Multivariate biochemical characterization of rice bean (Vigna umbellata) seeds for nutritional enhancement

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A B S T R A C T :

Legume seeds offer the primary source of dietary proteins for the people of many countries including India. To bear out high dietary protein demand, auxiliary legumes needs to be exploited. Rice bean (*Vigna umbellata*) is one of the underutilized legumes hereby confirm the nutritional potential for human consumption. Fifty diverse rice bean accessions were screened in the study for various biochemical signatures to identify promising accessions. The crude protein contents varied low and high (2.88 ± 0.00 to 31.79 ± 2.15 mg/g) on a dry matter basis. The highest *in vitro* protein and starch digestibility was exhibited by rice bean accessions equal to 63.84% and 44.60%. Significant variation for antioxidant activity (9.36 ± 1.20 to 18.56 ± 0.01%) was detected. Multivariate analysis revealed the positive correlation of antioxidant activity with phenolic compounds. Accessions EC-16136, IC-8565-3, IC-2909, IC-521068, IC-18171, IC-18452, IC-521081, IC-12436, and IC-18184 showed promising antioxidant activity, protein content, increased *in vitro* protein and starch digestibility and reduced oligosaccharides, as demonstrated by hierarchical cluster analysis. For designing future breeding programs, aforesaid accessions can be used as a donor parent and recommended for human consumption as well. Present study seems to be the first kind of comprehensive report on the biochemical analysis of rice bean seeds.

1. Introduction

To relieve protein deficiency, non-conventional food legumes with high nutritive value are being given greater attention. In India, rice bean is cultivated in the North-Eastern (N-E) hilly area under shifting cultivation systems as a minor crop (Iangrai et al., 2017). It is an annual pulse belongs to family *Fabaceae* of the genus *Vigna* that grows well on a diverse range of soils. Rice bean is a small scandent plant characterized by hairy appearance bearing bright yellow flowers developed into edible beans. The pods and the green seeds are consumed as vegetables. Seeds though vary in color are usually red and yellow.

Majority of the studies described morpho-agronomic status of rice bean (Muthusamy et al., 2008; Pattanayak et al., 2018). Previously, Arora et al. (1980) experimented 300 accessions and observed this crop resistant for the yellow mosaic virus. Gupta et al. (2009) reported hybridization in rice bean based on selection of genetic parameters. Recently, Pattanayak et al. (2018) generated a data on 65 Indian accessions that are confined to N-E hills. Their study demonstrated various statistical approaches that can be linked to assess morphological and molecular diversity. Moreover, reports on genetic characterisation of rice bean using DNA markers are published on large scale. This includes

use of RAPD, ISSR, SSR and microsatellites markers (Muthusamy et al., 2008; Tian et al., 2013; Wang et al., 2015; Iangrai et al., 2017). Seeds of rice bean contain diverse saccharides however low in flatulence causing compared to other pulses (Bepary and Wadikar, 2019). Few reports on nutritional analysis of rice bean are too available in literature domain however confined to selected number of accessions only (Rodriguez and Mendoza, 1991; Kaur and Kapoor, 1992; Mohan and Janardhanan, 1994; Katoch, 2013; Bajaj, 2014; Dhillon and Tanwar, 2018). A systematic research on biochemical composition in rice bean on a greater scale was lacking.

Though referred as an underutilized legume, rice bean contain about 60% digestible proteins enriched with essential amino acid like methionine, tryptophan, lysine, tyrosine, and valine (Kaur et al., 2013). National Bureau of Plant genetic Resources (NBPGR), New Delhi, India is the front-runner in maintaining more than 1700 accessions (NBPGR, 2007). Accessions with broad genetic variability are the primary need to develop stable high yielding varieties. This crop is investigated to a small number for its various biochemical composition and available information in the literature domain is insubstancial. Till date only 16 different varieties of rice bean are evaluated for nutritional characteristics (Katoch, 2013; Dhillon and Tanwar, 2018). Herein, we report the

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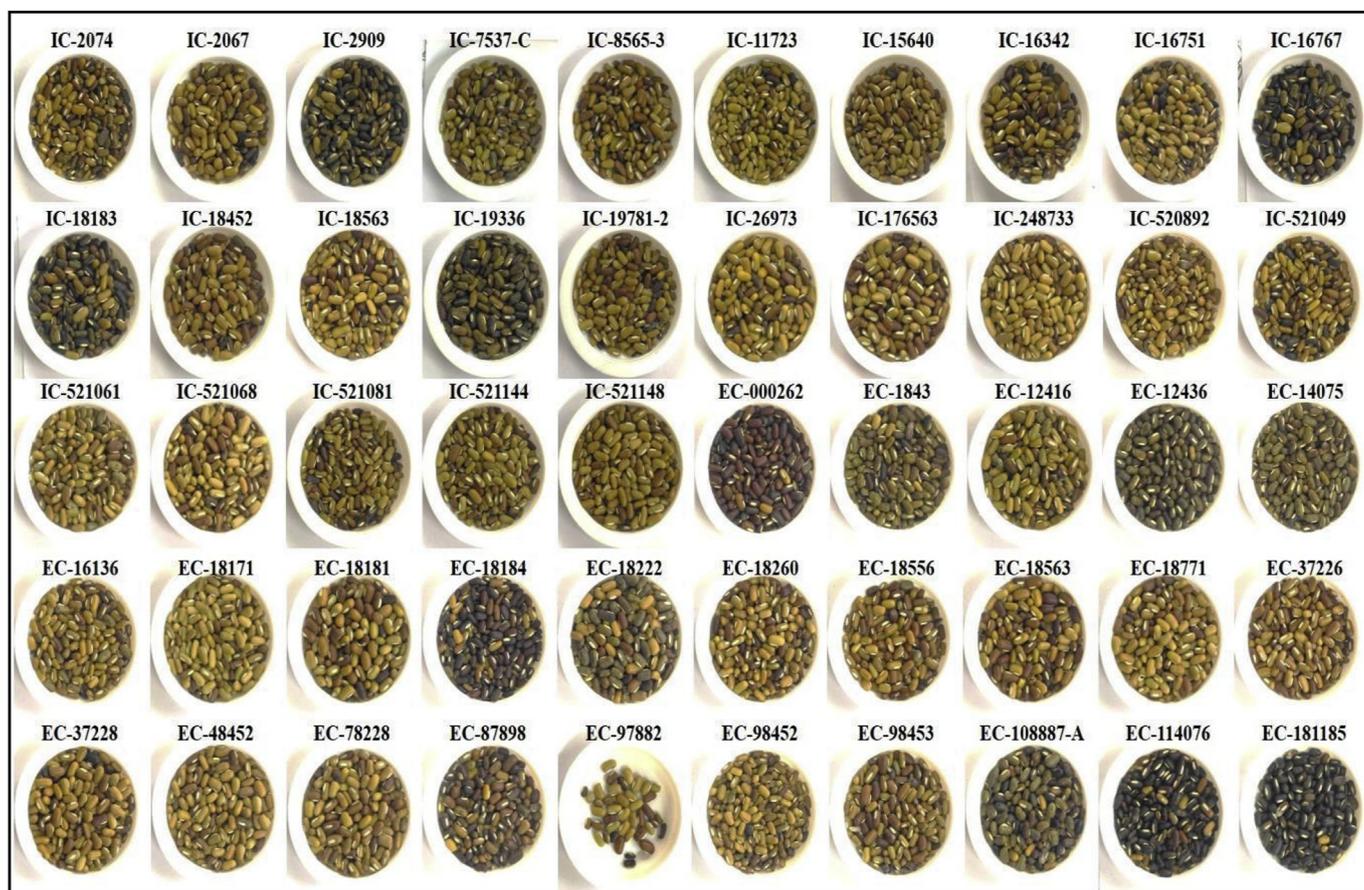 *E-mail address:* sameerbhagyawant@gmail.com (S.S. Bhagyawant).

Fig. 1. Representation of 50 accessions of rice bean used in the study.

biochemical signatures in 50 accessions of rice bean for the large number of parameters (16) in order to identify the elite accessions for nutritional enhancement. Present study will assist in implication of nutritional security, crop improvement and large-scale planting of the promising accessions. This appears to be a wide-ranging study and first kind of reports to date.

2. Material and methods

2.1. Plant material

Fifty different accessions of rice bean were procured from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The seeds were harvested after 160 days of sowing and the seed morphology of these accessions is represented in the Fig. 1. The seed material was thoroughly cleaned, ground and passed through 2 mm mesh to make a uniform powder and stored in air tight vials.

2.2. Analysis of antioxidant composition

2.2.1. Determination of antioxidant activity

The antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method of Sultana et al. (2007) based on the principle of accepting a hydrogen atom from the scavenger molecule that reduces DPPH to $DPPH_2$. Fifty milligram rice bean seed powder was extracted in 1 mL methanol. To this methanolic extract, 3 mL of 0.1 mM DPPH was added and incubated at room temperature in dark for 30 min. The absorbance was measured against the reagent blank at 517 nm using UV–Visible spectrophotometer (UV-1800, Schimadzu, Kyoto, Japan). Ascorbic acid was used as a standard and DPPH radical scavenging activity was calculated using the equation:

$$DPPH\% = (A_{control}-A_{sample})/(A_{control}) \text{ x } 100$$

Where, $A_{control}$ is absorbance of control and A_{sample} is absorbance of test sample respectively.

2.2.2. Determination of proline content

The proline content of rice bean seeds was determined following the method of Bates et al. (1973). The sulphosalicyclic acid precipitates protein as a complex, which on reaction with ninhydrin form red color chromophore that can be read at 520 nm. The seed sample (100 mg) was homogenized in 3% aqueous sulphosalicyclic acid and centrifuged at 5000 rpm for 10 min. To this, glacial acetic acid and ninhydrin was added. The solution was boiled to 100 °C for 1 h and placed on ice bath to terminate the reaction. Toluene was added and left to stand in dark for 10 min. The absorbance was measured at 520 nm using UV–visible spectrophotometer (UV-1800, Schimadzu, Kyoto, Japan) and compared with standard proline.

