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Nutritional Composition, Phenolic Content and Antioxidant Capacity of Seeds from A Collection of Ricebean [*Vigna umbellata* (Thumb.) Ohwi et Ohashi] Introduced in Burkina Faso

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A B S T R A C T

Recent years have seen a resurgence of interest in under-utilized legumes with high nutritional and therapeutic properties. Ricebean are an appropriate option for inclusion in the list of existing legumes. Ricebean seeds are a well-balanced source of constituents that are beneficial to health and combat malnutrition. Although very important from a dietary point of view in Burkina Faso, there is as yet no research into the nutrient composition and antioxidant properties of rice beans. The aim of this study was to analyze the biochemical composition of a ricebean collection. Total of 24 ricebean seed accessions were evaluated for various biochemical traits using official and standard analytical methods. Results showed great variability in protein content (11.493 to 33.852 mg/g) and sugar content (4.207 to 8.48 mg/g). Concentrations of phenolic compounds such as phenols and flavonoids varied respectively from 1.140 to 3.446 mg EQ/g and from 0.559 to 1.322 mg EAG/g. DPPH ranged from 3.285% to 27.018% and FRAP from 2.042 to 35.21 mM EAA/g DM. Correlation coefficient between parameters was calculated to understand the relationship between variables. First three principal components contributed 62.7% of the variation. CAH formed four groups based on levels of macromolecules, ascorbic acid, pigments, phenolic compounds and antioxidants. Accessions RB27, RB10 RB59, RB60 RB127 RB79 showed interesting antioxidant and phenolic activity, high protein, ascorbic acid and sugar content. Results of this study suggest the use of these potential accessions in breeding programs with the main aim of improving the nutritional quality of ricebean.

Introduction

Sustainable Development Goal has emphasized a holistic approach to achieving zero hunger, good health and well-being by 2030 (Baruah, 2018). Dietary diversifications and fortification can be employed to prevent hunger, starvation and micronutrient deficiencies in the long term. An effective way of solving these problems through food is to create new value-added products such as pulses. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) are focusing on the exploitation of under-exploited legumes. According to Ayilara *et al.,* (2022), they contain large quantities of phytochemicals, antioxidants and phenols. What's more, they are capable of promoting a physiological state that could help prevent

various human diseases. Several under-utilized legumes have been identified with a healthy nutritional profile and remarkable production potential. Ricebean, is currently of interest due to its production potential and nutritional profile rich in protein, amino acids, micronutrients and antioxidants (Katoch, 2011). However, few publications on nutritional studies of ricebean are available, and those that do exist concern only a limited number of accessions (Bajaj, 2014; Dhillon and Tanwar, 2018). In Burkina Faso, there is as yet no scientific research into the nutritional and antioxidant composition of ricebean. A research system on the biochemical characterization of rice beans could play an important role in improving the diet and health of populations. With this in mind, this study was carried out to investigate the complete nutritional and phytochemical profile of different accessions. Specifically, the aim was to (i) quantify the content of nutritional compounds (ii) assess polyphenol content and antioxidant activity (iii) and identify promising high-quality accessions for involvement in nutritional security, extension and crop improvement programs.

Materials and Methods

Sample analysis laboratory

This work was carried out within the Training and Research Unit (SVT) in the Laboratory of Biochemistry, Food Technology and Nutrition at the Université Joseph KI-ZERBO in Ouagadougou, Burkina Faso.

Plant material

Seeds from twenty-four (24) rice bean accessions introduced in Burkina Faso were collected for the study (Table 1). These accessions were obtained in partnership with the Nelson Mandela - Agricultural Institute of Sciences and Technologies (NM-AIST).

Sample preparation

Accession-dried seeds were carefully cleaned, ground in a blender (MICROTRON®MB800) and passed through a 2 mm mesh sieve. The flour obtained was packed in bags, then stored and subjected to the various extractions.

Determination of macromolecule composition

Determination of protein content

Protein content was assessed using the method described by Bradford with slight modifications (Bradford, 1976; Gonçalves *et al.,* 2020). Flour samples from the seeds (500 mg) were homogenized in 10 mL 0.1 M NaCl and the mixture stirred (at 1500 rpm/min) for 5 h at 25°C. The extract was collected after centrifugation at 4400 rpm for 30 min at 4°C. To 50 µL of each extract was added 250 µL of Bradford reagent. After incubation for 2 min, absorbances were read at 595 nm. A standard curve (y =1.313 8 x + 0.011 9; R2 =0.999) constructed using BSA (Bovine Serum Albumin) as the standard (0 - 25 µg/ml) was used to determine the protein concentration of the various samples.

Determination of Total soluble sugar

Soluble sugars are determined using the Phenol-sulfuric acid method described by Dubois *et al.,* (2009).

Extraction of soluble carbohydrates

0.5 g of powder from each sample was homogenized in Falcon tubes containing 10 ml of 80% ethanol. After 24 h incubation at room temperature, the samples were centrifuged at 5,000 rpm for 10 min. The supernatant collected was used for assays.

