g/100 g for Indian cultivars and El Hag et al. (2002) for non-Indian cultivars, who found a range of 0.943–1.076 g/100 g. The results suggest that PM grains have a significantly lower phytic acid content when compared to cereals like soybeans, rice, wheat and oats. Thus their routine consumption cannot possibly cause a decrease in mineral bioavailability and bioaccessibility.

RFOs is another category of antinutritional factors that are metabolized in the lower intestine causing the production of hydrogen, carbon dioxide and methane causing diarrhoea and flatulence. RFO constitute nearly 25 % of the sugars in PM grains. The RFO content ranged from 0.27 ± 0.01 mmol/100 g in IC-420,336 to 2.08 ± 0.08 mmol/100 g in RHB-234. A similar range of 1.2–2.5%, 0.67 to 0.84 % and 0.787 % was reported by Hadimani et al. (2001); Subramanian et al. (1981), and Mouquet-Rivier et al. (2008) respectively.

Polyphenols comprise the biggest class of phytochemicals which are directly linked to a multitude of health benefits (Tian et al., 2020b). Several studies have shown that PM is rich in phenolic compounds which have rich antioxidant and metal chelating activities (Saleh et al., 2013). Phenol content ranged from 0.04 ± 0.001 GAE g/100 g in IC-420, 336 to 0.21 ± 0.0088 GAE g/100 g in PHB-2168. A similar content of 0.303 g/100 g was reported by El Hag et al. (2002) and 0.294 to 0.314 g/100 g by Elyas et al. (2002) for non-Indian cultivars. Higher phenol content in PM makes them optimum for maintaining the cellular redox potential and quenching the ROS species. Grain moisture content in our study ranged from 9.2 ± 0.25 % in IC-420,336 to 11.9 ± 0.46 % in PB-1705. A similar content of 9.60–12.81% was reported by Suma and Urooj (2015) and 10.36–12.39% by Obadina et al. (2016) for non-Indian cultivars. The grain moisture content determines the storability and rheological properties of PM grains. The ash content reflects the mineral content of a sample. The ash content in the present study ranged from 0.63 ± 0.02 g/100 g in JKBH-1486 to 1.69 ± 0.05 g/100 g in IC-420360. Similar ash content of 1.68–2.21 g/100 g was reported by Obadina et al. (2016) and 1.64 g/100 g by Amadou et al. (2013) for non-Indian cultivars.

Mineral nutrients are indispensable for human health and the efficient functioning of metabolic pathways. Human nutrition requires at least 49 nutrients to fulfil organic metabolic requirements (Mohammad et al., 2017). Out of these, 23 minerals are vital for biochemical and physiological activities. phosphorous forms an important component of teeth, bones, and phospholipids. It helps to maintain physiological pH, nucleotide synthesis and several energy transfer reactions (Mohammad

et al., 2017). The phosphorous content in the present study ranged from 151.14 ± 5.87 ppm in Kaveri Super Boss to 403.46 ± 11.02 ppm in Kudri. Similar content of 304–681 ppm was reported by Badau et al. (2005) and 268 ppm by Obadina et al. (2016) for non-Indian cultivars. Calcium forms the basic structural framework of teeth and bones. It is also vital for intracellular communication, blood coagulation, contractility and excitability of striated muscles, neuromuscular transmission and maintenance of cell permeability. The calcium content in the present study ranged from 192.5 ± 8.08 ppm in MP-7792 to 405.59 ± 16.68 ppm MPMH-17. Similar content of 500 ppm was reported by Jukanti et al. (2016) for Indian cultivars and 170–340 ppm Bailey et al. (1979). Iron primarily regulates electron and oxygen transport, participates in cellular metabolism, a component of numerous enzymes and haem/-myoglobin. The iron content in the present study ranged from 36.56 ± 1.11 ppm in Nandi-72 to 124.89 ± 4.55 ppm in IC-537960. Similar content of 55 ppm was reported by Oshodi et al. (1999) and 121 ppm by Owheruo et al. (2019) for non-Indian cultivars. Potassium helps to maintain inter and intracellular fluid volume, partially regulates blood pressure, regulates and generates nerve impulses. The potassium content in the present study ranged from 2495.57 ± 102.63 ppm in MPMH-17 to 5257.51 ± 192.35 ppm in Chanana Bajri-1. Similar content of 4500 ppm was reported by Owheruo et al. (2019) and 5004.5 ppm by Adebiyi et al. (2017) for non-Indian cultivars. Magnesium forms an important component of bones, regulates muscle relaxation, governs salt, water and acid-base balance. It also regulates nerve transmission and governs enzyme-mediated energy metabolism. The magnesium content in the present study ranged from 1424.02 ± 43.44 ppm in HHB-146 to 2663.47 ± 92.71 ppm in IC-537983. Similar content of 1028.6 ppm was reported by Owheruo et al. (2019) and 1370 ppm by Adetola et al. (2019) for non-Indian cultivars. Sodium retains the cellular fluid volume, controls pH, regulates muscle contraction, and nerve transmission. The sodium content in the present study ranged from 23.25 ± 0.69 ppm in DR-3 to 49.97 ± 1.51 ppm in RHB-121. A similar content of 60.89 ppm was reported by Ragaee et al. (2006) and 182 ppm by Oshodi et al. (1999) for non-Indian cultivars. Zinc regulates protein synthesis, immunity, sexual maturation, macronutrient metabolism enzymes, flavour sensation, DNA metabolism and gene expression. The zinc content in the present study ranged from 15.62 ± 0.57 ppm in Chanana Bajri-1 to 62.3 ± 1.9 ppm in KBH-58. Similar content of 53–71 ppm was reported by Abdalla et al. (1998) and 66 ppm by Adetola et al. (2019) for non-Indian cultivars. Manganese works as a cofactor for enzymes involved in carbohydrate, amino acid, cholesterol metabolism. The manganese content in the present study ranged from 8.21 ± 0.24 ppm in Peeli Bajri to 17.77 ± 0.65 ppm in Chanana Bajri-3. Similar results of 8 ppm were indicated by Oshodi et al. (1999) and 31.9 ppm by Adebiyi et al. (2017) for non-Indian cultivars. Copper participates in elastin and collagen synthesis, works as an antioxidant, forms haemoglobin and red blood cells. It is also the primary cofactor of several metalloenzymes (Zn/Cu superoxide dismutase, lysyl oxidase) participating in iron metabolism and cellular respiration. The copper content in the present study ranged from 1.29 ± 0.04 ppm in PUSA-1601 to 7.29 ± 0.23 ppm in KBH-3580. Similar content of 3.45 ppm was reported by Ragaee et al. (2006) and 4.4–6.0 ppm by Badau et al. (2005). Cobalt forms the central part of vitamin cyanocobalamin. The cobalt content in the present study ranged from 0.19 ± 0.006 ppm in CZP16−923 to 0.51 ± 0.016 ppm in HHB-226. Similar content of 0.54 to 0.89 ppm was reported by Abdelrahaman et al. (2007). Nickel serves as a metalloenzyme cofactor and enhances iron bioavailability. nickel in the present study ranged from 0.29 ± 0.012 ppm in MP-7792 to 1.91 ± 0.079 ppm in MPMH-17. Molybdenum serves as a cofactor for enzymes participating in sulfur amino acid catabolism, metabolism of pyrimidines and purines, and enzymes in the electron transport system. The molybdenum content in the present study ranged from 0.59 ± 0.016 ppm in PUSA-605 to 0.95 ± 0.029 ppm in AHB-1200. Selenium acts as a cellular antioxidant, regulates thyroid hormone, maintains the oxidative states of vitamin C. It also acts as a cofactor for tyrosine deiodinase, glutathione peroxidase and regulates the expression of T lymphocyte receptor. selenium content in the present study ranged from 0.001 ± 0 ppm in NANDI-52 to 0.53 ± 0.015 ppm in PC-383. The relative mean nutritional composition of each germplasm is indicated through a heat map in Fig. 3 and supplementary Table S4, S5 and S6. The geographical regions of the cultivars and their nutritional mean composition are indicated through a heat map, Fig. 4. The varieties used in the present study and their place of cultivation is present in Supplementary Table S1. The agronomic aspects of PM germplasm grown in different states is as follows. The field was ploughed once or twice, followed by harrowing for creating fine tilth. The grains were sowed following (1) flat surface, or (2) ridge and furrow system, or (3) broad-bed and furrow system. The seeds were sown at 2–3 cm depth at a seed rate of 3–4 kg/ha. The Kharif pearl millet sowing was done with the onset of monsoon i.e. first fortnight of July in Rajasthan, Gujarat, Uttar Pradesh, New Delhi, Punjab, Haryana, Madhya Pradesh and Uttarakhand. The first fortnight of October was appropriate rabi season in Tamil Nadu and Telangana. Gap filling was done where needed by transplanting the seedlings 2–3 weeks after sowing. Dry sowing, before the first monsoon rains, was done in Maharashtra. Summer pearl millet was sown from the last week of January to the 1st week of February. Seeds were treated by thiram 75 % dust @ 3 g kg^{-1} seed or biopesticides (*Trichoderma harzianum* @ 4 g kg^{-1} seed) for preventing soilborne diseases. Seeds were treated with 300-mesh sulfur powder @ 4 g kg^{-1} seeds for controlling smut infection. Seeds were soaked in 10 % salt solution for eliminating ergot affected seeds. Seeds were treated with metalaxyl (Apron 35 SD) @ 6 g kg^{-1} seed for controlling downy mildew. Seeds were treated by *Phosphobacterium* and *Azospirillum* (600 g) for enhancing phosphorous and nitrogen availability. For arid-western plains of Gujarat, Haryana and Rajasthan PM were planted in 60 cm apart rows, maintain a plant population of 1.00–1.25 lac/ha. For areas with rainfall more than 450 mm, the crop was planted at 45 × 10–15 cm, maintaining a plant population of 1.75–2.0 lakhs/ha. Sixty kg N/ha +30 kg P_2O_5/ha for semi-arid and 40 kg N + 20 kg P_2O_5/ha was used for arid regions, for sole pearl millet as well as intercropping system. In the regions with sandy loamy soils, the applied nitrogen can be lost by leaching due to heavy rains, thus only half the recommended N dose must be applied during seedbed preparation. The other half of the N dose was side-dressed after the crop was 25 days old. On soils that do not easily leach, the complete nitrogen dose should be applied during seedbed preparation. Since PM is sensitive to fertilizer burn, the fertilizer was not applied to the furrows with seeds or near the seeds and was applied as side-dressing. Biofertilizer (*Azospirillum* and phosphate solubilizing bacteria) was used to economize the P and N application. A zinc dose of 10 kg $ZnSO_4$/ha was used for zinc deficient soils, also 0.2 % $ZnSO_4$ spray at tillering to the pre-flowering stage was used. For weed control (weeding and hoeing) 15 and 30 DAS (days after sowing) was used for effectively controlling weeds along with the pre-emergent application of atrazine @ 0.5 kg/ha. Pearl millet-based cropping systems in Kharif for Rajasthan (Pearl millet + Sesame/ moth bean/ green gram/ cowpea/ cluster bean), Haryana (Pearl millet + Cowpea/ cluster bean/ sesame / green gram), Gujarat (Pearl millet + Cowpea/ sesame/ green gram), Uttar Pradesh (Pearl millet + Cowpea/ sesame/ green gram), Madhya Pradesh (Pearl millet + Cowpea/ Black gram/ pigeon pea/ soybean), New Delhi (Pearl millet + Castor/ pigeon pea/ groundnut), Punjab (Pearl millet + Wheat/ fodder sorghum/ chickpea), Maharashtra (Pearl millet + sunflower/ cowpea /green gram, black gram/ soybean/ pigeon pea/ moth bean), Karnataka (Pearl millet + Green gram, Pigeon pea / soybean/ sunflower), Tamil Nadu (Pearl millet + Green gram, Pigeon pea / cowpea/ soybean/ sunflower), Telangana (Pearl millet + Green gram/ groundnut/ soybean / sunflower / pigeon pea). During prolonged dry spells, irrigation was applied at certain critical crop growth stages tillering, flowering and grain developmental stages. During summers, PM was irrigated regularly (0.75–1.0 ratio of the net depth of irrigation water (IW) to cumulative pan evaporation (CPE) with 40 mm). The crop was harvested after attaining its physiological maturity, which was determined by a black