2.2.3. Determination of total phenolic content (TPC)

TPC was determined by Folin-Ciocalteu (FC) method of Khoo et al. (2013). Phenols react with phosphomolybidic acid in FC reagent in alkaline medium producing molybedum blue complex. The 50 μL of methanolic extract of the seed sample was mixed with 50 μL of FC phenol reagent. Then after, 200 μL of sodium carbonate was added and the final volume was made to 1 mL with distilled water. The contents were boiled for 1 min and left at room temperature for 2 h; the absorbance was measured at 650 nm. The results were estimated using a standard curve prepared using gallic acid and were expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

2.2.4. Determination of total flavonoid content (TFC)

Following Zou et al. (2004), total flavonoid content in methanolic extract of rice bean seeds was estimated using aluminium chloride employing quercetin as a standard. The aluminium chloride forms acid stable complex with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Briefly, methanolic extract (50 μL) was added to distilled water (450 μL) followed by 2% aluminium chloride (500 μL). Incubated at room temperature for 5 min, 1% of sodium hydroxide was added and absorbance was measured at 420 nm. The results were expressed as milligrams of quercetin equivalent (QE) per gram of sample.

2.3. Analysis of nutritional composition

2.3.1. Determination of proteins

Total protein of samples was extracted by the method described by Hameed et al. (2009). Hundred milligrams (100 mg) of powdered seed sample was vortexed with 1 mL of 50 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 20 min. The supernatant was collected and the total seed protein was determined using BSA as a standard (Lowry et al., 1951). The tyrosine and tryptophan present in the protein reduces the components of FC reagent and develop blue color.

2.3.2. Determination of total amino acids

The total amino acids were estimated by following the ninhydrin method of Moore and Stein (1948). The amino acids are decarboxylated by ninhydrin giving purple color product. Hundred milligram (100 mg) seed powder was suspended in 1 mL of 80% ethanol for extraction of free amino acid. The 1 mL of ninhydrin solution (0.1%) was added to 0.1 mL of supernatant and volume was made up to 2 mL of distilled water. This solution was heated in a water bath for 20 min and thereafter 5 mL of n-propanol was added as diluent solution. The absorbance was measured at 570 nm.

2.3.3. Determination of carbohydrates

The total sugar was extracted and estimated by the method described by Dubois et al. (1956) in 80% ethanol in which glucose is dehydrated to hydroxymethyl furfural producing green color. To 100 μL of sugar extract, 0.5 mL saturated phenol (5%) and 0.5 mL of H_2SO_4 (96%) were added. After incubation at 30 °C for 20 min, the absorbance was measured at 490 nm. The amount of sugar was calculated from a standard curve of sugars equivalent to 100 mg of glucose per millilitre. The sugar free residue was dried at 60 °C and used for the starch estimation (Gupta et al., 1993).

2.3.4. Determination of in vitro protein digestibility (IVPD)

In vitro protein digestibility of rice bean accessions was carried out by the method of Hsu et al. (1977). The proteolytic enzymes digest the protein and the pH changes due to release of amino acids. The rate of change in pH is correlated with protein digestibility and that there is a direct relationship between the observed pH drop and the extent of protein hydrolysis. The protein samples (previously prepared for total seed protein) were treated with two enzymes i.e. α-chymotrypsin and trypsin at 37 °C. The drop in the pH of a protein suspension from pH 8 after 10 min was recorded. The protein digestibility was estimated by the following regression equation:

% digestibility (Y) = 234.84 − [(22.56)X]

Where, X = pH drop in the protein sample after addition of enzyme.

2.3.5. Determination of in vitro starch digestibility (IVSD)

The *in vitro* starch digestibility was determined by the method of Englyst et al. (1992). Inhibition of digestive enzyme α-amylase is used as activity assay for determining IVSD. The samples were vortexed with 0.02 M phosphate buffer (pH 6.9) for 1 h at room temperature and centrifuged at 10,000 rpm for 20 min. The amylase (1260 U/mg) solution was added to collected rice bean supernatant, followed by incubation for 2 h. 3, 5- Dinitrosalicylic acid (DNSA) was added to terminate the reaction. The absorbance was measured at 550 nm. The digested starch was expressed as milligram maltose released per gram.

$$I_{\alpha\text{-amylase}} = (A_{control} - A_{sample}) / A_{control} \; X \; 100$$

Where, $A_{control}$ is absorbance of control and A_{sample} is absorbance of test sample respectively.

2.4. Analysis of antinutritional composition

2.4.1. Determination of oligosaccharides

Following the method of Somiari and Balogh (1993), oligosaccharides were fractioned on thin layer chromatography (TLC) on the basis of their size and mobility. Hundred milligram seed powder was added to 1 mL ethanol (80%) and the contents were centrifuged subsequently, the supernatant was used for TLC. Briefly, 10 μL of the sample was spotted. The chromatogram was developed using mobile phase of n-propanol, ethyl acetate and distilled water (6:1:3) and developed spots were matched with standard sugars (raffinose, stachyose and verbascose). Then after, sugar spots were eluted in 1 mL of distilled water and kept overnight. The samples were centrifuged at 8000 rpm for 10 min and the supernatant was estimated for oligosaccharides by the method of Tanaka et al. (1975). The 0.5 mL of eluted sugar solution was treated with 0.5 mL of 0.2 M thiobarbituric acid (TBA) and 0.5 mL of conc. HCl. The mixture was heated for 5 min in a water bath and after cooling, absorbance was taken at 432 nm.

2.4.2. Determination of tannins

The amount of tannins was determined by the procedure of Schandrei (1970), based on Folin-Denis (FD) reagent and sodium carbonate reaction. Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce blue colour. The intensity of colour is proportional to the amount of tannins measured at 700 nm. The tannins were estimated as milligram tannic acid equivalent (TAE) per gram of dry extract.

2.4.3. Determination of phytic acid

Phytic acid content was determined by the method of Wilcox et al. (2000) based on the reduction of phosphomolybdate complex. To 50 mg of the powdered seed sample, 1 mL of 0.4 mM HCl was added. Then mixture was given small spin to obtain clear supernatant. Then, 100 μL of supernatant was mixed with 900 μL Chen's reagent (Chen's reagent- 3 M sulphuric acid; 2.5% ammonium molybdate; 10% ascorbic acid; distilled water (1:1:1:2)). The mixture was incubated at room temperature and absorbance was measured at 650 nm.

2.4.4. Extraction of lectin and hemagglutination assay

The hemagglutination assay is based on the binding of sugar to lectin that results in agglutination of erythrocytes. To 100 mg rice bean powder, 1 mL of 10 mM Tris-HCl, containing 150 mM NaCl (pH 7.2) was added. This suspension was stirred for 16 h, centrifuged at 10,000 rpm for 20 min. The obtained supernatant was used as a source of lectin. The hemagglutination assay was performed using trypsinised rabbit erythrocytes. The tests were performed in a standard micro titre plate by the two-fold serial dilution method (Liener, 1962). The unit of hemagglutination activity was expressed as the reciprocal of the highest dilution of lectin that showed complete agglutination. The specific activity of the lectin is measured as units of hemagglutination per mg of protein.

2.5. Categorization of rice bean accessions into equal classes

On the basis of class interval, the rice bean accessions were

classified into three groups: low, medium and high for all the parameters. The class interval (d) was obtained by subtracting the minimum value from the maximum value and divided by 3 in order to get three classes.

2.6. Statistical analysis

All the results were presented as mean ± standard deviation. The data was subjected to one way analysis of variance, correlation coefficients and Tukey's test. P < 0.05 was considered statistically significant. The multivariate analysis and the similarity matrix were accessed by UPGMA using NTSYS pc 2.02 (Rohlf, 1997).

3. Results and discussion

There is a growing interest in characterizing phytochemical composition of food legumes like phenolic contents, tannins, phytic acid, and antioxidants for human consumption and their application in breeding programmes. Seed composition to aim at the development of cultivars suitable for food industry is therefore fundamental. This information thus can provide insights for germplasm selection. The variation in nutritional and anti-nutritional composition of 50 rice bean accessions is presented in the Tables 1–3.

3.1. Antioxidant content of rice bean accessions

3.1.1. Antioxidant activity

DPPH free radical scavenging activity was observed in a range of 9.36 ± 1.20 to 18.57 ± 0.01% (Table 1). It was observed highest in EC-16136 and lowest in IC-521144. The mean of free radical scavenging activity across all the accessions was found to be 13.14%. It was observed above average in the seeds of EC-37226, EC-48452, EC-78228, EC-108887-A, EC-78228, EC-87898, and IC-521148. There was a significant difference in the antioxidant potentials of different rice bean accessions which may be due to the presence of condensed tannins and phytic acids. Tannins show high antioxidant activity as they possess high molecular weight with high degree of hydroxylation to aromatic rings. It provides free radical scavenging activity against superoxide radicals, hydroxyl radicals and nitric oxide. It has the ability to donate electron to a free radical and make it stable (Koleckar et al., 2008; Barrett et al., 2018). In addition, environmental factors including soil, time of harvest, temperature and post-harvest management affect the antioxidant capacity and phenolic compounds in the plants (Alirezalu et al., 2018; Gholizadeh-Moghadam et al., 2019). Also, earlier studies on different plant species demonstrated their antioxidant activity and reported numerous. While, the phytic acid is a recognized source of preserving juice (Du et al., 2012) and meat products (Pacheco et al., 2012). Presence of phytic acid is well characterised in cereals, nuts, vegetables and natural oils. Numerous studies have revealed the benefits of phytic acid being natural antioxidants on health and food preservation (Silva and Bracarense, 2016). Phytic acid protects the seeds by inhibiting the radical oxygen species (ROS) production and thus functions as a natural antioxidant in legumes (Bhowmik et al., 2017; Bhagyawant et al., 2018a).

Antioxidants are an important part of the human defence system and help to cope with oxidative stress caused by reactive oxygen species. DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity based on the principal of reduction of 2, 2-diphenyl-1-picrylhydrazyl, which gives an electron to absorption maximum 517 nm. The radical scavenging activity was related to substitution of hydroxyl groups in the aromatic rings of phenolic thus contributing to their hydrogen donating ability (Zhang et al., 2011). Higher the DPPH scavenging activity, greater will be the inhibition of free radicals i.e. higher antioxidant power. There was a significant difference in the antioxidant potentials of different rice bean accessions (p ≥ 0.05) which represents the variation in percent antioxidant

Table 1
Distribution of DPPH activity, proline, total phenolic and total flavonoid content in the seeds of rice bean.