Determination of soluble carbohydrate content

To one milliliter of sample was added 1 ml of freshly prepared 5% phenol solution and 5 ml of 95% sulfuric acid. The mixture was vortexed for 30 s. A blank was prepared under the same conditions with 1 ml of 80% ethanol in place of the extract. Tubes were incubated in a water bath at 30°C for 20 min. Spectrometer readings were taken at 490 nm. Absorbance readings were extrapolated to a glucose standard curve (0-400 µg/ml). Soluble sugar content was expressed as (%) glucose equivalent of dry matter (DM).

Composition of pigments (β-carotene and lycopene) and ascorbic acid (Vitamin C)

Determination of β-carotene and lycopene content

The extraction technique used was maceration. This involved weighing 100 mg of seed crush, then adding 5 ml of acetone/hexane (70/30). The resulting mixture was stirred for 1 min, then centrifuged at 4500 rpm for 10 min. The supernatant was then collected for carotenoid assay following the method of Nagata and Yamashita,

(1992). After extraction, sample absorbances were read on a UV-Visible spectrophotometer (HELIOS EPSILON, THERMO Scientific) at wavelengths of 453, 505 and 663 nm. β-carotene and lycopene contents were determined using the following formulas:

$$\beta - Carotène = 0{,}216 \times A663 - 0{,}304 \times A505 + 0{,}452 \times A453$$

$$Lycopène = -0{,}0458 \times A663 + 0{,}372 \times A505 - 0{,}0806 \times A453$$

Determination of ascorbic acid content (Vitamin C)

Ascorbic acid extraction involved homogenizing 100 mg of accession flour for 1 min in 2 ml distilled water, followed by centrifugation at 2000 rpm for 5 min. The supernatant was used for ascorbic acid determination.

Ascorbic acid quantification was performed according to the method described by Mehta *et al.,* (2018) with minor modifications. To 50 µl of rice bean extracts (50 mg/ml) were added 150 µl of DCPIP (0.2 mM).

After 15 s incubation, absorbance was read on a spectrophotometer (Epoch; BioTeK) at 515 nm against a blank consisting of 150 µl DCPIP and 50 µl distilled water.

A calibration curve was established with ascorbic acid in the concentration range 10 µg/ml to 100 µg/ml. Each test was performed in triplicate.

Analysis of phenolic compound composition

Determination of total flavonoids

Determination of total flavonoid content was carried out using Dowd's aluminum trichloride (AlCl3) colorimetric method adapted by Arvouet-grand *et al.,* (1994). A 125 µl volume of each extract (1 mg/ml) is mixed with 125 µl of 2% (w/v) AlCl3.

The mixture was stirred and incubated in the dark at room temperature for 30 min. A blank was made by replacing the AlCl3 with 95% methanol and absorbance was measured at 415 nm with a spectrophotometer (Epoch; BioTeK). Quercetin (0 - 100 µg/L) was used as the standard in establishing the calibration curve. Results are expressed in mg quercetin equivalent/100 mg/MS dry plant matter, with reference to the quercetin calibration curve established under the same conditions.

Determination of total phenolic compounds

Total phenolics were determined using the Folin-Ciocalteu reagent method described by Singleton *et al.,* (1999) with some modifications by Dicko *et al.,* (2002). To this end, 100 µl of extract was mixed with 500 µl of FC reagent and 400 µl of 7.5% (w/v) sodium carbonate (Na2CO3). The mixture was stirred and incubated in the dark at room temperature for 2 h, and absorbance was measured at 760 nm by a spectrophotometer (Epoch; BioTeK). Phenolic content was determined against a gallic acid calibration curve established under the same conditions.

Determination of hydrolysable tannins

The determination of hydrolysable tannins was carried out according to the protocol of Mole and Waterman, (1987). A volume of 1 ml of extract (5 mg/ml) was added to 3.5 ml of a solution prepared from 0.01 M ferric trichloride (FeCl3) in 0.001 M hydrochloric acid (HCl). After 15 seconds of incubation, the absorbance of the mixture was read at 660 nm. Results were expressed as mg Gallic Acid Equivalent (GAE) per g dry extract (mg GAE/g).

Determination of condensed tannins

Quantification of condensed tannins was carried out according to the method of Broadhurst and Jones (1978) with minor modifications. To this end, 1 ml of extract (5 mg/ml) was added to 2 ml of a 1% vanillin solution (1 g vanillin in 100 ml 70% sulfuric acid). The whole mixture was placed in a water bath for 15 min at 20°C, protected from light. The absorbance of the mixture was read at 500 nm. Condensed tannin content was calculated and expressed as mg Cyanidine Equivalent (CE) per g dry extract (mg CE/g MS).

Assessment of antioxidant activity

Evaluation of antioxidant activity by the Ferric Reducing Antioxidant Power (FRAP) test

Evaluation of antioxidant activity by the iron reduction method was carried out as described by Hinneburg *et al.,* (2006). In a test tube containing 0.5 ml sample solution

(0.1 mg/ml), 1.25 ml phosphate buffer (0.2 M, pH 6.6) was added, followed by 1.25 ml potassium hexacyanoferrate [K_3 Fe $(CN)_6$] 1% in water. The mixture was heated to 50°C in a water bath for 30 minutes. Next, 1.25 ml trichloroacetic acid (10%) was added and the mixture centrifuged at 2000 rpm for 10 minutes.