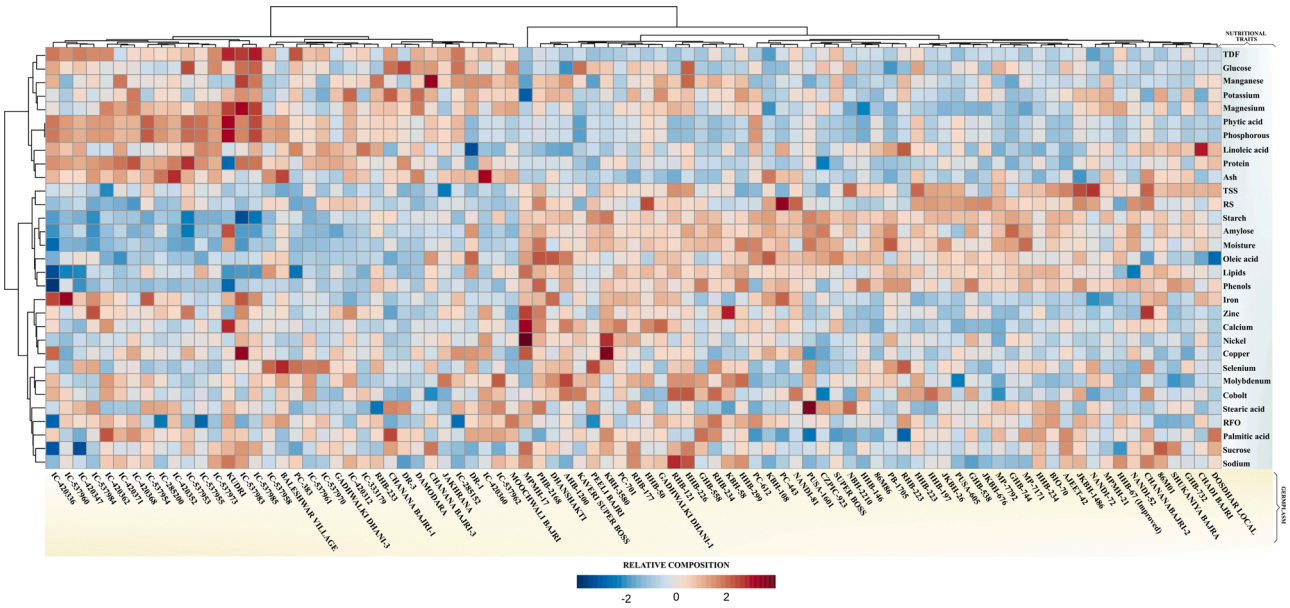

Fig. 3. The relative mean nutritional composition of each germplasm using a heat map. The mean values of the relative nutritional attributes of these clusters are graphically represented as individual values contained in the matrix and represented as colours. The data is scaled between +2 and -2.