Accession	DPPH radical scavenging activity (%)	Proline (mg/g)	TPC (mg/g)	TFC (mg/g)
IC-2074	12.00 ± 0.57[a]	17.87 ± 1.07[a]	2.30 ± 0.19[b]	0.38 ± 0.01[a]
IC-2067	11.11 ± 0.63[a]	17.93 ± 0.63[b]	1.92 ± 0.69[a]	0.45 ± 0.02[b]
IC-2909	11.81 ± 0.59[a]	17.65 ± 0.48[b]	1.65 ± 0.38[a]	0.36 ± 0.01[a]
IC-7537-C	12.50 ± 0.71[b]	17.12 ± 2.06[b]	2.15 ± 0.68[b]	0.74 ± 0.08[c]
IC-8565-3	11.92 ± 0.15[a]	17.52 ± 2.20[b]	2.67 ± 0.33[b]	0.53 ± 0.02[b]
IC-11723	12.49 ± 0.64[b]	15.96 ± 0.46[b]	2.15 ± 0.71[b]	0.33 ± 0.04[a]
IC-15640	11.07 ± 0.88[a]	18.65 ± 0.82[b]	1.85 ± 0.36[a]	0.39 ± 0.01[a]
IC-16342	11.15 ± 1.30[a]	18.84 ± 0.75[b]	1.91 ± 0.12[a]	0.44 ± 0.07[b]
IC-16751	11.79 ± 0.38[a]	16.71 ± 1.01[b]	2.57 ± 0.85[b]	0.27 ± 0.02[a]
IC-16767	11.27 ± 1.42[a]	11.72 ± 0.73[b]	3.49 ± 0.44[c]	0.74 ± 0.05[c]
IC-18183	11.93 ± 0.51[a]	19.06 ± 2.51[b]	2.54 ± 0.05[b]	0.34 ± 0.03[a]
IC-18452	11.77 ± 0.32[a]	29.84 ± 1.35[c]	1.68 ± 0.27[a]	0.54 ± 0.02[b]
IC-18563	11.14 ± 0.08[a]	27.27 ± 1.91[c]	2.50 ± 0.02[b]	0.36 ± 0.01[a]
IC-19336	10.74 ± 0.82[a]	20.44 ± 1.33[c]	2.23 ± 0.08[b]	0.48 ± 0.04[b]
IC-19781-2	9.79 ± 0.86[a]	21.97 ± 1.80[c]	1.93 ± 0.20[a]	0.49 ± 0.02[b]
IC-26973	9.49 ± 1.30[a]	23.57 ± 2.12[c]	2.68 ± 0.10[b]	0.51 ± 0.02[b]
IC-176563	10.53 ± 0.23[a]	19.97 ± 0.97[b]	2.82 ± 0.11[b]	0.27 ± 0.01[a]
IC-248733	10.40 ± 1.09[a]	21.63 ± 0.33[c]	1.13 ± 0.04[a]	0.38 ± 0.02[a]
IC-520892	10.42 ± 0.48[a]	21.29 ± 1.66[c]	1.20 ± 0.11[a]	0.29 ± 0.01[a]
IC-521049	11.31 ± 1.36[a]	21.76 ± 2.50[c]	2.75 ± 0.06[b]	0.43 ± 0.03[b]
IC-521061	10.40 ± 0.47[a]	21.82 ± 0.59[c]	2.67 ± 0.11[b]	0.44 ± 0.02[b]
IC-521068	9.36 ± 1.20[a]	21.50 ± 2.47[c]	1.77 ± 0.51[a]	0.45 ± 0.08[b]
IC-521081	10.21 ± 0.73[a]	23.76 ± 0.80[c]	2.46 ± 0.19[b]	0.50 ± 0.02[b]
IC-521144	9.58 ± 0.44[a]	23.07 ± 1.73[c]	8.27 ± 0.37[c]	0.45 ± 0.05[b]
IC-521148	15.58 ± 0.25[c]	20.94 ± 1.66[c]	3.09 ± 0.27[c]	0.27 ± 0.03[a]
EC-000262	14.35 ± 1.21[b]	21.82 ± 0.73[c]	4.25 ± 0.38[c]	0.55 ± 0.04[b]
EC-1843	13.69 ± 0.22[b]	22.13 ± 1.84[c]	3.36 ± 0.19[c]	0.44 ± 0.02[b]
EC-12416	14.31 ± 1.11[b]	22.79 ± 1.52[c]	2.02 ± 0.14[b]	0.50 ± 0.08[b]
EC-12436	14.05 ± 0.29[b]	24.80 ± 2.18[c]	3.27 ± 0.29[c]	0.51 ± 0.15[b]
EC-14075	14.38 ± 0.85[b]	14.20 ± 1.90[b]	2.30 ± 0.25[b]	0.54 ± 0.08[b]
EC-16136	18.56 ± 0.01[c]	25.20 ± 2.19[c]	2.74 ± 0.33[b]	0.56 ± 0.01[b]
EC-18171	13.23 ± 0.25[b]	16.87 ± 2.43[b]	2.48 ± 0.77[b]	0.42 ± 0.03[b]
EC-18181	15.29 ± 0.26[c]	26.14 ± 1.54[c]	3.18 ± 0.20[c]	0.32 ± 0.09[a]
EC-18184	14.96 ± 0.5[b]	26.39 ± 0.30[c]	2.60 ± 0.09[b]	0.52 ± 0.15[b]
EC-18222	14.62 ± 1.46[b]	26.43 ± 0.09[c]	2.98 ± 0.16[b]	0.45 ± 0.12[b]
EC-18260	13.66 ± 0.84[b]	26.27 ± 0.89[c]	3.02 ± 0.29[c]	0.70 ± 0.34[c]
EC-18556	14.84 ± 0.65[b]	27.96 ± 0.43[c]	1.28 ± 0.16[a]	0.69 ± 0.09[c]
EC-18563	14.09 ± 0.31[b]	31.79 ± 2.15[c]	0.73 ± 0.04[a]	0.77 ± 0.16[c]
EC-18771	14.32 ± 0.59[b]	12.01 ± 1.79[b]	3.28 ± 0.24[c]	0.43 ± 0.08[b]
EC-37226	18.02 ± 0.35[c]	21.57 ± 1.36[c]	2.60 ± 0.16[b]	0.27 ± 0.01[a]
EC-37228	14.69 ± 0.17[b]	19.56 ± 0.91[b]	2.25 ± 0.43[b]	0.33 ± 0.05[a]
EC-48452	17.16 ± 0.63[c]	17.71 ± 2.47[b]	2.86 ± 0.79[b]	0.21 ± 0.03[a]
EC-78228	16.29 ± 0.18[c]	2.88 ± 0.84[a]	1.87 ± 0.78[a]	0.21 ± 0.01[a]
EC-87898	15.70 ± 0.72[c]	8.50 ± 6.67[a]	2.04 ± 0.13[b]	0.70 ± 0.06[c]
EC-97882	16.02 ± 0.27[c]	8.24 ± 0.29[a]	3.69 ± 0.21[c]	0.27 ± 0.03[a]
EC-98452	15.13 ± 0.20[c]	12.32 ± 2.00[b]	4.42 ± 0.34[c]	0.27 ± 0.01[a]
EC-98453	15.06 ± 0.77[c]	4.08 ± 1.09[a]	2.12 ± 0.15[b]	0.32 ± 0.02[a]
EC-108887-A	16.17 ± 0.30[c]	15.58 ± 1.52[b]	4.31 ± 0.17[c]	0.27 ± 0.02[a]
EC-114076	14.35 ± 0.36[b]	16.27 ± 0.25[b]	4.25 ± 0.44[c]	0.32 ± 0.02[a]
EC-181185	14.20 ± 1.89[b]	17.27 ± 0.98[b]	4.45 ± 0.32[c]	0.20 ± 0.01[a]
Minimum	9.36 ± 1.20	2.88 ± 0.84	0.73 ± 0.04	0.20 ± 0.01
Maximum	18.56 ± 0.01	31.79 ± 2.15	8.27 ± 0.37	0.77 ± 0.16
Mean	13.14 ± 2.35	19.51 ± 6.08	2.69 ± 1.17	0.45 ± 0.19

Results are represented as the mean value of triplicates ± standard deviation. Different letters in the same column represent statistically different mean values (p < 0.05). DPPH = 2,2-diphenyl-1-picrylhydrazyl, TPC = Total phenolic content, TFC = Total flavonoid content, % = percentage, mg/g = milligram per gram.

scavenging capacity. Yao et al. (2010) observed the antioxidant activity in the grains was positively correlated with TPC. Pulses with the high phenolic contents demonstrate high antioxidant capacity. Antioxidant properties of food exhibit disease curing properties against cancer, diabetes and various respiratory diseases (Cai et al., 2004).

3.1.2. Proline content

The mean content across the rice bean accessions was observed to be 19.51 ± 6.08 mg/g (Table 1). The content was highest in EC-18563

Table 2
Distribution of proteins, total amino acids, starch, sugar, IVPD and IVSD in the seeds of rice bean.