After cooling, three 0.625 ml aliquots were made in 3 Eppendorf tubes, to which 0.625 ml distilled water was added, followed by 0.125 ml freshly prepared 1% FeCl3 in water. A blank without sample was prepared under the same conditions and read at 700 nm. A calibration curve was established with ascorbic acid. The concentration of reducing compounds (antioxidants) in the extract is expressed in mg Ascorbic Acid Equivalent (EAA) per 100 mg extract.

Evaluation of the Antioxidant Activity by the DPPH Reduction Test (DPPH inhibition)

Assessment of antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging was carried out as described by Sánchez-Rangel *et al.,* (2013). For each sample, three tests are performed by mixing 75 µl of crude extract (1 mg/ml) with 150 µl of DPPH (20 mg/l). The absorbance of the mixture was read after 15 minutes incubation at 517 nm on the spectrometer against a blank (75 µl sample and 150 µl ethanol). Antioxidant activity was expressed as percentage inhibition (% Inh).

Statistical analysis

The data collected was entered and the various concentrations were calculated using Excel 2016. Analyses consisted in determining means and standard deviations. Significant differences between samples were calculated using a one-way ANOVA, followed by the Newman-Keuls multiple comparison test at the 5% level ($p \leq 0.05$) using CoStat software (version 6.20.4). Linear correlations coupled with principal component analysis (PCA) were performed with mean parameter values to establish relationships between variables and similarities between individuals. Ward's aggregation method using Euclidean distance was used to group accessions by hierarchical ascending classification (HAC). Finally, a discriminant factor analysis (DFA) was performed to characterize the groups resulting from CAH. XLSTAT software (version 2021.1) was used for these analyses.

Results and Discussion

Analysis of the macromolecule composition of rice bean seed

Table 2 shows the macromolecule compositions of the seeds from each rice bean accession. Analysis of variance showed a significant difference ($p < 0.05$) between accessions for nutritional parameters.

Seed protein concentration ranged from 11.493 ± 3.711 mg/g (accession RB148) to 33.852 ± 1.628 mg/g (accession RB170). Total soluble sugar (TSS) content for seeds ranged from 4.207±1.004 mg/g (RB110 accession) to 8.48±0.7 mg/g (RB60 accession).

The average sugar value for all accessions was 6.159 mg/g. The coefficient of variation (CV) was 30% for protein and 16% for total soluble sugars.

Pigment and ascorbic acid composition (vitamin C)

Ricebean seeds showed totally variable levels of pigment microelements and ascorbic acid. The β-carotene, lycopene and ascorbic acid composition of the accessions analyzed is presented in Table 3. ANOVA revealed a significant difference only for ascorbic acid.

Seed β-carotene and lycopene contents ranged respectively from 13.541±1.270 mg/100g to 17.307±0.388 mg/100g (RB59) and from 9.016 ± 0.867 mg/100g to 11.366 ± 0.259 mg/100g. The RB59 accession had the highest levels of β-carotene and lycopene, while the RB127 accession had the lowest levels of these two elements.

Ascorbic acid levels ranged from 4.400±0.849 mg/100g (RB70) to 42.509±0.719 mg/100g (RB61), with an average of 18.918 mg/100g. The CV was 61% for ascorbic acid and 6% for β-carotene and lycopene.

Analysis of phenolic compound composition

Total polyphenol, flavonoid and tannin contents were quantified, and the results are presented in Table 4. Phenolic compound concentration showed significant differences for all phenolic compounds except polyphenols. Flavonoid contents ranged from 0.559±0.049 mg EAG/g DM (RB110) to 1.322±0.222 mg

EAG/g (RB60), with an average of 0.879 mg EAG/g DM.

For polyphenols, concentrations ranged from 1.140±0.164 (RB148) to 3.446±2.420 (RB28) mg EQ/g with an average of 1.901 mg EAG/g DM. The CV was 24% for flavonoids and 30% for polyphenols. Condensed tannins and hydrolysable tannins of rice bean accessions varied in the seeds. Condensed tannin content ranged from 0.398±0.028 mg EC/g DM (RB111) to 1.859±0.445 mg EC/g DM (RB110), with an average of 1.056 mg EC/g DM. Hydrolysable tannin content ranged from 0.257±0.006 mg EAG/g (RB10) to 0.465±0.043 mg EAG/g (RB148) with an average of 0.336 mg EAG/g. The CV was 37% for condensed tannins and 19% for hydrolysable tannins.

Assessment of antioxidant activity

The antioxidant activity of seeds assessed by FRAP and DPPH methods is presented in Table 5. The antioxidant activities of seeds assessed by DPPH and FRAP methods generally showed a significant variation in the different activities. ANOVA results indicated a statistically significant difference for all parameters.