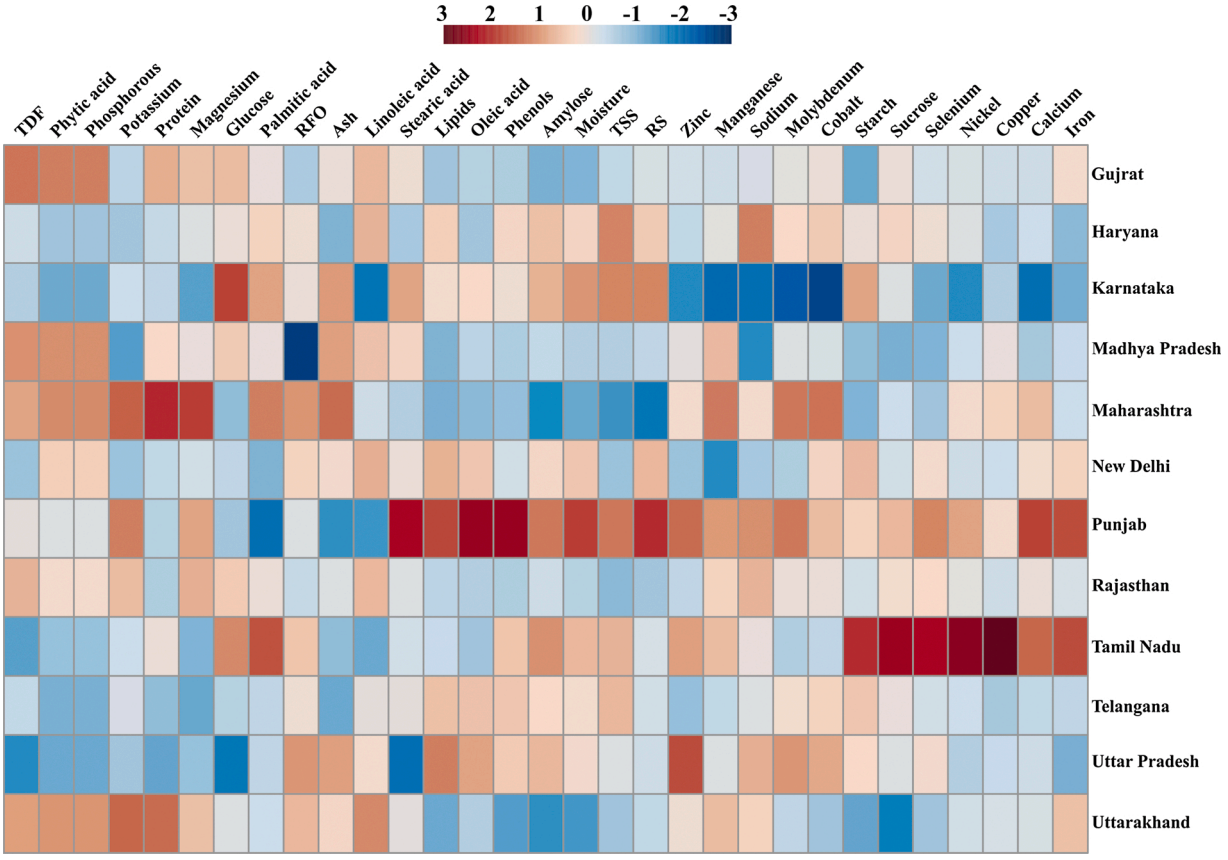

Fig. 4. The geographical regions of the cultivars and their nutritional mean composition. The mean values of the relative nutritional attributes of these clusters are graphically represented as individual values contained in the matrix and represented as colours. The data is scaled between +3 and -3. Here TDF: Total dietary fibre; RFO: Raffinose family oligosaccharides; TSS: total soluble sugars; RS: Resistant starch.

spot in the hilar region at the bottom of the grain. The leaves attain a yellowish colour with a dried up appearance. The earheads were cut first and the stalks later. The stalks (straw) were cut after one week, allowed to dry and later stacked.

The results indicate that germplasm derived from Gujrat, Madhya Pradesh, Maharashtra and Rajasthan showed high content of total dietary fibre ($p = 0.011$). Those derived from Haryana, Karnataka, Tamil Nadu, Telangana and Uttar Pradesh showed lower phytic acid content (p

= 0.017) and those from Gujarat, Madhya Pradesh and Rajasthan had lower RFO contents (p = 0.262). Those derived from Gujarat, Maharashtra and Uttarakhand showed higher protein content (p = 0.002). Those derived from Haryana, Karnataka, New Delhi, Punjab, Tamil Nadu and Uttar Pradesh showed high amylose (p = 0.002) and starch values (p = 0.001). Those derived from New Delhi, Punjab, Tamil Nadu and Uttarakhand showed high Iron content (p = 0.599). Those derived from Maharashtra, Punjab and Tamil Nadu had higher copper content (p = 0.001). Those derived from Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh had higher zinc contents (p = 0.002). Those derived from Maharashtra, Punjab, New Delhi, and Tamil Nadu showed higher calcium contents (p = 0.001). Those derived from Punjab and Tamil Nadu had higher sucrose content (p = 0.607). Those derived from Haryana, Karnataka and Punjab showed higher TSS (p = 0.011) and RS (p = 0.006) values. Haryana, Karnataka, New Delhi, Punjab, and Uttar Pradesh had higher lipid content. This variation in the nutritional content of these germplasms could result from soil nutrient availability, root absorption capacity, interactions between soil nutrients and plants, interactions with microbial communities in the plant rhizosphere, photosynthesis rate, climatic conditions etc (Briat et al., 2020). The state-wise results with their mean difference at $p \leq 0.05$ are indicated in Supplementary Table S7 (nutritional composition), S8 (mineral composition) and S9 (lipid and fatty acids composition).

3.3. Correlation analysis

The correlation coefficient was studied among carbohydrates, protein, total dietary fibre, lipids, antinutritional factors, phenols, ash, moisture and minerals. Pearson's correlation test was used to determine significant correlations between the nutritional parameters. With $p < 0.05$, Pearson's $r > 0$ indicate significant positive correlation, $r < 0$ indicate significant negative correlation. Factors like genetic variation, growth conditions, agronomic practices and soil type can cause significant changes in the grain nutritional and biochemical composition. The values of Pearson's r is indicated through a heat map in Fig. 5, and the exact values are depicted in Supplementary Table S10. Grain protein content was negatively correlated with starch ($r = -0.698$ and $p < 0.001$) and amylose ($r = -0.782$ and $p < 0.001$). Yu et al. (2017) also obtained similar results, where they explained that the protein content of grains increases by suppressing starch synthesis. The catalytic