Accession	Proteins (mg/g)	Total amino acids (mg/g)	Starch (mg/g)	Sugar (mg/g)	IVPD (%)	IVSD (%)
IC-2074	21.59 ± 1.00[b]	6.08 ± 0.19[b]	50.69 ± 2.83[b]	5.61 ± 0.13[c]	60.90 ± 0.32[b]	27.53 ± 2.13[b]
IC-2067	25.46 ± 1.02[c]	7.54 ± 0.36[b]	40.63 ± 1.41[a]	3.39 ± 0.31[b]	62.82 ± 0.48[b]	26.87 ± 2.80[b]
IC-2909	22.85 ± 1.34[b]	6.08 ± 0.07[b]	43.24 ± 1.18[a]	7.08 ± 0.60[c]	63.84 ± 0.32[c]	34.51 ± 1.60[c]
IC-7537-C	23.76 ± 0.96[b]	6.10 ± 0.26[b]	62.21 ± 3.49[c]	5.06 ± 0.41[c]	58.53 ± 0.48[b]	33.47 ± 2.53[c]
IC-8565-3	32.64 ± 0.41[c]	6.71 ± 0.69[b]	44.47 ± 3.02[b]	3.41 ± 0.48[b]	62.71 ± 2.23[b]	37.06 ± 3.60[c]
IC-11723	29.29 ± 0.71[c]	6.88 ± 0.63[b]	44.01 ± 1.51[b]	3.83 ± 0.24[b]	60.00 ± 1.28[b]	34.98 ± 0.13[c]
IC-15640	24.64 ± 1.10[c]	6.00 ± 0.94[b]	45.70 ± 2.01[b]	6.33 ± 0.24[c]	59.89 ± 0.16[b]	25.64 ± 1.60[b]
IC-16342	23.83 ± 2.25[b]	6.17 ± 0.36[b]	46.16 ± 1.85[b]	4.91 ± 0.22[c]	59.77 ± 0.32[b]	35.45 ± 1.60[c]
IC-16751	29.32 ± 1.02[c]	6.96 ± 0.58[b]	41.71 ± 2.07[a]	5.82 ± 0.34[c]	61.35 ± 1.91[b]	37.99 ± 1.19[c]
IC-16767	18.61 ± 0.54[a]	8.04 ± 0.19[b]	42.55 ± 2.53[a]	3.76 ± 0.25[b]	62.48 ± 0.00[b]	40.08 ± 2.80[c]
IC-18183	22.37 ± 2.54[b]	12.42 ± 0.4[c]	51.38 ± 2.57[b]	3.74 ± 0.20[b]	60.68 ± 0.96[b]	37.72 ± 1.87[c]
IC-18452	25.02 ± 2.03[c]	17.13 ± 0.5[c]	68.13 ± 1.74[c]	5.34 ± 0.10[c]	57.86 ± 1.12[b]	43.09 ± 0.67[c]
IC-18563	22.81 ± 0.65[b]	6.58 ± 0.19[b]	66.82 ± 2.44[c]	1.84 ± 0.25[a]	60.90 ± 0.64[b]	42.25 ± 1.33[c]
IC-19336	23.25 ± 0.69[b]	11.83 ± 0.3[c]	50.08 ± 3.27[b]	2.39 ± 0.18[a]	61.02 ± 0.16[b]	44.04 ± 1.47[b]
IC-19781-2	27.39 ± 0.69[c]	5.33 ± 0.56[b]	54.61 ± 3.10[b]	1.14 ± 0.18[a]	56.39 ± 0.32[a]	21.02 ± 2.27[a]
IC-26973	24.03 ± 1.56[c]	4.92 ± 0.62[a]	56.22 ± 1.89[c]	1.60 ± 0.08[a]	58.08 ± 0.80[a]	28.00 ± 2.80[a]
IC-176563	24.64 ± 0.36[c]	4.88 ± 0.00[a]	48.92 ± 2.36[b]	1.97 ± 0.12[a]	62.37 ± 0.80[b]	24.23 ± 2.53[b]
IC-248733	23.90 ± 0.93[b]	6.63 ± 1.13[b]	53.00 ± 0.80[b]	2.22 ± 0.23[a]	62.26 ± 0.96[b]	26.40 ± 0.53[b]
IC-520892	25.29 ± 2.34[c]	7.75 ± 1.13[b]	51.84 ± 3.58[b]	1.60 ± 0.13[a]	60.45 ± 0.64[b]	28.00 ± 2.80[b]
IC-521049	18.92 ± 2.12[a]	6.50 ± 0.57[b]	51.00 ± 2.13[b]	2.58 ± 0.35[b]	60.11 ± 0.48[b]	39.51 ± 2.53[c]
IC-521061	22.14 ± 0.71[b]	7.13 ± 0.88[b]	53.46 ± 1.44[b]	2.72 ± 0.24[b]	58.20 ± 0.32[b]	33.85 ± 2.00[c]
IC-521068	27.69 ± 1.06[c]	8.04 ± 0.59[b]	51.15 ± 3.41[b]	2.27 ± 0.22[b]	60.23 ± 0.32[b]	44.60 ± 2.80[c]
IC-521081	28.03 ± 1.43[c]	8.21 ± 0.40[b]	58.22 ± 1.96[c]	5.38 ± 0.20[c]	57.63 ± 0.48[b]	32.25 ± 2.13[b]
IC-521144	22.61 ± 0.72[b]	9.08 ± 0.47[b]	58.06 ± 1.89[c]	4.80 ± 0.42[c]	63.05 ± 0.16[b]	19.42 ± 0.80[a]
IC-521148	18.31 ± 2.03[a]	6.38 ± 0.25[b]	50.54 ± 2.75[b]	2.89 ± 0.56[b]	55.49 ± 0.64[a]	20.74 ± 0.80[a]
EC-000262	26.71 ± 2.09[c]	6.67 ± 0.51[b]	53.46 ± 1.80[b]	2.45 ± 0.41[b]	55.26 ± 0.32[a]	26.49 ± 1.73[b]
EC-1843	23.86 ± 1.34[b]	7.42 ± 0.14[b]	53.46 ± 3.25[b]	2.55 ± 0.18[b]	54.92 ± 0.16[a]	19.04 ± 0.53[a]
EC-12416	21.25 ± 1.74[b]	6.71 ± 0.19[b]	55.84 ± 3.72[c]	3.24 ± 0.53[b]	54.70 ± 0.16[a]	17.34 ± 1.60[a]
EC-12436	27.15 ± 1.77[c]	6.92 ± 0.29[b]	61.52 ± 1.83[c]	2.44 ± 0.12[b]	63.16 ± 0.64[c]	29.79 ± 2.94[b]
EC-14075	28.54 ± 1.72[c]	7.96 ± 0.19[b]	40.94 ± 1.96[a]	3.24 ± 0.25[b]	63.16 ± 0.00[c]	12.81 ± 0.53[a]
EC-16136	24.81 ± 0.98[c]	6.58 ± 0.47[b]	56.37 ± 2.79[c]	2.79 ± 0.52[b]	55.49 ± 0.64[a]	38.47 ± 2.67[c]
EC-18171	27.90 ± 1.63[c]	5.13 ± 0.54[b]	43.24 ± 2.78[a]	2.76 ± 0.28[b]	61.02 ± 0.16[b]	30.83 ± 2.53[c]
EC-18181	22.27 ± 0.64[b]	9.33 ± 0.40[b]	65.36 ± 2.59[c]	2.40 ± 0.43[b]	58.87 ± 0.64[b]	30.45 ± 2.00[b]
EC-18184	26.95 ± 1.86[c]	6.79 ± 0.29[b]	64.67 ± 0.74[c]	2.37 ± 0.28[b]	63.30 ± 0.32[c]	30.26 ± 1.20[b]
EC-18222	23.76 ± 1.53[b]	5.42 ± 1.02[b]	64.75 ± 0.23[c]	1.15 ± 0.10[a]	60.45 ± 0.32[b]	26.77 ± 2.40[b]
EC-18260	23.73 ± 0.86[b]	7.04 ± 0.58[b]	64.36 ± 2.18[c]	2.07 ± 0.69[b]	55.60 ± 0.48[a]	18.09 ± 1.07[a]
EC-18556	21.15 ± 0.47[b]	6.21 ± 0.95[b]	68.51 ± 1.06[c]	1.72 ± 0.16[a]	61.02 ± 0.16[b]	22.72 ± 2.27[a]
EC-18563	26.41 ± 1.94[c]	5.00 ± 1.07[a]	66.21 ± 2.90[c]	2.23 ± 0.37[b]	56.84 ± 0.32[a]	18.75 ± 0.93[a]
EC-18771	23.15 ± 1.38[b]	5.38 ± 0.66[b]	40.17 ± 2.88[a]	2.09 ± 0.07[b]	54.70 ± 0.16[a]	13.28 ± 3.60[a]
EC-37226	19.86 ± 0.50[a]	5.83 ± 0.81[b]	56.07 ± 2.77[c]	2.48 ± 0.07[b]	59.55 ± 0.32[b]	30.83 ± 1.73[b]
EC-37228	27.69 ± 1.33[c]	6.96 ± 0.40[b]	47.93 ± 2.22[b]	1.64 ± 0.08[a]	54.7 ± 0.16[a]	23.09 ± 1.20[b]
EC-48452	27.63 ± 2.12[c]	4.50 ± 0.33[a]	45.70 ± 2.07[b]	1.74 ± 0.11[a]	55.26 ± 0.32[a]	11.02 ± 2.53[a]
EC-78228	26.78 ± 0.97[c]	3.17 ± 0.59[a]	37.79 ± 2.79[a]	2.36 ± 0.14[b]	57.29 ± 0.00[a]	16.58 ± 1.60[a]
EC-87898	22.75 ± 1.44[b]	5.17 ± 0.88[b]	43.32 ± 2.88[a]	1.92 ± 0.45[a]	55.04 ± 0.64[a]	16.58 ± 1.87[a]
EC-97882	21.56 ± 0.97[b]	6.33 ± 0.58[b]	43.24 ± 0.70[a]	1.33 ± 0.21[a]	56.39 ± 1.60[a]	10.74 ± 1.33[a]
EC-98452	19.59 ± 1.45[a]	7.13 ± 0.01[b]	35.56 ± 2.88[a]	1.86 ± 0.33[a]	54.70 ± 0.16[a]	15.55 ± 2.53[a]
EC-98453	21.05 ± 0.18[b]	5.67 ± 0.38[b]	36.10 ± 3.04[a]	2.84 ± 0.47[b]	61.92 ± 0.16[b]	17.91 ± 2.40[a]
EC-108887-A	26.37 ± 1.13[c]	6.46 ± 0.69[b]	38.17 ± 3.72[a]	1.56 ± 0.23[a]	58.20 ± 0.32[b]	20.92 ± 2.94[a]
EC-114076	28.95 ± 1.09[c]	9.63 ± 0.54[b]	39.86 ± 0.61[a]	4.92 ± 0.38[c]	57.52 ± 0.32[b]	16.87 ± 2.27[a]
EC-181185	18.81 ± 0.87[a]	6.21 ± 0.89[b]	42.32 ± 2.40[a]	2.95 ± 0.13[b]	60.00 ± 0.64[b]	19.23 ± 2.40[a]
Minimum	18.31 ± 2.03	3.17 ± 0.59	35.56 ± 2.88	1.14 ± 0.18	54.70 ± 0.16	10.74 ± 1.33
Maximum	32.64 ± 0.41	17.13 ± 0.50	68.51 ± 1.06	7.08 ± 0.60	63.84 ± 0.32	44.60 ± 2.80
Mean	24.37 ± 3.25	6.98 ± 2.20	50.99 ± 9.39	3.02 ± 1.45	59.12 ± 2.86	27.08 ± 9.32

Results are represented as the mean value of triplicates ± standard deviation. Different letters in the same column represent statistically different mean values ($p < 0.05$). IVPD = *in vitro* protein digestibility, IVSD = *in vitro* starch digestibility, % = percentage, mg/g = milligram per gram.