The CV was 39% for DPPH radical scavenging capacity and 69% for FRAP iron reducing activity. The highest percentage of inhibition of DPPH radical scavenging capacity was observed in accession RB10 (35.214±9.633%), with an average of 14.158%.

According to the FRAP method, the highest FRAP iron-reducing activity values were recorded with accessions RB127 (27.018±2.771 mM EAA/g MS) and RB167 (26.882±3.745 mM EAA/g MS) with an average of 20.500 mM EAA/g MS

Relationships between biochemical characteristics of seeds

The similarity matrix (Table 6) revealed highly significant correlations at the 5% level between certain biochemical seed variables. A positive correlation exists between Lycopene and β-carotene. Condensed tannins are negatively correlated with vitamin C and positively correlated with polyphenols. FRAP negatively correlated with all pigments quantified (β-carotene, lycopene).

Structuring biochemical variability in seeds

Principal component analysis (PCA) identified only three principal components (F1, F2 and F3) that contributed significantly to 58.510% of the total variation in seed biochemical parameters. The eigenvalues, variability contribution rate and cumulative contribution rate are presented in Table 7.

The F1 factor was described as having the highest variance (25.183%), followed by F2 and F3, which accounted for 19.26% and 14.066% of the total biochemical variation respectively.

The first component (F1) explains 25.183% of this variability. It is defined by the variables of quantified pigments (β-carotene, lycopene), condensed tannins and FRAP ferric ion reducing activity.

The second factor (F2) accounts for 19.26% of total variability. This component is mainly influenced by ascorbic acid and DPPH radical scavenging capacity.

The third axis (F3) expresses 14.066% of total variability. This component is strongly correlated with flavonoid and polyphenol composition.

F1 can be defined as pigment accumulation, F2 as ascorbic acid composition and antioxidant activity (DPPH). F3 is characterized by phenolic compound composition.

Accession grouping

Hierarchical ascending classification (HAC) was used to assess the multivariate association between the biochemical characteristics of the seeds. Accessions were divided into four groups and are presented in dendrogram form in Figure 1. These four classes are made up of 3, 3, 11 and 7 accessions respectively.

Table 8 describes the accessions, which are divided into four groups, together with the mean values of their nutritional properties. Analysis of Fisher's F statistic values indicates that all parameters are the most discriminating, with relatively high F and R values^2 with the exception of proteins, polyphenols and DPPH.

Group I consist of 3 accessions (RB61, RB79, RB59) with high levels of B-Carotene and Lycopene, ascorbic

acid, flavonoids and medium levels of polyphenols and low levels of FRAP.

Group II comprises 3 accessions. These accessions showed the highest concentrations of hydrolyzable tannins and low values for flavonoids, polyphenols and condensed tannins (RB148, RB85, RB111).
Group III contains 11 accessions (RB110, RB14, RB101, RB127, RB144, RB50, RB27, RB42, RB70, RB10, RB170) characterized by high levels of protein, polyphenols, FRAP DPPH condensed tannins and low to medium levels of ascorbic acid.

Group IV contains 7 (RB28, RB167, RB80, RB52, RB5, RB58, RB60) accessions characterized by a high number of Total Sugars, flavonoids.

Characterization of biochemical groups

To assess the differences and similarities between the biochemical groups defined by the seeds, a discriminant factor analysis (DFA) was carried out. Figure 2 shows the distribution of accessions according to the first two discriminant factors, explaining 64.43% and 35.57% of the total variance respectively.

Group I accessions are positively correlated with the F2 axis and negatively with the F1 axis, characterized by high pigment and ascorbic acid values. Group II is positively correlated with the F1 and F2 axes, and includes accessions rich in hydrolyzable tannins. Group III is negatively correlated with the F2 axis and groups together accessions with high phenolic and antioxidant activity. Finally, group IV is negatively correlated with the F1 axis and negatively correlated with the F2 axis. This group is distinguished from the others by high values for total sugars, flavonoids, polyphenols and FRAP.

The biochemical study of rice bean seeds highlighted the diversity within this species in Burkina Faso. Examination of the macromolecule data revealed variability in protein content within the seeds. This variation in nutritional composition may be due to differences between accessions, nutritional analysis methods and growing conditions (Katoch, 2013). The values obtained are in agreement with those previously reported by Bhagyawant *et al.,* (2019), on ricebean (32.64 mg/g to 18.31 mg/g). This content is quite comparable to other commonly consumed and marketed legumes, such as common bean (19.91 mg/g), pigeon pea (20.27 mg/g), lentil (22.49 mg/g), mungbean (22.53 mg/g), cowpea (33 mg/g) and groundnut (20 to 30 mg/g) (Pattanayak *et al.,* 2019; Ddamulira and Santos, 2015; Toomer, 2018). Ricebean can make a significant contribution to human daily protein requirements due to their relatively high content. The presence of high protein will help to achieve Sustainable Development Goal 2 - zero hunger - by combating diseases linked to malnutrition. It can be used to produce protein-rich foods and food additives that could benefit infants in particular. In addition, it would be possible to set up an effective rice bean breeding program by selecting the best accessions, such as RB170 (33.85 mg/g), RB28 (32.99 mg/g), RB27 (30.32 mg/g).