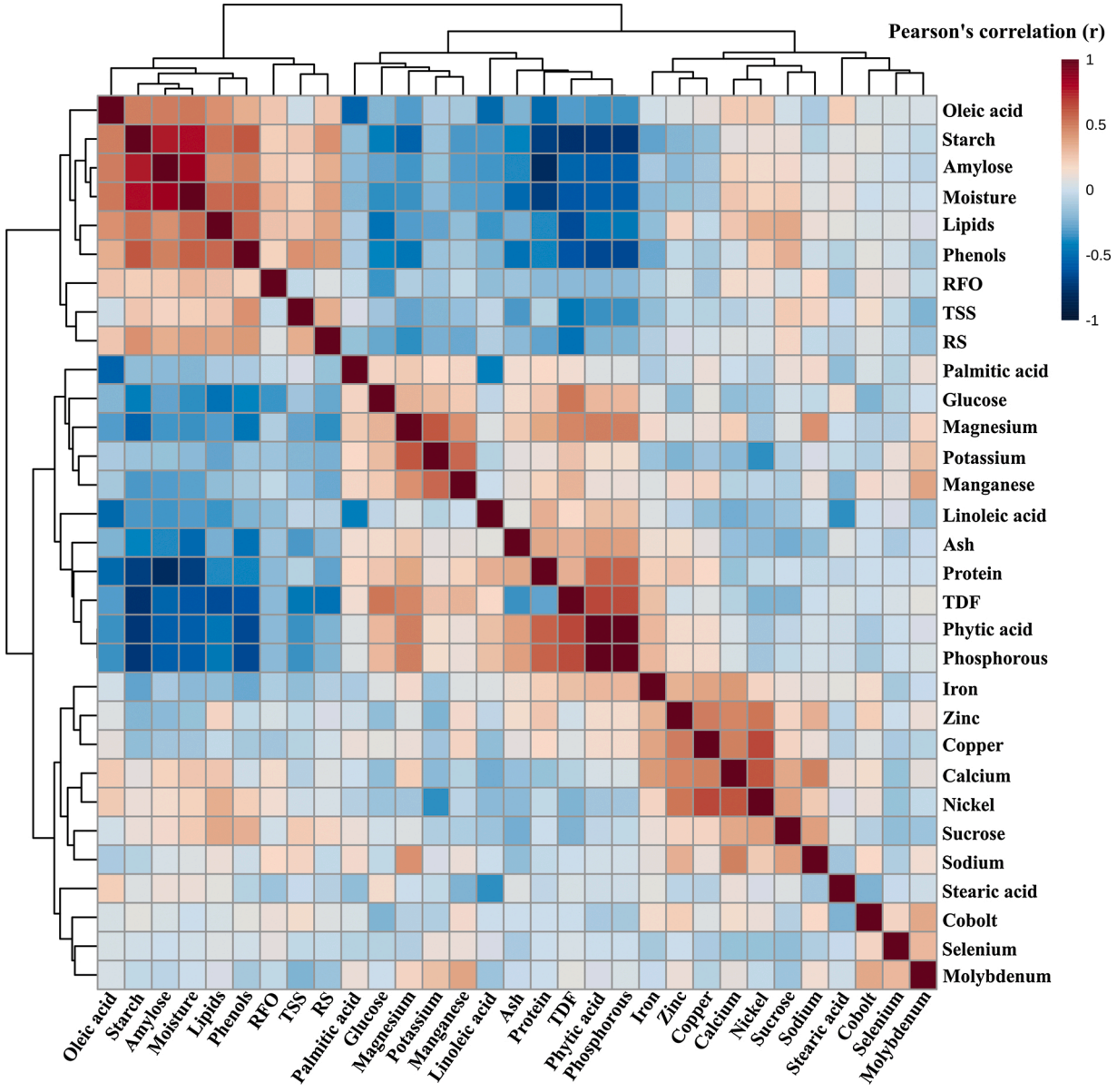

Fig. 5. The values of Pearson's r is indicated through a heat map. The Pearson's r (+1 to -1) is indicated through colours.

activities of starch synthases show variation with protein content, causing altered starch content. The nature of the starch-protein matrix can cause such correlations. The authors also explained that activities of starch branching enzymes can be subsequently reduced by an increase in grain protein content, explaining the inverse correlation between amylose and protein content (Bao et al., 2020).

RS was positively correlated with starch ($r = 0.425$ and $p < 0.001$), amylose ($r = 0.341$ and $p < 0.001$) and negatively correlated with proteins ($r = –0.286$ and $p = 0.007$). It is widely known that grain starches having higher amylose content, show higher RS (Tao et al., 2019). This could be due to the presence of a positive correlation between starch synthesizing enzymes, granule-bound starch synthase I (synthesize amylose), starch synthase IIIa (synthesize long amylopectin chains), amylopectin chain length and RS content (Bao et al., 2020). Also, the negative correlation between RS and proteins could be explained by the negative correlation between starch and proteins. TDF was negatively correlated with TSS ($r = –0.435$ and $p < 0.001$). This could be explained by the fact that α-amylase in grain catalyzes the conversion of starch to soluble sugars. The non-starch polysaccharides forming the crucial proportion of TDFs can impede the α-amylase activity, inhibiting its interaction with starch (Štěrbová et al., 2016). Amylose was positively correlated with starch ($r = 0.753$ and $p < 0.001$) as amylose having α-1,4-linked linear glucose chains constitute < 35 % of PM starch (Annor et al., 2014). Glucose was found to be negatively correlated to starch ($r = –0.401$ and $p < 0.001$), amylose ($r = –0.261$ and $p = 0.015$) and RS ($r = –0.265$ and $p = 0.013$). ADP-glucose pyrophosphorylase (AGPase) catalyzed the synthesis of ADP-glucose from glucose. ADP-glucose is the primary substrate for the biosynthesis of amylose, starch, and RS (Ballicora et al., 2004). The negative correlation exists due to the depletion in glucose levels caused by its channelization towards starch, amylose and RS biosynthesis (Luo and Huang, 2011).

Protein and lipid content showed a negative correlation ($r = –0.376$ and $p < 0.001$). Studies have indicated that lipid and storage proteins compete with each other for the same pool of carbon skeletons (Wang et al., 2019). The germplasm with high protein content consumes an excess of carbon skeletons, with an insufficient number of carbon skeletons left for lipid biosynthesis. The lipid content was also positively associated with TSS ($r = 0.241$ and $p = 0.024$) and sucrose ($r = 0.296$ and $p = 0.005$), which fuel the lipid biosynthesis through metabolic processes like glycolysis. The positive correlation of lipid with starch ($r = 0.523$ and $p < 0.001$), amylose ($r = 0.418$ and $p < 0.001$) and RS ($r = 0.373$ and $p < 0.001$) could be attributable to the involvement of starch biosynthesis in establishing the sink activity of seeds needed for the onset of lipid biosynthesis and accumulation. Synthesis of starch during the early seed development stage facilitates the accumulation of incoming photosynthetic carbon for lipid synthesis.

During fatty acid biosynthesis, malonyl-CoA and acetyl-CoA are sequentially linked through acetyl group transfers to form palmitic acid. This is the precursor of several long-chained fatty acids like oleic acid. This was also depicted by a negative correlation between oleic and palmitic acid ($r = –0.521$ and $p < 0.001$), due to the flow of palmitic acid for oleic acid biosynthesis. Lipid content was positively correlated with oleic acid ($r = 0.395$ and $p < 0.001$). Similar results were found by Coetzee et al. (2008). Stearic acid was negatively correlated with linoleic acid ($r = –0.343$ and $p = 0.001$) and linoleic acid was negatively correlated with oleic acid ($r = –0.500$ and $p < 0.001$). These correlations could be explained through the sequential conversion of stearic acid to oleic acid through Δ9 desaturase (decreasing stearic acid and increasing oleic acid) and oleic acid to linoleic acid through Δ12 desaturase (decreasing oleic acid and increasing linoleic acid) (Liu et al., 2002; Burton et al., 2004). RFO was positively correlated with lipid content ($r = 0.233$ and $p = 0.03$). Studies suggest that carbon derived from lipids could participate in central metabolism and initiate RFO biosynthesis (Kambhampati et al., 2020).

Phytic acid was positively correlated with phosphorous ($r = 1.000$ and $p < 0.001$) and proteins ($r = 0.560$ and $p < 0.001$). The highly positive correlation can be explained by the fact that one phytic acid molecule, contains six phosphorous atoms (Marathe et al., 2018). Also phosphorous was positively correlated with proteins ($r = 0.560$ and $p < 0.001$). This correlation could be attributable to the important role of phosphorous in plant nitrogen, protein metabolism and phosphorylation of storage and signalling proteins (Liu et al., 2015). phosphorous is also the constituent of respiratory enzymes like cytochrome oxidase, flavin enzyme, dehydrogenases producing organic acids like oxaloacetic acid, fumaric acid, α-ketoglutaric acid and pyruvic acid. These organic acids serve as receptors of ammonia for amino acid biosynthesis (Han et al., 2014). Proteins also showed positive correlation with minerals like iron ($r = 0.224$ and $p = 0.037$), magnesium ($r = 0.356$ and $p < 0.001$) and zinc ($r = 0.304$ and $p = 0.004$). This could be because these minerals are important components and cofactors for several enzymatic proteins. iron is an important part of catalase, ferritin, superoxide dismutase, cytochromes and electron transfer proteins in respiration and photosynthesis, heme and iron-sulfur cluster proteins, respiration and nitrogen assimilation (Shokrollahi et al., 2018). magnesium is an integral part of phosphate transfer enzymes, DNA/RNA polymerases, carbohydrate metabolism enzymes, photophosphorylation, protein synthesis (Xie et al., 2020). zinc is the primary component of enzymes like carbonic anhydrase, glutamic dehydrogenase, alcohol dehydrogenase, zinc-finger transcription factors, protein synthesis, carbohydrate metabolizing enzymes (Zaheer et al., 2019).