(31.79 ± 2.15 mg/g) and lowest in EC-78228 (2.88 ± 0.84 mg/g). Proline content above average levels was found in IC-18452, EC-18556, IC-18563, EC-18222, EC-18184, EC-18260, and EC-18181. Proline is a primary antioxidant to ward of plant stress and one of the important markers (Lee et al., 2012) and is reported to be regulated by various physiological responses during stress. It is assumed to be a protective mechanism (Rejeb et al., 2014). Proline also mitigates ROS generation and re-establishes cellular redox balance (Ghaffari et al., 2019; Szabados and Savoure, 2010). Further, proline has been reported to activate detoxification pathways in plants subjected to various stresses (Khedr et al., 2003). Therefore, proline in the present investigation has found negatively correlated with DPPH activity (Table 4). Thus, a close relation between high seed proline and DPPH radical scavenging activity was observed for most of the accessions (r = 0.102*, $p < 0.05$).

In plants, proline synthesis is coupled with pentose phosphate pathway that produces phenolic metabolites. This stimulates the synthesis of $NADPH_2$ and sugar phosphates required for anabolic pathways together with phenolic and antioxidant response pathways.

3.1.3. Total phenolic content and total flavonoid content

During the growth of plants, the biotic and abiotic stress produces different phenolic compounds to defend their predators (Khang et al., 2016). Phenols are natural plant antioxidants involved in regulation of ROS. TPC was observed in a range of 0.73 ± 0.04 to 8.27 ± 0.37 mg/g across all the accessions (Table 1). IC-521144, EC-181185, EC-98452, EC-108887-A, and EC-114076 showed significantly higher content of total phenols. EC-18563 exhibited the lowest phenol content. The result was in agreement with the study of Khabiruddin et al. (2002); Katoch

Table 3
Distribution of oligosaccharides and antinutritional factors in the seeds of rice bean.

Accession	Oligosaccharides			Anti-nutrients		
	Raffinose (mg/g)	Stachyose (mg/g)	Verbascose (mg/g)	Tannins (mg/g)	Phytic acids (mg/g)	Lectins (HU)
IC-2074	22.07 ± 0.92^c	11.51 ± 0.23^a	23.39 ± 0.4^c	5.09 ± 0.77^a	9.88 ± 0.02^b	0.32 ± 0.02^b
IC-2067	20.87 ± 0.41^c	18.21 ± 0.41^b	18.82 ± 0.87^c	11.75 ± 0.85^c	9.15 ± 0.02^b	0.38 ± 0.02^c
IC-2909	22.63 ± 0.16^c	21.74 ± 0.49^c	8.86 ± 0.88^b	10.09 ± 0.70^c	9.73 ± 0.02^b	0.30 ± 0.02^b
IC-7537-C	20.71 ± 0.80^c	19.22 ± 0.56^c	17.01 ± 0.69^c	8.40 ± 1.09^b	10.29 ± 0.03^b	0.29 ± 0.01^b
IC-8565-3	20.57 ± 0.49^c	16.95 ± 0.87^b	10.48 ± 0.32^b	5.64 ± 0.39^a	9.82 ± 0.01^b	0.21 ± 0.00^a
IC-11723	22.91 ± 0.68^c	16.67 ± 0.39^b	14.05 ± 0.59^c	7.04 ± 0.48^b	10.29 ± 0.02^b	0.23 ± 0.01^a
IC-15640	22.45 ± 0.98^c	17.65 ± 0.96^b	16.32 ± 0.56^c	6.70 ± 0.83^b	9.11 ± 0.03^b	0.28 ± 0.01^b
IC-16342	23.53 ± 0.83^c	26.74 ± 0.35^c	14.47 ± 0.82^c	7.08 ± 0.26^b	9.18 ± 0.05^b	0.29 ± 0.03^b
IC-16751	11.35 ± 0.86^a	15.08 ± 0.81^b	8.50 ± 0.55^b	12.21 ± 0.38^c	9.71 ± 0.01^b	0.23 ± 0.01^a
IC-16767	17.44 ± 0.45^b	8.87 ± 0.94^a	8.23 ± 0.51^b	11.54 ± 1.08^c	9.28 ± 0.05^b	0.15 ± 0.00^a
IC-18183	17.95 ± 0.65^b	8.04 ± 0.66^a	9.34 ± 0.87^b	9.20 ± 1.03^c	9.83 ± 0.02^b	0.31 ± 0.04^b
IC-18452	17.31 ± 0.52^b	8.38 ± 0.92^a	2.76 ± 0.33^a	10.09 ± 0.70^c	9.70 ± 0.01^b	0.27 ± 0.02^b
IC-18563	16.32 ± 0.67^b	8.01 ± 0.91^a	12.24 ± 0.23^b	2.04 ± 0.34^a	9.17 ± 0.00^b	0.21 ± 0.01^a
IC-19336	16.96 ± 0.55^b	6.58 ± 0.62^a	12.12 ± 0.32^b	11.28 ± 0.32^c	8.84 ± 0.03^a	0.21 ± 0.01^a
IC-19781-2	17.57 ± 0.83^b	9.33 ± 0.29^a	5.92 ± 0.70^a	10.64 ± 0.51^c	8.06 ± 0.03^a	0.18 ± 0.00^a
IC-26973	21.95 ± 0.40^c	28.43 ± 0.64^c	6.49 ± 0.78^a	8.69 ± 1.47^b	7.82 ± 0.05^a	0.20 ± 0.01^a
IC-176563	23.53 ± 0.35^c	23.32 ± 0.56^c	10.89 ± 0.47^b	9.50 ± 0.45^c	8.67 ± 0.01^a	0.19 ± 0.00^a
IC-248733	22.22 ± 0.40^c	13.74 ± 0.52^b	13.35 ± 0.49^c	10.01 ± 0.26^c	8.57 ± 0.04^a	0.20 ± 0.01^a
IC-520892	21.38 ± 0.67^c	18.47 ± 0.76^c	5.36 ± 0.20^a	6.91 ± 0.19^b	8.58 ± 0.02^a	0.19 ± 0.02^a
IC-521049	20.89 ± 0.65^c	18.40 ± 0.83^c	7.01 ± 0.29^b	6.87 ± 0.99^b	9.32 ± 0.05^a	0.26 ± 0.03^b
IC-521061	18.62 ± 0.34^b	13.40 ± 0.96^b	4.95 ± 0.30^a	11.96 ± 0.51^c	8.57 ± 0.02^a	0.31 ± 0.01^c
IC-521068	17.96 ± 0.47^b	12.17 ± 0.77^b	5.63 ± 0.37^a	10.18 ± 0.79^c	7.71 ± 0.04^a	0.22 ± 0.01^a
IC-521081	19.20 ± 0.42^b	13.25 ± 0.99^b	7.13 ± 0.39^b	9.88 ± 0.85^c	8.41 ± 0.03^a	0.21 ± 0.01^a
IC-521144	21.23 ± 0.15^c	6.98 ± 0.52^a	9.48 ± 0.40^b	10.86 ± 0.51^c	7.89 ± 0.02^a	0.27 ± 0.01^a
IC-521148	22.59 ± 0.57^c	8.65 ± 0.51^a	7.09 ± 0.61^b	11.54 ± 0.45^c	12.83 ± 0.01^c	0.38 ± 0.04^c
EC-000262	16.58 ± 0.54^b	15.60 ± 0.57^b	9.71 ± 0.51^b	8.99 ± 0.53^b	11.82 ± 0.04^b	0.19 ± 0.01^a
EC-1843	17.44 ± 0.67^b	10.28 ± 0.40^a	5.86 ± 0.59^a	3.99 ± 0.39^a	11.27 ± 0.01^b	0.34 ± 0.02^b
EC-12416	17.96 ± 0.81^b	12.30 ± 0.38^b	6.57 ± 0.81^a	9.84 ± 1.29^c	11.79 ± 0.04^b	0.32 ± 0.03^b
EC-12436	15.63 ± 0.32^a	13.16 ± 0.62^b	7.40 ± 0.90^b	3.05 ± 0.34^a	11.57 ± 0.01^b	0.25 ± 0.02^a
EC-14075	16.54 ± 0.40^b	11.11 ± 0.42^a	12.62 ± 0.67^c	10.90 ± 0.51^c	11.84 ± 0.03^b	0.24 ± 0.01^a
EC-16136	17.08 ± 0.57^b	6.92 ± 0.66^a	7.86 ± 0.76^b	10.81 ± 1.47^c	15.28 ± 0.00^c	0.28 ± 0.01^b
EC-18171	18.77 ± 0.34^a	8.16 ± 0.97^a	5.86 ± 0.37^a	8.52 ± 0.64^b	10.89 ± 0.01^b	0.35 ± 0.02^b
EC-18181	21.94 ± 0.50^c	13.45 ± 0.32^b	8.38 ± 0.59^b	6.74 ± 1.02^b	12.59 ± 0.01^c	0.15 ± 0.00^a
EC-18184	22.04 ± 0.58^c	13.55 ± 0.83^b	7.59 ± 0.94^b	9.54 ± 0.13^c	12.32 ± 0.02^c	0.25 ± 0.02^a
EC-18222	22.00 ± 0.64^c	6.85 ± 0.21^a	12.72 ± 0.38^b	11.20 ± 0.34^c	12.04 ± 0.05^c	0.29 ± 0.02^b
EC-18260	18.62 ± 0.39^b	5.97 ± 0.85^a	7.75 ± 0.15^b	10.60 ± 0.82^c	11.25 ± 0.03^b	0.41 ± 0.01^c
EC-18556	16.65 ± 0.79^b	5.15 ± 0.75^a	9.09 ± 0.88^b	10.35 ± 0.92^c	12.22 ± 0.02^c	0.32 ± 0.01^b
EC-18563	16.96 ± 0.59^b	4.74 ± 0.71^a	13.43 ± 0.88^b	7.04 ± 0.75^b	11.60 ± 0.01^b	0.25 ± 0.02^a
EC-18771	17.26 ± 0.83^b	5.30 ± 0.96^a	7.53 ± 0.18^b	9.92 ± 0.89^c	11.79 ± 0.02^b	0.30 ± 0.02^b
EC-37226	18.65 ± 0.40^b	7.86 ± 0.76^a	8.67 ± 0.93^b	3.48 ± 0.87^a	14.84 ± 0.01^c	0.34 ± 0.01^b
EC-37228	21.43 ± 0.73^c	9.05 ± 0.23^a	6.72 ± 0.70^a	5.81 ± 0.45^a	12.10 ± 0.01^c	0.25 ± 0.01^a
EC-48452	18.65 ± 0.46^b	10.50 ± 0.65^a	8.63 ± 0.64^b	4.28 ± 0.07^a	14.12 ± 0.02^c	0.25 ± 0.02^a
EC-78228	16.01 ± 0.65^b	10.17 ± 0.11^a	11.14 ± 0.67^b	4.88 ± 0.39^a	13.41 ± 0.01^c	0.25 ± 0.01^a
EC-87898	18.01 ± 0.40^b	8.14 ± 0.96^a	8.13 ± 0.95^b	5.26 ± 0.70^a	12.93 ± 0.03^c	0.30 ± 0.02^b
EC-97882	17.96 ± 0.43^b	8.35 ± 0.57^a	9.19 ± 0.96^b	5.94 ± 0.41^a	13.19 ± 0.01^c	0.32 ± 0.01^b
EC-98452	17.42 ± 0.41^b	23.83 ± 0.13^c	5.95 ± 0.74^a	3.22 ± 0.92^a	12.46 ± 0.00^c	0.35 ± 0.02^b
EC-98453	16.44 ± 0.45^b	18.20 ± 0.12^c	11.71 ± 0.71^b	3.27 ± 0.26^a	12.40 ± 0.03^c	0.32 ± 0.00^b
EC-108887-A	17.57 ± 0.33^b	14.78 ± 0.28^b	5.92 ± 0.58^a	4.07 ± 0.25^a	13.31 ± 0.01^c	0.26 ± 0.01^a
EC-114076	17.85 ± 0.55^b	30.51 ± 0.64^c	9.75 ± 0.50^b	5.22 ± 0.51^a	11.82 ± 0.01^c	0.14 ± 0.01^a
EC-181185	15.68 ± 0.32^b	8.03 ± 0.47^a	6.82 ± 0.81^a	5.39 ± 0.63^a	11.69 ± 0.07^c	0.21 ± 0.01^a
Minimum	11.35 ± 0.86	4.74 ± 0.71	2.76 ± 0.33	2.04 ± 0.34	7.71 ± 0.04	0.14 ± 0.01
Maximum	23.53 ± 0.35	30.51 ± 0.64	23.39 ± 0.41	12.21 ± 0.38	15.28 ± 0.00	0.41 ± 0.01
Mean	19.04 ± 2.67	13.05 ± 6.27	9.48 ± 3.85	7.95 ± 2.88	10.82 ± 1.98	0.26 ± 0.06