Total soluble sugars generally play an essential role in carbohydrate metabolism. A higher concentration of TSS was found compared to that reported (5.0 to 5.6 mg/g) by Khabiruddin *et al.,* (2002); Katoch (2013) on ricebean. Nevertheless, the values observed in the experiment are similar to those reported by Weng *et al.,* (2018), ranging from 3.26 to 8.60 mg/g, with an average value of 5.45 mg/g in cowpea. The presence of high levels of TSS helps to cope with abiotic stress factors and improve seed preservation by increasing resistance to desiccation. TSS contribute to the pleasant taste and availability of fermentable sugars. This characteristic can be explored in selection and genetic improvement programs for rice bean accessions, all of which will meet market and consumer needs.

Quantification of pigment composition (β-carotene, lycopene) revealed that the seeds possess a non-negligible proportion of β-carotene and lycopene. Indeed, the β-carotene contents found are similar to those reported in previous studies by Dhillon and Tanwar (2018). β-carotene has the advantage of acting as a precursor to vitamin A, which is a powerful antioxidant. According to Grassi *et al.,* (2013), the positive correlation observed between β-carotene levels and lycopene is due to the bioaccumulation process of lycopene. For these authors, the synthesis of an enzyme, lycopene β-cyclase, is associated with this process, transforming lycopene into β-carotene. Furthermore, carotenoids (lycopene and β-carotene) are cited as powerful antioxidants that have a protective effect against oxidative stress and are beneficial to human health (Edwards *et al.,* 2003; Veljović *et al.,* 2012).

Table.1 Characteristics of the ricebean accessions studied.

N°	Accessions	Origin	N°	Accessions	Origin
1	RB10	NBPGR-India	13	RB42	NBPGR-India
2	RB101	NBPGR-India	14	RB5	NBPGR-India
3	RB110	NBPGR-India	15	RB50	NBPGR-India
4	RB111	NBPGR-India	16	RB52	NBPGR-India
5	RB127	NBPGR-India	17	RB58	NBPGR-India
6	RB14	NBPGR-India	18	RB59	NBPGR-India
7	RB144	NBPGR-India	19	RB60	NBPGR-India
8	RB148	NBPGR-India	20	RB61	NBPGR-India
9	RB167	NBPGR-India	21	RB70	NBPGR-India
10	RB170	NBPGR-India	22	RB79	NBPGR-India
11	RB27	NBPGR-India	23	RB80	NBPGR-India
12	RB28	NBPGR-India	24	RB85	NBPGR-India

Figure.1 Dendrogram showing grouping scheme for rice bean accessions based on biochemical parameters