Phytic acid was positively correlated with proteins ($r = 0.560$ and $p < 0.001$) as the anionic phytic acid groups strongly binds to cationic protein groups. Researchers have indicated that ε-NH_2 of lysine and α-NH_2 terminal group of the protein chain, also guanidyl group of arginine and imidazole group of histidine have shown to be probable binding sites of phytic acid. Our results showed a positive correlation between phytic acid and iron ($r = 0.316$ and $p = 0.003$) and magnesium ($r = 0.487$ and $p < 0.001$). This could be due to the strong chelating activity of phytic acid for these cations. More phytic acid molecules bind a higher number of such metal cations, substantiating the positive correlation. TDF was negatively correlated with ash ($r = –0.316$ and $p = 0.003$), protein ($r = –0.325$ and $p = 0.002$) starch ($r = –0.744$ and $p < 0.001$), amylose ($r = –0.510$ and $p < 0.001$) and RS ($r = –0.460$ and $p < 0.001$). This correlation pattern was observed because the TDF was estimated by eliminating the carbohydrates and sugars using carbohydrases and degradation of proteins using proteases. The TDF was calculated by subtracting the weight of ash and proteins from the weight of filtered and dried fractions.

4. Conclusion

The present work suggests that PM grains exhibit immense potential as a food crop, due to their superior content of pertinent nutrients like carbohydrates, proteins, minerals, dietary fibres besides their low cultivation cost. Additionally, this crop displays appreciable relevance for ensuring food security, owing to its unique agronomic and physiological characteristics like Our findings indicate that PM germplasm displays a great nutritional diversity and thus can have diverse food applications. HCA revealed that Cluster I, II and III showed higher content of amylose, starch, moisture, cluster III had higher lipid content. cluster I, II, III and IV showed higher RS. Cluster II and III had higher TSS, cluster III showed higher sucrose content. Cluster V and VII were indicated by higher glucose and protein content. Cluster II, III, IV and VI showed phytic acid content and cluster III showed higher mineral content. The germplasms in these clusters can be used as functional food ingredients and food diversification. Clusters with high content of amylose, starch, moisture and lipids determine optimum techno-functional properties making these germplasms suitable for food processing industries and designing food products like cookies, bread and bakery products. Germplasms with high RS, TDF and low TSS can be used for designing low glycemic index diet foods for diabetics and individuals with cardiovascular diseases, hypercholesteremia and bowel

disorders. Clusters with high carbohydrate and protein content could increase the feasibility of these germplasms for designing foods for underdeveloped and poor countries and for addressing the problems of protein-energy malnutrition. Cluster with low RFO can be used for flatulence sensitive individuals.

The germplasms also display wide region-specific variations. Those derived from Gujarat, Maharashtra and Uttarakhand showed higher protein content. Those derived from Haryana, Karnataka, New Delhi, Punjab, Tamil Nadu and Uttar Pradesh showed high carbohydrates. Those from New Delhi, Punjab, Tamil Nadu and Uttarakhand showed high Iron content and high copper was found in germplasm from Maharashtra, Punjab and Tamil Nadu. High zinc was found in germplasms from Maharashtra, Punjab, Tamil Nadu and Uttar Pradesh. More calcium was found in germplasm from Maharashtra, Punjab, New Delhi, and Tamil Nadu. The nutritional composition depends on the genetic makeup of the cultivars, agricultural practices, soil fertility, irrigation practices, climatic variations, which might explain the difference among germplasm from various locations. The variations caused by plant-soil interactions include soil nutrient availability, root absorption capacity which is feedback controlled by plant growth. Interactions between plants and different soil nutrients imply that the availability of one nutrient determines the availability of other nutrients. The interaction between the plant rhizosphere microflora and soil also influences the nutrient dynamics. Factors like soil pH, redox potential, and the cation exchange capacity also govern nutrient availability, plant growth and consequently the nutritional quality of grains. The nutritional uptake also relies on the physicochemical properties of the soil mineral matrix, determining the equilibrium among different mineral forms which are more or less utilized by plants. Different geographical locations have different climatic conditions, which in turn determine the rate of carbohydrate accumulation through photosynthesis. Future studies are needed to launch novel cultivars having the desired blend of nutritional and functional attributes to make this crop a more viable alternative for consumers seeking cost-effective, sustainable and nutritious products.

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CRediT authorship contribution statement

Maharishi Tomar: Investigation, Writing - original draft, Visualization. **Rakesh Bhardwaj:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Manoj Kumar:** Formal analysis, Investigation, Writing - review & editing. **Sumer Pal Singh:** Writing - review & editing. **Veda Krishnan:** Writing - review & editing. **Rekha Kansal:** Writing - review & editing. **Reetu Verma:** Formal analysis, Investigation, Writing - review & editing. **Vijay Kumar Yadav:** Writing - review & editing. **Anil dahuja:** Writing - review & editing. **Sudhir Pal Ahlawat:** Writing - review & editing. **Jai Chand Rana:** Writing - review & editing. **Haritha Bollinedi:** Writing - review & editing. **Ranjeet Ranjan Kumar:** Writing - review & editing. **Suneha Goswami:** Writing - review & editing. **Vinutha T:** Writing - review & editing. **C. Tara Satyavathi:** Writing - review & editing. **Shelly Praveen:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Archana Sachdev:** Conceptualization, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2021.104086.