Results are represented as the mean value of triplicates ± standard deviation. Different letters in the same column represent statistically different mean values ($p < 0.05$). HU = Haemagglunating units, mg/g = milligram per gram.

(2013). The observed differences in total phenol content of seeds could be attributed to genetic background. Most of the polyphenols are effective scavengers of hydroxyl and peroxyl radicals. They can stabilize lipid oxidation and act as chelators of metals, DNA damage and cancer inhibition (Luo et al., 2002). The antioxidant ability of various seeds is positively correlated with phenolic content (Verma et al., 2008). The present results also showed that most of the rice bean accessions exhibit higher content of total phenols attained increased level of DPPH radical scavenging activity. The phenolic content compared to the other major legumes like chickpea, cowpea, green gram, horse gram, lablab bean, lentils and pigeon pea ranged from 0.325 to 6.378 mg/g (Marathe et al., 2011).

Flavonoids are the secondary metabolites of plants and act as a natural antioxidant (Bouaziz et al., 2005). TFC in rice bean varied between 0.20 ± 0.01 to 0.77 ± 0.16 mg/g with mean value 0.45 ± 0.19 mg/g (Table 1). EC-18563, IC-7537-C, IC-16767, EC-18260, and EC-87898 had the higher average flavonoid content across all the accessions while minimum was exhibited by EC-181185. According to epidemiological studies, the intake of flavonoid rich food prevent humans from diseases (Bakoyiannis et al., 2019; Ginwala et al., 2019). Flavonoid rich foods presents free radical scavenging activity and provide protection against oxidative stress.

Table 4
Correlation coefficient among the evaluated parameters in the seeds of rice bean.

Traits	DPPH	TPC	TFC	Proline	Protein	Total amino acids	IVPD	Sugar	Starch	IVSD	Tannins	Phytic acid	Verbascose	Stachyose	Raffinose	Lectins
DPPH																
TPC	0.102*															
TFC	-0.150	-0.198														
Proline	-0.216	-0.096	0.332*													
Protein	-0.103	-0.185	0.064*	0.069*												
Total amino acids	-0.261	0.095*	0.093*	0.296*	-0.014											
IVPD	-0.430	-0.054	-0.015	0.041*	0.076*	0.117*										
Sugar	-0.316	0.003*	0.031*	-0.045	0.074*	0.310*	0.310*									
Starch	-0.104	-0.130	0.472*	0.861*	-0.059	0.235*	0.000*	-0.117								
IVSD	-0.473	-0.252	0.173*	0.355*	0.058*	0.429*	0.425*	0.319*	0.289*							
Tannins	-0.396	-0.174	0.094	-0.051	0.020*	0.342*	0.342*	0.625*	-0.121	0.447*						
Phytic acid	1.000*	0.102*	-0.150	-0.216	-0.103	0.261	0.430	0.316	0.104	-0.473	-0.396					
Verbascose	-0.119	-0.202	0.072*	-0.142	0.043*	-0.203	0.341*	0.337*	-0.077	0.009*	0.335*	-0.119				
Stachyose	-0.290	-0.009	-0.222	-0.183	0.147*	0.087	0.171*	0.296*	0.330	0.066*	0.118*	0.290	0.140*			
Raffinose	-0.262	-0.082	-0.081	0.167*	-0.073	-0.122	0.201*	0.146*	0.116*	0.083*	0.034*	-0.262	0.297*	0.371*		
Lectins	0.309*	-0.049	0.034*	-0.090	-0.361	-0.123	-0.189	0.026*	0.028	-0.245	0.000	0.309*	0.081*	-0.237	0.039*	1

Significance at 0.05% probability. DPPH = 2,2-diphenyl-1-picrylhydrazyl, TPC = Total phenolic content, TFC = Total flavonoid content, IVPD = in vitro protein digestibility, IVSD = in vitro starch digestibility.

3.2. Nutritional composition of rice bean accessions

3.2.1. Total protein and total amino acid content

Grain legumes play an important role in human nutrition as they provide rich source of protein. Protein content of rice bean is higher than the common cereal grains (Campos-Vega et al., 2010). The wide range of variability in protein content was reported and was significantly higher are shown in Table 2. The highest protein content was recorded in IC-8565-3 (32.64 ± 0.41 mg/g) and the lowest in IC-521148 (18.31 ± 2.03 mg/g). The mean content of total protein across all the accessions was observed to be 24.37 ± 3.25 mg/g. The range of protein varied from 18.31 ± 2.03 to 32.64 ± 0.41 mg/g. Promising accessions found with higher value (> 28.00 mg/g) of protein were EC-12436, IC-11723, EC-114076, EC-14075 and IC-521081. Earlier studies on rice bean depicted similar values of protein (Kaur and Kapoor, 1992; Awasthi et al., 2011; Katoch, 2013). Thus, high amount of protein containing accessions can be used as an alternative of conventional edible legumes.

Total free amino acids were observed in a range of 3.17 ± 0.59 to 17.13 ± 0.50 mg/g across all the rice bean accessions (Table 2). The mean content was found to be 6.98 ± 2.20 mg/g. The maximum total amino acid was observed in IC-18452 (17.13 ± 0.50 mg/g). IC-18183, IC-19336, EC-114076, EC-18181, and IC-521144 contained highest values while minimum was exhibited by EC-78228 (3.17 ± 0.59 mg/g). Mature pulse seeds can accumulate normally high amount of protein and total amino acids (Bhagyawant et al., 2018b).

3.2.2. Carbohydrates

The total soluble sugars (TSS) content varied from 1.14 ± 0.18 mg/g (IC-19781-2) to 7.08 ± 0.60 mg/g (IC-2909) (Table 2). The mean sugar value across all the accessions was observed to be 3.02 ± 1.45 mg/g. The accessions IC-15640, IC-16751, IC-2074, IC-521081, and IC-48452 were observed to have higher sugar content. Earlier studies reported TSS content in rice bean ranged from 5.0 to 5.6 g/100 g (Kaur and Kapoor, 1992; Khabiruddin et al., 2002; Katoch, 2013).

Starch contents varied from 35.56 to 68.51 mg/g (Table 2). The highest starch content was reported in EC-18556 (68.51 ± 1.06 mg/g), and lowest in EC-98452 (35.56 ± 2.88 mg/g). The high starch and TSS contents of the bean made it a good source of calories. The TSS was increases with cooking due to degradation of starch into simple soluble sugars (Saikia et al., 1999).

3.2.3. Protein and starch digestibility

Rice bean possess a significant content of protein, but quality protein depends on amino acid composition and digestibility (Katoch, 2013). IVPD of rice bean is shown in Table 2. It was significant in IC-2909 (63.84 ± 0.32%), followed by EC-18184 (63.30 ± 0.32%) and lowest in EC-12416 (54.70 ± 0.16%). Higher the TPC lower will be the IVPD. The phytic acid content is also known to be negatively correlated with IVPD of pulses (Chitra et al., 1995) and constitutes an important indicator for physiological status of food.