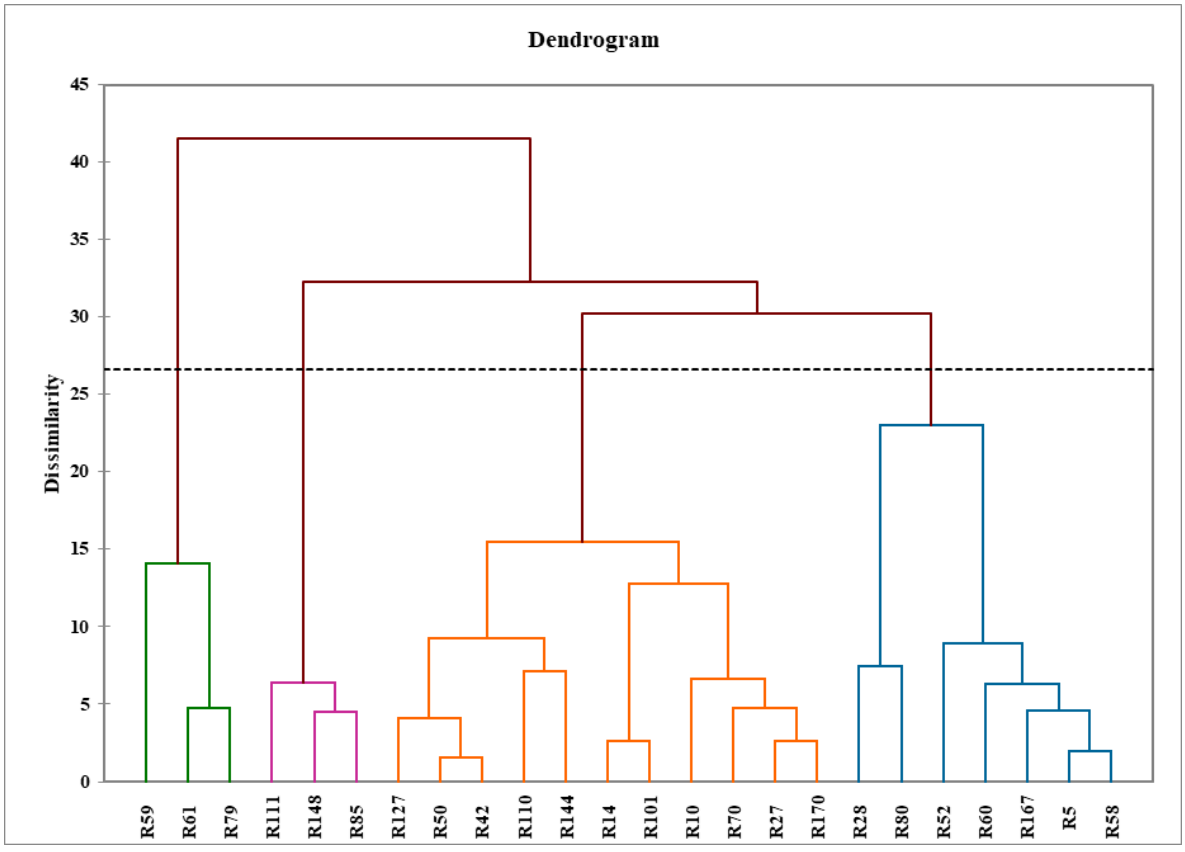


Table.2 Macromolecule compositions of ricebean accession seed

Accessions	Protein (mg/g)				Total soluble sugars (mg/g)			
RB10	29.495	±	0.714	a	5.865	±	0.49	abc
RB101	17.491	±	2.433	a	7.366	±	1.11	abc
RB110	26.157	±	15.384	a	4.207	±	1.004	abc
RB111	19.709	±	0.412	a	4.507	±	1.224	c
RB127	14.881	±	0.169	a	5.717	±	1.081	abc
RB14	14.658	±	12.443	a	6.402	±	0.887	abc
RB144	21.332	±	9.691	a	4.937	±	0.285	abc
RB148	11.493	±	3.711	a	6.473	±	0.752	abc
RB167	23.583	±	6.096	a	6.785	±	0.834	abc
RB170	33.852	±	1.628	a	5.97	±	1.137	abc
RB27	30.316	±	0.446	a	5.872	±	1.608	abc
RB28	32.989	±	1.026	a	5.807	±	0.78	abc
RB42	25.325	±	8.509	a	6.14	±	0.651	abc
RB5	17.340	±	13.776	a	6.24	±	0.06	abc
RB50	19.314	±	10.244	a	5.165	±	2.242	bc
RB52	16.486	±	10.993	a	7.961	±	0.359	abc
RB58	19.107	±	6.141	a	7.629	±	1.053	ab
RB59	13.063	±	8.597	a	5.872	±	0.657	abc
RB60	26.959	±	2.909	a	8.48	±	0.7	a
RB61	22.617	±	14.555	a	5.318	±	0.534	abc
RB70	18.521	±	10.422	a	6.566	±	0.791	abc
RB79	28.602	±	7.397	a	5.819	±	0.249	abc
RB80	22.807	±	9.913	a	6.533	±	0.524	abc
RB85	13.063	±	4.930	a	6.189	±	0.898	abc
Minimum	**11.493**				**4.207**			
Maximum	**33.852**				**8.48**			
Average	**21.632**				**6.159**			
CV (%)	**30**				**16**			
R^2	**0.513**				**0.495**			
Pr > F	**0.011***				**0.018***			

*Means followed by the same letter in each group are not significantly different at the 5% level; ns = not significant (P>0.05); * : significant difference at 5% ;** : significant difference at 1%, F: Fisher's F, R^2 : coefficient of determination, ns = not significant (P>0.05); * : significant difference at 5%, ** : significant difference at 1%.*

Table.3 β-carotene, lycopene and ascorbic acid composition of ricebean accessions

Accessions	β-carotene (mg/100g)				Lycopene (mg/100g)				Ascorbic acid (mg/100g)			
RB10	15.765	±	2.039	ab	10.41	±	1.345	a	16.096	±	3.517	bc
RB101	15.958	±	1.089	ab	10.498	±	0.585	a	7.561	±	0.62	bc
RB110	15.163	±	0.655	ab	9.959	±	0.449	a	14.5	±	0.707	bc
RB111	15.469	±	1.024	ab	10.373	±	0.696	a	11.722	±	0.394	bc
RB127	13.541	±	1.27	ab	9.016	±	0.867	a	6.563	±	0.618	c
RB14	16.056	±	0.791	ab	10.612	±	0.483	a	8.895	±	7.596	bc
RB144	15.117	±	0.293	ab	9.973	±	0.174	a	4.907	±	2.538	c
RB148	14.421	±	0.375	ab	9.548	±	0.239	a	24.867	±	1.227	abc
RB167	13.94	±	1.81	ab	9.072	±	1.163	a	8.892	±	0.152	bc
RB170	8.624	±	10.82	b	10.929	±	1.992	a	13.719	±	0.398	bc
RB27	15.391	±	0.501	ab	10.132	±	0.338	a	16.547	±	0.773	bc
RB28	14.323	±	0.446	ab	9.273	±	0.271	a	14.767	±	7.837	bc
RB42	14.231	±	0.4	ab	9.373	±	0.242	a	13.991	±	8.745	bc
RB5	14.818	±	0.196	ab	9.7	±	0.133	a	26.534	±	0.66	abc
RB50	14.644	±	0.233	ab	9.606	±	0.192	a	16.65	±	10.517	bc
RB52	13.94	±	1.601	ab	9.067	±	1.055	a	38.5	±	2.121	a
RB58	14.586	±	1.357	ab	9.547	±	0.917	a	26.4	±	6.11	abc
RB59	17.307	±	0.388	a	11.366	±	0.259	a	14.219	±	1.105	bc
RB60	15.475	±	0.57	ab	10.159	±	0.339	a	14.268	±	17.156	bc
RB61	15.613	±	1.453	ab	10.249	±	0.958	a	42.509	±	0.719	a
RB70	15.497	±	1.428	ab	10.252	±	0.974	a	4.4	±	0.849	c
RB79	16.731	±	0.579	a	11.033	±	0.408	a	39.681	±	0.451	a
RB80	15.393	±	1.32	ab	10.051	±	0.867	a	29	±	1.414	ab
RB85	15.962	±	1.348	ab	10.533	±	0.894	a	38.85	±	1.627	a
Minimum	**13.541**				**9.016**				**4.4**			
Maximum	**17.307**				**11.366**				**42.509**			
Average	**15.175**				**10.031**				**18.918**			
CV	**6**				**6**				**61**			
R^2	**0.311**				**0.275**				**0.515**			
Pr > F	**0.551 ns**				**0.726 ns**				**0.01***			