References

AACC - American Association of Cereal Chemists, 2010. Approved Methods of Analysis, 11th ed. AACC International., St. Paul, MN, USA.
Abdalla, A.A., Tinay, A.H.El, Abdalla, B.E.M.A.H., 1998. Proximate composition, starch, phytate and mineral contents of 10 pearl millet genotypes. Food Chem. 63, 243–246.
Abdelrahaman, S.M., Elmaki, H.B., Idris, W.H., Hassan, A.B., Babiker, E.E., El Tinay, A. H., 2007. Antinutritional factor content and hydrochloric acid extractability of minerals in pearl millet cultivars as affected by germination. Int. J. Food Sci. Nutr. https://doi.org/10.1080/09637480601093236.
Abraham, Z., Yadav, S., Latha, M., Mani, S., Mishra, S.K., 2010. Seed variability in Madhuca longifolia (Koenig) J. F. Macbride, a source of oil for use as bio-energy. Genet. Resour. Crop Evol. 57, 619–623. https://doi.org/10.1007/s10722-010-9536-x.
Adebiyi, J.A., Obadina, A.O., Adebo, O.A., Kayitesi, E., 2017. Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (Pennisetum glaucum) flour. Food Chem. 232, 210–217. https://doi.org/10.1016/j.foodchem.2017.04.020.
Adetola, O.Y., Kruger, J., White, Z., Taylor, J.R.N., 2019. Comparison between food-to-food fortification of pearl millet porridge with moringa leaves and baobab fruit and with adding ascorbic and citric acid on iron, zinc and other mineral bioaccessibility. Lwt 106, 92–97. https://doi.org/10.1016/j.lwt.2019.02.044.
Amadou, I., Gounga, M.E., Le, G.W., 2013. Millets: nutritional composition, some health benefits and processing - A review. Emirates J. Food Agric. 25, 501–508. https://doi.org/10.9755/ejfa.v25i7.12045.
Amponsah, G., Marcone, M., Corredig, M., Bertoft, E., 2015. Effects of the amount and type of fatty acids present in millets on their in vitro starch digestibility and expected glycemic index (eGI). J. Cereal Sci. 64, 76–81. https://doi.org/10.1016/j.jcs.2015.05.004.
Annor, G.A., Marcone, M., Bertoft, E., Seetharaman, K., 2014. Physical and molecular characterization of millet starches. Cereal Chem. 91, 286–292. https://doi.org/10.1094/CCHEM-08-13-0155-R.
Annor, G.A., Tyl, C., Marcone, M., Ragaee, S., Marti, A., 2017. Why do millets have slower starch and protein digestibility than other cereals? Trends Food Sci. Technol. 66, 73–83. https://doi.org/10.1016/j.tifs.2017.05.012.
AOAC - Association of Official Analytical Chemists, 2005. Official Methods of Analysis of the Association of Analytical Chemists, 18th ed. AOAC International., Gaithersburg, MD, USA.
Badau, M.H., Nkama, I., Jideani, I.A., 2005. Phytic acid content and hydrochloric acid extractability of minerals in pearl millet as affected by germination time and cultivar. Food Chem. 92, 425–435. https://doi.org/10.1016/j.foodchem.2004.08.006.
Bailey, A.V., Piccolo, B., Sumrell, G., Burton, G.W., 1979. Amino acid profiles, chemical scores, and mineral contents of some pearl millet inbred lines. J. Agric. Food Chem. https://doi.org/10.1021/jf60226a061.
Ballicora, M.A., Iglesias, A.A., Preiss, J., 2004. ADP-glucose pyrophosphorylase: a regulatory enzyme for plant starch synthesis. Photosynth. Res. 79, 1–24. https://doi.org/10.1023/B:PRES.0000011916.67519.58.
Bao, J., Ying, Y., Zhou, X., Xu, Y., Wu, P., Xu, F., Pang, Y., 2020. Relationships among starch biosynthesizing protein content, fine structure and functionality in rice. Carbohydr. Polym. 237, 116118 https://doi.org/10.1016/j.carbpol.2020.116118.
Bhupender, S.K., Rajneesh, B., Baljeet, S.Y., 2013. Physicochemical, functional, thermal and pasting properties of starches isolated from pearl millet cultivars. Int. Food Res. J. 20, 1555–1561.
Briat, J., Gojon, A., Plassard, C., Rouached, H., Lemaire, G., 2020. Reappraisal of the central role of soil nutrient availability in nutrient management in light of recent advances in plant nutrition at crop and molecular levels. Eur. J. Agron. 116, 126069 https://doi.org/10.1016/j.eja.2020.126069.
Burton, J.W., Miller, J.F., Vick, B.A., Holbrook, C.C., Scarth, R., 2004. Altering fatty acid composition in oil seed crops. Adv. Agron. 84, 273–306. https://doi.org/10.1016/S0065-2113(04)84006-9.
Chethan, S., Malleshi, N.G., 2007. Finger millet polyphenols: Optimization of extraction and the effect of pH on their stability. Food Chem. https://doi.org/10.1016/j.foodchem.2007.02.012.
Chong, J., Yamamoto, M., Xia, J., 2019. MetaboAnalystR 2.0: from raw spectra to biological insights. Metabolites 9. https://doi.org/10.3390/metabo9030057.

Coetzee, R., Labuschagne, M.T., Hugo, A., 2008. Fatty acid and oil variation in seed from kenaf (Hibiscus cannabinus L.). Ind. Crops Prod. 27, 104–109. https://doi.org/10.1016/j.indcrop.2007.08.005.

Cornejo-Ramírez, Y.I., Martínez-Cruz, O., Del Toro-Sánchez, C.L., Wong-Corral, F.J., Borboa-Flores, J., Cinco-Moroyoqui, F.J., 2018. The structural characteristics of starches and their functional properties. CYTA - J. Food 16, 1003–1017. https://doi.org/10.1080/19476337.2018.1518343.

Dhingra, D., Michael, M., Rajput, H., Patil, R.T., 2012. Dietary fibre in foods: a review. J. Food Sci. Technol. 49, 255–266. https://doi.org/10.1007/s13197-011-0365-5.

Dias-Martins, A.M., Pessanha, K.L.F., Pacheco, S., Rodrigues, J.A.S., Carvalho, C.W.P., 2018. Potential use of pearl millet (Pennisetum glaucum (L.) R. Br.) in Brazil: food security, processing, health benefits and nutritional products. Food Res. Int. 109, 175–186. https://doi.org/10.1016/j.foodres.2018.04.023.

Dykes, L., Rooney, L.W., 2007. Phenolic compounds in cereal grains and their health benefits. Cereal Foods World 52, 105–111. https://doi.org/10.1094/CFW-52-3-0105.

El Hag, M.E., El Tinay, A.H., Yousif, N.E., 2002. Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and in vitro protein digestibility of pearl millet. Food Chem. https://doi.org/10.1016/S0308-8146(01)00336-3.

Elyas, S.H.A., El Tinay, A.H., Yousif, N.E., Elsheikh, E.A.E., 2002. Effect of natural fermentation on nutritive value and in vitro protein digestibility of pearl millet. Food Chem. https://doi.org/10.1016/S0308-8146(01)00386-7.

Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P., Raes, K., 2018. Iron and zinc bioaccessibility of fermented maize, sorghum and millets from fi ve locations in Zimbabwe. Food Res. Int. 103, 361–370. https://doi.org/10.1016/j.foodres.2017.10.047.

Gong, L., Cao, W., Chi, H., Wang, J., Zhang, H., Liu, J., Sun, B., 2018. Whole cereal grains and potential health effects: involvement of the gut microbiota. Food Res. Int. 103, 84–102. https://doi.org/10.1016/j.foodres.2017.10.025.

Goñi, I., García-Diz, L., Mañas, E., Saura-Calixto, F., 1996. Analysis of resistant starch: a method for foods and food products. Food Chem. 56, 445–449. https://doi.org/10.1016/0308-8146(95)00222-7.

Granato, D., Santos, J.S., Escher, G.B., Ferreira, B.L., Maggio, R.M., 2018. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: a critical perspective. Trends Food Sci. Technol. 72, 83–90. https://doi.org/10.1016/j.tifs.2017.12.006.

Hadimani, N., Ali, S., Malleshi, N., 1995. Physico-chemical composition and processing characteristics of pearl millet varieties. J. Food Sci. Technol. 32 (3), 193e198.

Hadimani, N.A., Muralikrishna, G., Tharanathan, R.N., Malleshi, N.G., 2001. Nature of carbohydrates and proteins in three pearl millet varieties varying in processing characteristics and kernel texture. J. Cereal Sci. 33, 17–25. https://doi.org/10.1006/jcrs.2000.0342.

Han, J., Jie, W.G., Cai, B.Y., 2014. Effect of phosphorus on the composition and accumulation of 7S and 11S globulin subunits during seed development of three soybean varieties. J. Soil Sci. Plant Nutr. 14, 769–782. https://doi.org/10.4067/s0718-95162014005000062.

Han, N.R., Kim, H.Y., Yang, W.M., Jeong, H.J., Kim, H.M., 2015. Glutamic acid ameliorates estrogen deficiency-induced menopausal-like symptoms in ovariectomized mice. Nutr. Res. 35, 774–783. https://doi.org/10.1016/j.nutres.2015.06.006.

Hansen, J., Møller, I., 1975. Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal. Biochem. 68, 87–94. https://doi.org/10.1016/0003-2697(75)90682-X.