IVSD observed was in a range of 10.74 ± 1.33 to 44.60 ± 2.80% (Table 2). The mean value was found to be 27.08 ± 9.32% and the highest in IC-521068 (44.60 ± 2.80%). The starch digestibility of rice bean is 32.86 mg maltose/g which is lower than faba bean which releases 42 mg maltose/g (Kaur and Kapoor, 1990). In contrast to other major legumes like mung bean, pea, pigeon pea, soybean and cowpea, the rice bean has the lowest glycemic index (Marsosno et al., 2002). Enhancement of starch digestibility is attributed to the loss of phytic acid, tannins and polyphenols, which inhibit the activity of α-amylase (Alonso et al., 2000). Legume starches to a certain extent are refractory to enzymatic digestion and contribute flatulence. This can be overcomed by intake of whole legume wherein the dietary fibre helps in reducing the intestinal transit time and thus supports bowel motility. Reduced digestibility lowers glucose release into the blood stream is
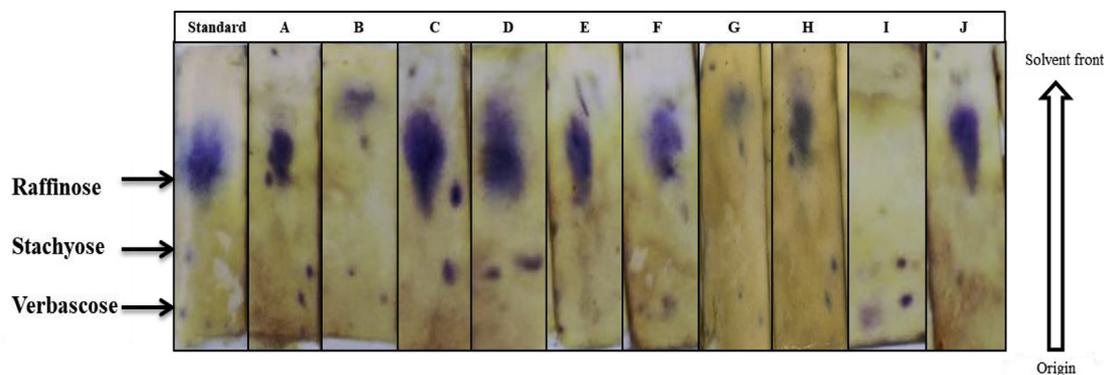
Fig. 2. TLC separation patterns of oligosaccharides in representative rice bean accessions. Raffinose, stachyose and verbascose were used as standard. Lane A: EC-1843; lane B: IC-521061; lane C: EC-18171, lane D: EC-37228, lane E: IC-521061, lane F: EC-181185, lane G: IC-521081, lane H: IC-19781-2, lane I: IC-7537-C, lane J: IC-16342.

advantageous to diabetic patients (Muhammad et al., 2000). Therefore, IVPD offers a hint for protein absorption, whereas IVSD depicts the susceptibility of starch to digestive enzymes. Differences in seed coat and texture influence digestibility.

3.3. Antinutritional composition of rice bean accessions

3.3.1. Oligosaccharides

In the present analysis, oligosaccharides in the rice bean seeds were fractionated using thin layer chromatography. This conventional chemical analysis illustrates a rapid, easy and cost effective method. The separation and variation in saccharide contents are clearly visible using TLC therefore used in the investigation. Representative TLC patterns is shown in Fig. 2. The maximum content of raffinose was observed in IC-16342 (23.53 ± 0.35 mg/g) and minimum in IC-16751 (11.35 ± 0.86 mg/g) with mean value 19.04 ± 2.67 mg/g (Table 3). The stachyose found highest in EC-114076 (30.51 ± 0.64 mg/g) and lowest in EC-18563 (4.74 ± 0.71 mg/g) with mean value 13.05 ± 6.27 mg/g (Table 3). The verbascose varied significantly between 2.76 ± 0.33 (IC-18452) mg/g to 23.39 ± 0.41 (IC-2074) mg/g with mean value of 9.48 ± 3.85 mg/g (Table 3). The oligosaccharides namely raffinose (0.3%–2.58%) along with stachyose (0.37%–1.98%) and verbascose (0.45–2.58%) are found in rice bean (Katoch, 2013). The contents of oligosaccharides in rice bean are lesser compared to soybean, black gram, green gram and cowpea (Bepary et al., 2016).

The scientific reports have reported that the oligosaccharides possess several functions such as mineral absorbability, anti-cardiovascular, anti-carcinogenic and anti-diabetic activity (Masao, 2002; Tajoddin et al., 2012). In legumes, oligosaccharides like raffinose, stachyose and verbascose are called as raffinose family oligosaccharides (RFOs) or α–galactosides (Sengupta et al., 2015). The human intestine is incapable to digest and absorb these oligosaccharides due to absence of α–galactosidase enzyme in digestive system. Therefore, they get accumulated in intestine and undergo anaerobic fermentation by enteric micro biome releasing the flatulence gases like carbon dioxide, hydrogen and methane. Apart from health-promoting benefits, these oligosaccharides are considered antinutrients as they causes flatulence, stomach rumblings, abdominal discomfort, pain, cramps and diarrhea (Andersen et al., 2005; Bepary and Wadikar, 2019).

3.3.2. Tannins and phytic acid

The tannin concentration in the legume seeds corroborate to seed coat color (Deshpande and Cheryan, 1983). Tannin content in rice bean varied from 2.04 ± 0.34 mg/g to 12.21 ± 0.38 mg/g (Table 3). The mean content of tannins was observed to be 7.95 ± 2.88 mg/g in the rice bean seeds. The seed coat of rice bean accessions (IC-521061, IC-2067, IC-521148, IC-16767 and IC-19336) is dark in color which

represents higher concentration of polyphenols in comparison with light color accessions (IC-18563, EC-12436 and EC-98452). Earlier studies by Katoch (2013) and Awasthi et al. (2011) observed similar tannin content in rice beans. Tannins form complexes with protein and to a lesser content with available carbohydrates and other polymers. Tannins have been reported to inhibit digestive enzymes and thereby lower the digestibility of important nutrients, basically protein and starch (Khattab et al., 2009). The tannin has been reported to possess antioxidant properties and also affect the compatibility by forming the complexes with salivary glycoproteins (Barrett et al., 2018; Soares et al., 2018).

Rice bean has fairly high content of phytic acid and its content among accessions varied significantly. The content of phytic acid in rice bean accessions ranged 7.71 ± 0.04 to 15.28 ± 0.00 mg/g with median value of 10.82 ± 1.98 mg/g (Table 3). The accessions had higher phytic acid content were EC-16136, EC-37226, EC-48452, EC-78228, EC-108887-A, EC-97882 and EC-87898. Phytic acid has been known as major storage form of phosphorus and in legumes it varied from 0.44 to 1.46% (Reddy et al., 1982). Saharan et al. (2002) had observed 2018 mg/100 g of phytic acid content in rice bean whereas Katoch (2013) reported phytic acid content in rice bean accessions between 3.2 and 6.4%.

Legumes contain antinutritional substances like phytic acid. Phytic acid is a strong chelator of important minerals which lowers their absorption and bioavailability for monogastric animals, including humans as they lack the phytase enzyme in their digestive track (Gupta et al., 2015). It affects the digestion and absorption of protein and carbohydrates by forming the complexes with them (Oatway et al., 2001). In rice bean, the content of phytic acid was lower in contrast to commonly consumed legumes like *V. mungo, Dolichos lablab var. vulgaris, Mucuna pruriens var. utilis,* and *Mucuna atropurpurea* (Kataria et al., 1988; Vijayakumari et al., 1995; Janardhanan et al., 2003; Kamatchi et al., 2010).

3.3.3. Hemagglutinating activity

The lectin content in rice bean accessions ranged between 0.14 ± 0.01 to 0.41 ± 0.01 haemagglutination unit (HU) per milligram (Table 3). The moderate content was found to be 0.26 ± 0.66 HU/mg. Earlier, haemagglutination activity in rice bean was reported by Kaur and Kapoor (1992). In the present study, trypsin treated rabbit erythrocytes were able to agglutinate. This may be due to molecular properties of the lectin, cell surface interactions, metabolic state of the cells and assay conditions as well. The seed type, cultivation area and method of harvest may also affect the variation in concentration of lectins (Mekbungwan, 2007). The amount of lectin varies in legume and has potent clinical applications. Lectin from kidney bean seeds inhibits HIV-1 reverse transcriptase (Ng et al., 2002). Compared to other
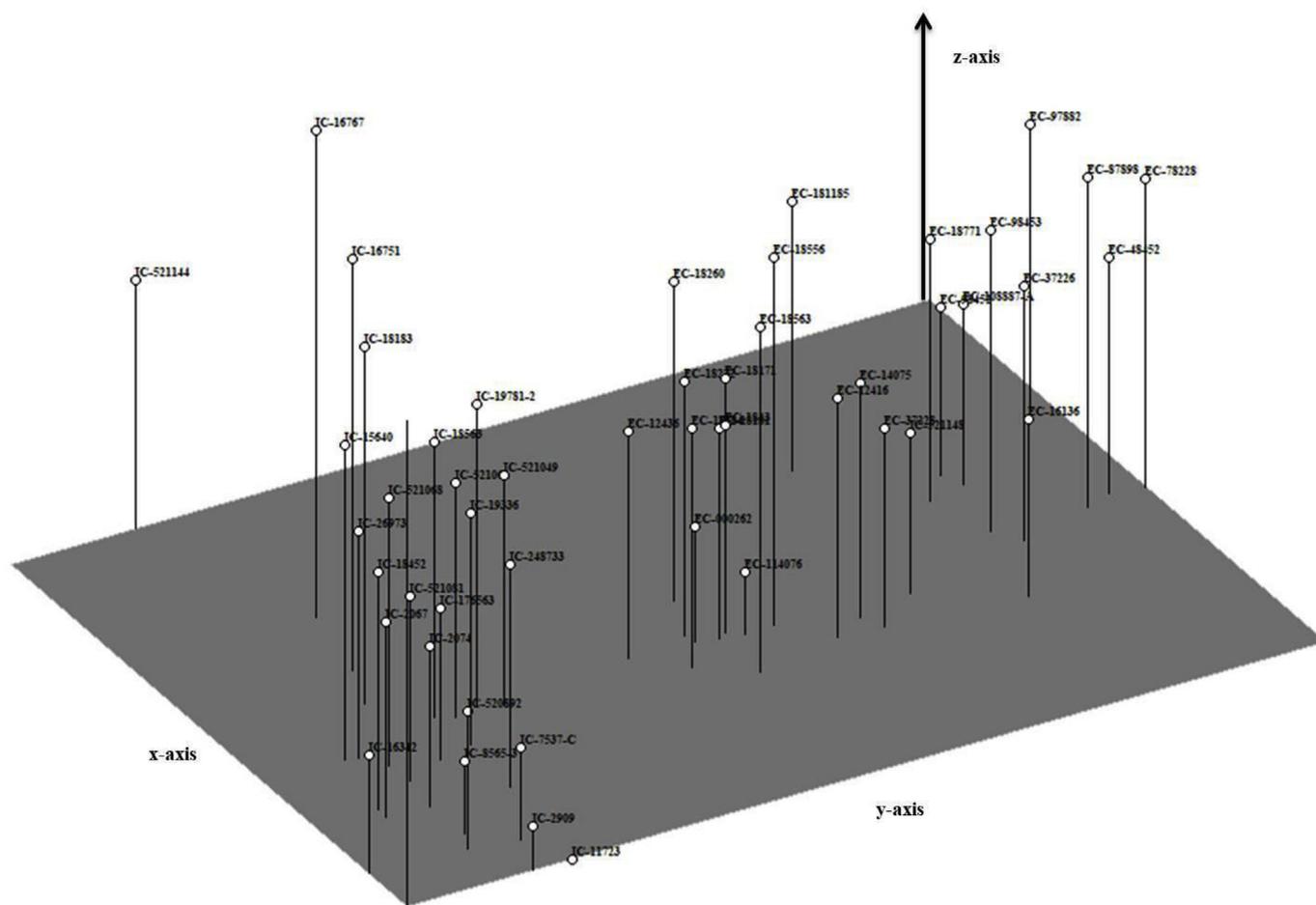
Fig. 3. Multivariate analyses of 50 rice bean accessions based on evaluated parameters.