*Means followed by the same letter in each group are not significantly different at the 5% level; ns = not significant (P>0.05); * : significant difference at 5% ;** : significant difference at 1%, F: Fisher's F, R^2 : coefficient of determination, ns = not significant (P>0.05); * : significant difference at 5%, ** : significant difference at 1%.*

Table.4 Total polyphenol, flavonoid and tannin content of ricebean

Accessions	Flavonoids (mg EQ/g)				Polyphenols (mg EAG/g)				Condensed tannins (mg EC/g MS)				Hydrolysable tannins (mg EAG/g)			
RB10	0.774	±	0.041	cd	1.314	±	0.515	a	0.894	±	0.254	abc	0.257	±	0.006	c
RB101	0.825	±	0.103	cd	2.478	±	0.438	a	1.477	±	0.277	abc	0.338	±	0.044	abc
RB110	0.559	±	0.049	d	2.364	±	1.46	a	1.859	±	0.445	a	0.29	±	0.018	abc
RB111	0.683	±	0.172	cd	1.234	±	0.37	a	0.398	±	0.028	c	0.459	±	0.135	ab
RB127	0.681	±	0.08	cd	1.532	±	0.138	a	1.386	±	0.781	abc	0.316	±	0.064	abc
RB14	0.973	±	0.118	bc	1.745	±	0.791	a	1.755	±	0.388	ab	0.299	±	0.018	abc
RB144	0.802	±	0.073	cd	1.545	±	0.429	a	1.305	±	0.68	abc	0.278	±	0.005	bc
RB148	0.695	±	0.04	cd	1.14	±	0.164	a	0.751	±	0.11	abc	0.465	±	0.043	a
RB167	1.167	±	0.052	ab	2.128	±	0.364	a	1.126	±	0.455	abc	0.294	±	0.024	abc
RB170	0.751	±	0.103	cd	1.383	±	0.98	a	0.819	±	0.126	abc	0.281	±	0.011	bc
RB27	1.003	±	0.01	bc	1.615	±	0.928	a	1.227	±	0.593	abc	0.312	±	0.006	abc
RB28	0.999	±	0.168	bc	3.436	±	2.42	a	1.737	±	0.121	ab	0.41	±	0.109	abc
RB42	0.727	±	0.056	cd	2.018	±	0.552	a	1.097	±	0.098	abc	0.295	±	0.011	abc
RB5	1.172	±	0.08	ab	2.069	±	0.283	a	1.024	±	0.121	abc	0.353	±	0.075	abc
RB50	0.782	±	0.082	cd	1.916	±	0.359	a	1.248	±	1.084	abc	0.346	±	0.064	abc
RB52	0.756	±	0.11	cd	2.11	±	0.294	a	1.056	±	0.437	abc	0.273	±	0.024	bc
RB58	1.026	±	0.157	bc	1.568	±	0.219	a	0.749	±	0.143	abc	0.364	±	0.068	abc
RB59	1.222	±	0.123	ab	1.742	±	0.035	a	0.653	±	0.153	abc	0.278	±	0.011	bc
RB60	1.322	±	0.222	a	1.803	±	0.339	a	0.699	±	0.005	abc	0.353	±	0.05	abc
RB61	1.187	±	0.297	ab	2.054	±	0.134	a	0.767	±	0.083	abc	0.355	±	0.023	abc
RB70	0.691	±	0.073	cd	1.321	±	0.455	a	0.975	±	0.505	abc	0.28	±	0.02	bc
RB79	0.775	±	0.019	cd	2.192	±	0.249	a	0.705	±	0.171	abc	0.292	±	0.038	abc
RB80	0.821	±	0.07	cd	3.075	±	1.352	a	1.097	±	0.504	abc	0.43	±	0.163	abc
RB85	0.709	±	0.156	cd	1.849	±	0.315	a	0.549	±	0.005	bc	0.445	±	0.007	ab
Minimum	**0.559**				**1.14**				**0.398**				**0.257**			
Maximum	**1.322**				**3.436**				**1.859**				**0.465**			
Average	**0.879**				**1.901**				**1.056**				**0.336**			
CV	**24**				**30**				**37**				**19**			
R^2	**0.616**				**0.402**				**0.498**				**0.563**			
Pr > F	**< 0,0001****				**0.16 ns**				**0.017***				**0.002***			