Hoover, R., Swamidas, G., Kok, L.S., Vasanthan, T., 1996. Composition and physicochemical properties of starch from pearl millet grains. Food Chem. 56, 355–367. https://doi.org/10.1016/0308-8146(95)00099-2.

Jambunathan, R., Subramanian, V., 1988. Grain quality and utilization of sorghum and Pearl millet. Biotechnology in Tropical Crop Improvement: Proceedings of the International Biotechnology Workshop.

Jellum, M.D., Powell, J.B., 1971. Fatty acid composition of oil from pearl millet seed 1. Agron. J. 63, 29–33. https://doi.org/10.2134/agronj1971.00021962006300010011x.

Jukanti, A.K., Gowda, C.L.L., Rai, K.N., Manga, V.K., Bhatt, R.K., 2016. Crops that feed the world 11. Pearl Millet (Pennisetum glaucum L.): an important source of food security, nutrition and health in the arid and semi-arid tropics. Food Secur. 8, 307–329. https://doi.org/10.1007/s12571-016-0557-y.

Kaiser, H.F., 1960. The application of electronic computers to factor analysis. Educ. Psychol. Meas. https://doi.org/10.1177/001316446002000116.

Kamath, M.V., Belavady, B., 1980. Unavailable carbohydrates of commonly consumed Indian foods. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.2740310211.

Kambhampati, S., Aznar-Moreno, J.A., Hostetler, C., Caso, T., Bailey, S.R., Hubbard, A. H., Durrett, T.P., Allen, D.K., 2020. On the inverse correlation of protein and oil: examining the effects of altered central carbon metabolism on seed composition using soybean fast neutron mutants. Metabolites 10, 1–15. https://doi.org/10.3390/metabo10010018.

Krishnan, R., Meera, M.S., 2018. Pearl millet minerals: effect of processing on bioaccessibility. J. Food Sci. Technol. 55, 3362–3372. https://doi.org/10.1007/s13197-018-3305-9.

Kumar, M., 2019. Paruthi Paal, a nutrient-rich healthy drink from cottonseed: an Indian delicacy. J. Ethn. Foods 6, 1–6. https://doi.org/10.1186/s42779-019-0035-1.

Kumar, M., Tomar, M., Jayashree, P., Reetu, V., Punia, S., Mahapatra, A., Belwal, T., Dahuja, A., Joshi, S., Berwal, M.K., Satankar, V., Bhoite, A.G., Amarowicz, R., Kaur, C., Kennedy, J.F., 2021. Advances in the plant protein extraction: mechanism and recommendations. Food Hydrocoll. 115, 106595 https://doi.org/10.1016/j.foodhyd.2021.106595.

Kumar Dixit, A., Kumar, V., Rani, A., Manjaya, J.G., Bhatnagar, D., 2011. Effect of gamma irradiation on lipoxygenases, trypsin inhibitor, raffinose family oligosaccharides and nutritional factors of different seed coat colored soybean (Glycine max L.). Radiat. Phys. Chem. 80, 597–603. https://doi.org/10.1016/j.radphyschem.2010.12.014.

Lai, C.C., Varriano-Marston, E., 1980. Lipid content and fatty acid composition of free and bound lipids in pearl millets. Cereal Chem.

Lehmann, U., Robin, F., 2007. Slowly digestible starch - its structure and health implications: a review. Trends Food Sci. Technol. https://doi.org/10.1016/j.tifs.2007.02.009.

Liu, Q., Singh, S.P., Green, A.G., 2002. High-stearic and high-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. Plant Physiol. 129, 1732–1743. https://doi.org/10.1104/pp.001933.

Liu, C., Wang, Y., Pan, K., Jin, Y., Li, W., Zhang, L., 2015. Effects of phosphorus application on photosynthetic carbon and nitrogen metabolism, water use efficiency and growth of dwarf bamboo (Fargesia rufa) subjected to water deficit. Plant Physiol. Biochem. 96, 20–28. https://doi.org/10.1016/j.plaphy.2015.07.018.

Luo, X., Huang, Q., 2011. Relationships between leaf and stem soluble sugar content and tuberous root starch accumulation in cassava. J. Agric. Sci. 3, 64–72. https://doi.org/10.5539/jas.v3n2p64.

Marathe, A., Krishnan, V., Vinutha, T., Dahuja, A., Jolly, M., Sachdev, A., 2018. Exploring the role of Inositol 1,3,4-trisphosphate 5/6 kinase-2 (GmITPK2)as a dehydration and salinity stress regulator in Glycine max (L.) Merr. Through heterologous expression in E. Coli. Plant Physiol. Biochem. 123, 331–341. https://doi.org/10.1016/j.plaphy.2017.12.026.

McDonough, C.M., Rooney, L.W., Serna-saldivar, S.O., 2000. Handbook of cereal science and technology. In: Kulp, K., Ponte, J.G. (Eds.), The Millets. Marcel Dekker, New York, pp. 177–201.

McKie, V.A., McCleary, B.V., 2016. A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. J. AOAC Int. 99, 738–743. https://doi.org/10.5740/jaoacint.16-0029.

Mohammad, S., Gharibzahedi, T., Mahdi, S., 2017. Trends in Food Science & Technology the importance of minerals in human nutrition : bioavailability, food forti fi cation, processing effects and nanoencapsulation. Trends Food Sci. Technol. 62, 119–132. https://doi.org/10.1016/j.tifs.2017.02.017.

Mouquet-Rivier, C., Icard-Vernière, C., Guyot, J.P., Tou, E.H., Rochette, I., Trèche, S., 2008. Consumption pattern, biochemical composition and nutritional value of fermented pearl millet gruels in Burkina Faso. Int. J. Food Sci. Nutr. 59, 716–729. https://doi.org/10.1080/09637480802206389.

Nambiar, V.S., Dhaduk, J.J., Sareen, N., Shahu, T., Desai, R., 2011. Potential functional implications of pearl millet (Pennisetum glaucum) in health and disease. Int. J. Appl. Pharm. Sci. Res. 1, 62–67.

Obadina, A.O., Ishola, I.O., Adekoya, I.O., Soares, A.G., de Carvalho, C.W.P., Barboza, H. T., 2016. Nutritional and physico-chemical properties of flour from native and roasted whole grain pearl millet (Pennisetum glaucum [L.]R. Br.). J. Cereal Sci. 70, 247–252. https://doi.org/10.1016/j.jcs.2016.06.005.

Oshodi, A.A., Ogungbenle, H.N., Oladimeji, M.O., 1999. Chemical composition, nutritionally valuable minerals and functional properties of benniseed (Sesamum radiatum), pearl millet (Pennisetum typhoides) and quinoa (Chenopodium quinoa) flours. Int. J. Food Sci. Nutr. 50, 325–331. https://doi.org/10.1080/096374899101058.

Osman, M.A., 2011. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. J. Saudi Soc. Agric. Sci. 10, 1–6. https://doi.org/10.1016/j.jssas.2010.06.001.

Owheruo, J.O., Ifesan, B.O.T., Kolawole, A.O., 2019. Physicochemical properties of malted finger millet (Eleusine coracana) and pearl millet (Pennisetum glaucum). Food Sci. Nutr. 7, 476–482. https://doi.org/10.1002/fsn3.816.

Perez, C.M., Juliano, B.O., 1978. Modification of the simplified amylose test for milled rice. Starch - Stärke 30, 424–426. https://doi.org/10.1002/star.19780301206.

Punia, S., Kumar, M., Siroha, A.K., Kennedy, J.F., Dhull, S.B., Whiteside, W.S., 2021. Pearl millet grain as an emerging source of starch: a review on its structure, physicochemical properties, functionalization, and industrial applications. Carbohydr. Polym. 117776. https://doi.org/10.1016/j.carbpol.2021.117776.