legumes like pea and lentil, rice bean has lower lectin contents as revealed in the present study (Datta et al., 1988). Published report depicted that plant lectin show pharmacological properties *vis-à-vis* anti-fungal and *anti*-HIV-1 reverse transcriptase activity (Gupta et al., 2018).

Legumes contain bioactive proteins including carbohydrate binding proteins-lectins and phenolic compounds showing antioxidant activity. In our earlier published report, a positive correlation was depicted in DPPH activity and lectin concentration (Gautam et al., 2018). In the present investigation, antioxidant activity of rice bean seed extract positively correlated with DPPH radical scavenging activity as shown in Table 4. Thus, current study revealed that rice bean seeds could not only serve as a rich source of food proteins but also a prominent source of high antioxidant potential (Yao et al., 2012).

3.4. Correlation analysis

Correlation analysis describe the relationship and/or association between quantitative variables. it also measures the extent/strength of association between the analysed variables. Therefore, correlation analysis for all aforesaid biochemical parameters was performed (Table 4). DPPH percentage showed positive association with total phenolic content (TPC), phytic acid and lectin. Proline was positively associated with total amino acids and IVSD. Similarly, sugar was significantly showing positive correlation with oligosaccharides. Protein showed significant positive correlation with sugar, IVPD and IVSD while significant negative correlation was observed with phytic acid and lectin. TPC was negatively correlated with IVPD and IVSD.

Starch was positively correlated with IVSD, while significant negative correlation was observed with tannins and phytic acid.

Correspondingly, oligosaccharides showed significant positive correlation with tannins while negatively correlated with phytic acid. Phytic acid and IVPD were negatively correlated to each other. Lectins exhibited positive correlation with DPPH and significant negative correlation with tannins.

3.5. Principal component analysis

Principal component analysis (PCA) was done by considering all the 16 variables simultaneously. The pattern of variations illustrated by the PCA described by correlation coefficients determined for pair-wise association of the traits (Fig. 3). The PCA correlation depicted that the accessions viz., EC-97882, EC-87898, EC-78228, EC-48452, EC-98453, EC-16136, EC-18771, EC-14075 and EC-12416 possessed higher antioxidant activity and maximum protein content with lower levels anti-nutrient contents of tannin and lectin occupying unique position towards the right side of the graph (Fig. 3). The accessions viz., IC-16767, IC-16751, IC-18183, 18452, IC-15640, IC-19781-2, IC-26973, IC-16342, IC-520892, IC-7537-C, IC-2909, IC-11723 occupied specific left position in the graph with maximum values for *in vitro* starch digestibility and lower phytic acid content. EC-181185, EC-18260, EC-114076, EC-000262, EC-12436 possess centre of the graph with higher phenolic values. The accession IC-521144 came as out group showing the maximum diversity. It was further noticed that accessions existing towards right (Fig. 3) showed maximum value for antioxidant activity while those towards the left (Fig. 3) showed maximum proline value.
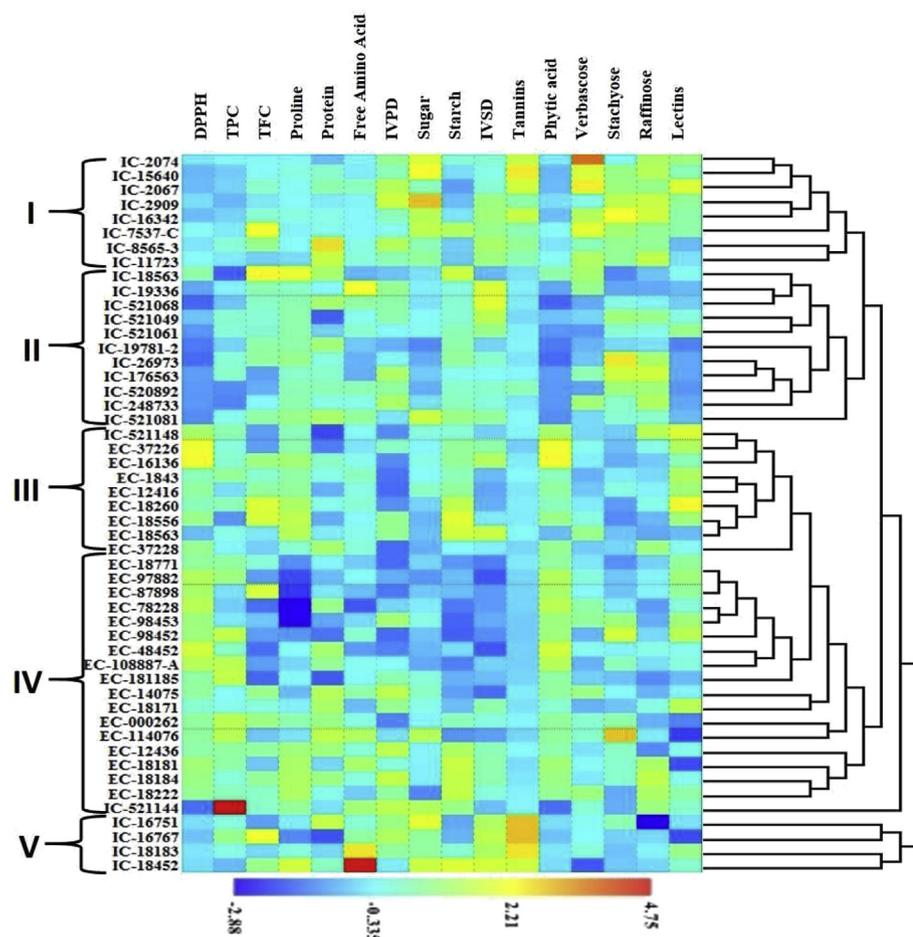
Fig. 4. Hierarchical clustering analyses of 50 rice bean accessions. The columns represent the different nutritional and antinutritional parameters. The row represents the individual accessions. The high and low content are indicated in red and blue color. The intensity of color increases as content increases as shown in the bar at the bottom. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.6. Hierarchical cluster analysis

According to the hierarchical cluster analysis and the content values listed in Tables 1–3; the dynamic expression profile were determined and are shown in Fig. 4. Multivariate analysis based on diversity was performed using the UPGMA. The mean value of accessions falling in each cluster was presented in the generated dendrogram for all the accessions distinguished into five clusters (I, II, III, IV, and V).

Cluster I consisted of 8 accessions (IC-2074, IC-15640, IC-2064, IC-2909, IC-16342, IC-7537-C, IC-8565-3, IC-11723) exhibit high variability for protein and IVSD while low variability was observed for antioxidant activity and TPC. Cluster II comprises of 11 accessions showed highest concentration of protein and proline and possesses low values for phytic acid and lectin (IC-521068, IC-26973, IC-520892, and IC-521081). Cluster III comprised of 9 accessions exhibited higher antioxidant activity, proline and starch content. EC-16136 possess high value for antioxidant activity, phenolic content, protein, starch, IVSD whereas EC-18563 possess lower value for sugar, stahycose, total amino acids and lectin. The accession EC-37228 was came as out group showing the maximum diversity. Cluster IV is grouped of 18 accessions showing higher antioxidant activity, total phenolic content, protein, and IVPD. The higher antioxidant activity present in the seeds mainly contributed by phenolic content. Cluster V consists of 4 accessions (IC-16751, IC-16767, IC-18183, 184252) representing maximum total amino acid and protein values with higher IVSD percentage.

The food security, protein-deficiency, malnutrition and hidden hunger are the major challenges for the developing nations (Riley, 2016; Harouna et al., 2018). Rice bean seeds show promising nutritive composition (Sharma et al., 2019). And yet, information on various biochemical compositions in the seeds of rice bean among available Indian accessions is inadequate (Katoch, 2013; Dhillon and Tanwar, 2018). Present study revealed a wide range of variability in rice bean seed accessions projecting it as a significant crop for food security.

4. Conclusion

Indian breeders are constantly looking for alternatives to presently consumed pulses. Exploiting agricultural potential of this less known pulse, it was imperative to analyse seed biochemical signatures. Rice bean seeds constitute nutritional benefits including rich protein content, good antioxidant potential, high protein digestibility and lower phytic acid and lectin content are appropriate for human consumption. Accession EC-16136 is elite in terms of biochemical composition. In conclusion, the production and consumption of rice bean should be enhanced and fortified nutritionally.Acknowledgement

Conflicts of interest

Authors do not have any conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101193.

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