Pushparaj, F.S., Urooj, A., 2014. Antioxidant activity in two pearl millet (Pennisetum typhoideum) cultivars as influenced by processing. Antioxidants 3, 55–66. https://doi.org/10.3390/antiox3010055.

Ragaee, S., Abdel-Aal, E.S.M., Noaman, M., 2006. Antioxidant activity and nutrient composition of selected cereals for food use. Food Chem. 98, 32–38. https://doi.org/10.1016/j.foodchem.2005.04.039.

Sáez-Plaza, P., Michałowski, T., Navas, M.J., Asuero, A.G., Wybraniec, S., 2013. An Overview of the Kjeldahl Method of Nitrogen Determination. Part I. Early History, Chemistry of the Procedure, and Titrimetric Finish. Crit. Rev. Anal. Chem.

Saleh, A.S.M., Zhang, Q., Chen, J., Shen, Q., 2013. Millet grains: nutritional quality, processing, and potential health benefits. Compr. Rev. Food Sci. Food Saf. 12, 281–295. https://doi.org/10.1111/1541-4337.12012.

Sandhu, K.S., Siroha, A.K., 2017. Relationships between physicochemical, thermal, rheological and in vitro digestibility properties of starches from pearl millet cultivars. LWT - Food Sci. Technol. 83, 213–224. https://doi.org/10.1016/j.lwt.2017.05.015.

Sawaya, W.N., Khalil, J.K., Safi, W.J., 1984. Nutritional quality of pearl millet flour and bread. Qual. Plant. Plant Foods Hum. Nutr. 34, 117–125. https://doi.org/10.1007/BF01094839.

Sharma, M., Yadav, D.N., Singh, A.K., 2015a. Rheological and functional properties of heat moisture treated pearl millet starch. J. Food Sci. Technol. 52, 6502–6510. https://doi.org/10.1007/s13197-015-1735-1.

Sharma, M., Yadav, D.N., Singh, A.K., Tomar, S.K., 2015b. Effect of heat-moisture treatment on resistant starch content as well as heat and shear stability of pearl millet starch. Agric. Res. 4, 411–419. https://doi.org/10.1007/s40003-015-0177-3.

Shokrollahi, S., Ghanati, F., Sajedi, R.H., Sharifi, M., 2018. Possible role of iron containing proteins in physiological responses of soybean to static magnetic field. J. Plant Physiol. 226, 163–171. https://doi.org/10.1016/j.jplph.2018.04.018.

Shukla, A., Srivastava, N., Suneja, P., Yadav, S.K., Hussain, Z., Rana, J.C., Yadav, S., 2018. Genetic diversity analysis in buckwheat germplasm for nutritional traits. Indian J. Exp. Biol. 56, 827–837.

Singh, J., Dartois, A., Kaur, L., 2010. Starch digestibility in food matrix: a review. Trends Food Sci. Technol. 21, 168–180. https://doi.org/10.1016/j.tifs.2009.12.001.

Siroha, A.K., Sandhu, K.S., Kaur, M., Kaur, V., 2019. Physicochemical, rheological, morphological and in vitro digestibility properties of pearl millet starch modified at varying levels of acetylation. Int. J. Biol. Macromol. 131, 1077–1083. https://doi.org/10.1016/j.ijbiomac.2019.03.179.

Slama, A., Cherif, A., Sakouhi, F., Boukhchina, S., Radhouane, L., 2020. Fatty acids, phytochemical composition and antioxidant potential of pearl millet oil. J. Verbrauch. Lebensm. 15, 145–151. https://doi.org/10.1007/s00003-019-01250-4.

Sluiter, A., Hyman, D., Payne, C., Wolfe, J., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Nrel, J.W., Selig, M., Weiss, N., Ji, Y., Union, I., Pure, O.F., Chemistry, A., Hames, B., Scarlata, C., Nrel, a S., Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Nrel, D.C., 2008. Determination of structural carbohydrates and lignin in biomass determination of structural carbohydrates and lignin in biomass. Natl. Renew. Energy Lab.

Štěrbová, L., Bradová, J., Sedláček, T., Holasová, M., Fiedlerová, V., Dvořáček, V., Smrčková, P., 2016. Influence of technological processing of wheat grain on starch digestibility and resistant starch content. StarchStaerke 68, 593–602. https://doi.org/10.1002/star.201500162.

Subramanian, V., Jambunathan, R., Suryaprakash, S., 1981. Sugars of pearl millet [Pennisetum. J. Food Sci. 46, 0–1.

Suma, P.F., Urooj, A., 2015. Isolation and characterization of starch from pearl millet (Pennisetum typhoidium) flours. Int. J. Food Prop. 18, 2675–2687. https://doi.org/10.1080/10942912.2014.981640.

Tahir, M., Båga, M., Vandenberg, A., Chibbar, R.N., 2012. An assessment of raffinose family oligosaccharides and sucrose concentration in genus Lens. Crop Sci. 52, 1713–1720. https://doi.org/10.2135/cropsci2011.08.0447.

Tao, K., Yu, W., Prakash, S., Gilbert, R.G., 2019. High-amylose rice: starch molecular structural features controlling cooked rice texture and preference. Carbohydr. Polym. 219, 251–260. https://doi.org/10.1016/j.carbpol.2019.05.031.

Tian, W., Chen, G., Gui, Y., Zhang, G., Li, Y., 2020a. Rapid quantification of total phenolics and ferulic acid in whole wheat using UV–Vis spectrophotometry. Food Control 107691. https://doi.org/10.1016/j.foodcont.2020.107691.

Tian, W., Chen, G., Zhang, G., Wang, D., Tilley, M., Li, Y., 2020b. Rapid determination of total phenolic content of whole wheat flour using near-infrared spectroscopy and chemometrics. Food Chem. 128633. https://doi.org/10.1016/j.foodchem.2020.128633.

Vasudev, S., Yadava, D.K., Malik, D., Tanwar, R.S., Prabhu, K.V., 2008. A simplified method for preparation of fatty acid methyl esters of Brassica oil. Indian J. Genet. Plant Breed. 68, 456–458.

Wang, J., Zhou, P., Shi, X., Yang, N., Yan, L., Zhao, Q., Yang, C., Guan, Y., 2019. Primary metabolite contents are correlated with seed protein and oil traits in near-isogenic lines of soybean. Crop J. 7, 651–659. https://doi.org/10.1016/j.cj.2019.04.002.

Xie, K., Cakmak, I., Wang, S., Zhang, F., Guo, S., 2020. Synergistic and antagonistic interactions between potassium and magnesium in higher plants. Crop J. https://doi.org/10.1016/j.cj.2020.10.005.

Yadav, D.N., Anand, T., Kaur, J., Singh, A.K., 2012. Improved storage stability of pearl millet flour through microwave treatment. Agric. Res. 1, 399–404. https://doi.org/10.1007/s40003-012-0040-8.

Yu, W., Tan, X., Zou, W., Hu, Z., Fox, G.P., Gidley, M.J., Gilbert, R.G., 2017. Relationships between protein content, starch molecular structure and grain size in barley. Carbohydr. Polym. 155, 271–279. https://doi.org/10.1016/j.carbpol.2016.08.078.

Zaheer, A., Malik, A., Sher, A., Mansoor Qaisrani, M., Mehmood, A., Ullah Khan, S., Ashraf, M., Mirza, Z., Karim, S., Rasool, M., 2019. Isolation, characterization, and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (Cicer arietinum L.) growth. Saudi J. Biol. Sci. 26, 1061–1067. https://doi.org/10.1016/j.sjbs.2019.04.004.

Zhao, S., Zhang, D., 2014. Supercritical CO_2 extraction of Eucalyptus leaves oil and comparison with Soxhlet extraction and hydro-distillation methods. Sep. Purif. Technol. 133, 443–451. https://doi.org/10.1016/j.seppur.2014.07.018.