*Means followed by the same letter in each group are not significantly different at the 5% level; ns = not significant (P>0.05); * : significant difference at 5% ;** : significant difference at 1%, F: Fisher's F, R^2 : coefficient of determination, ns = not significant (P>0.05); * : significant difference at 5%, ** : significant difference at 1%.*

Table.5 Antioxidant activity of seeds assessed by FRAP and DPPH methods

Accessions	FRAP (mM EAA/g MS)				DPPH (%)			
RB10	23.179	±	2.691	a	35.214	±	9.633	a
RB101	24.605	±	2.565	a	10.724	±	0.390	cd
RB110	22.983	±	1.365	a	18.480	±	3.180	bcd
RB111	23.738	±	0.398	a	13.609	±	3.131	cd
RB127	27.018	±	2.771	a	29.089	±	8.422	abc
RB14	22.312	±	1.732	b	19.841	±	4.556	bcd
RB144	3.753	±	1.322	a	9.323	±	6.504	d
RB148	23.934	±	1.472	a	2.042	±	0.059	d
RB167	26.882	±	3.745	a	4.222	±	1.251	d
RB170	22.863	±	0.860	a	7.126	±	2.185	d
RB27	22.373	±	1.889	a	8.900	±	3.511	d
RB28	25.510	±	1.811	a	35.102	±	17.307	a
RB42	24.190	±	0.627	a	15.721	±	3.116	bcd
RB5	22.282	±	3.562	a	11.073	±	6.943	cd
RB50	24.190	±	2.072	a	17.719	±	12.474	bcd
RB52	25.095	±	1.921	a	3.753	±	0.140	d
RB58	23.383	±	1.249	b	9.563	±	6.504	cd
RB59	3.285	±	0.490	a	31.936	±	5.088	ab
RB60	22.637	±	0.804	b	14.284	±	2.077	cd
RB61	3.572	±	0.223	a	3.354	±	1.592	d
RB70	24.824	±	2.991	b	6.714	±	0.404	d
RB79	3.330	±	0.474	a	9.348	±	0.922	cd
RB80	23.157	±	2.227	a	16.721	±	10.315	bcd
RB85	22.916	±	1.211	a	5.932	±	1.708	d
Minimum	**3.285**				**2.042**			
Maximum	**27.018**				**35.214**			
Average	**20.500**				**14.158**			
CV	**39**				**70**			
R^2	**0.710**				**0.572**			
Pr > F	**< 0,0001****				**< 0,0001****			

*Means followed by the same letter in each group are not significantly different at the 5% level; ns = not significant (P>0.05); * : significant difference at 5% ;** : significant difference at 1%, F: Fisher's F, R^2 : coefficient of determination, ns = not significant (P>0.05); * : significant difference at 5%, ** : significant difference at 1%.*

Table.6 Correlation matrix for biochemical parameters of the ricebean collection

Variables	Prot	SST	β-caro	Lyco	A. ascor	Flavo	Poly	T. cond	T. hydro	FRAP	DPPH
Prot	1										
SST	-0.15	1									
β-caro	-0.048	-0.121	1								
Lyco	0.071	-0.17	**0.924**	1							
A. ascor	-0.075	0.14	0.129	0.054	1						
Flavo	0.063	0.39	0.18	0.068	0.076	1					
Poly	0.279	0.066	-0.051	-0.2	0.213	0.167	1				
T cond	0.144	-0.169	-0.278	-0.339	**-0.427**	-0.144	**0.480**	1			
T hydro	-0.258	-0.015	-0.08	-0.128	0.301	-0.042	0.16	-0.285	1		
FRAP	-0.004	0.306	**-0.596**	**-0.532**	-0.288	-0.271	0.041	0.263	0.234	1	
DPPH	0.168	-0.249	0.125	0.059	-0.365	0.039	0.227	0.332	-0.142	0.045	1

*ns = not significant (P>0.05); * : significant difference at 5%, ** : significant difference at 1%, CV : coefficient of variation, R^2 (%) : coefficient of determination, Proteins : Prot, Total soluble sugars : SST, B-Carotene : β-caro, Lycopene : Lyco, Ascorbic acid : A. ascor, Flavonoids: Flavo, Polyphenols: Poly, Condensed tannins: T cond, Hydrolysable tannins: T hydro, FRAP: FRAP, DPPH: DPPH.*

Table.7 Eigen values and percentage of variation expressed for the first three axes based on biochemical parameters in principal component analysis

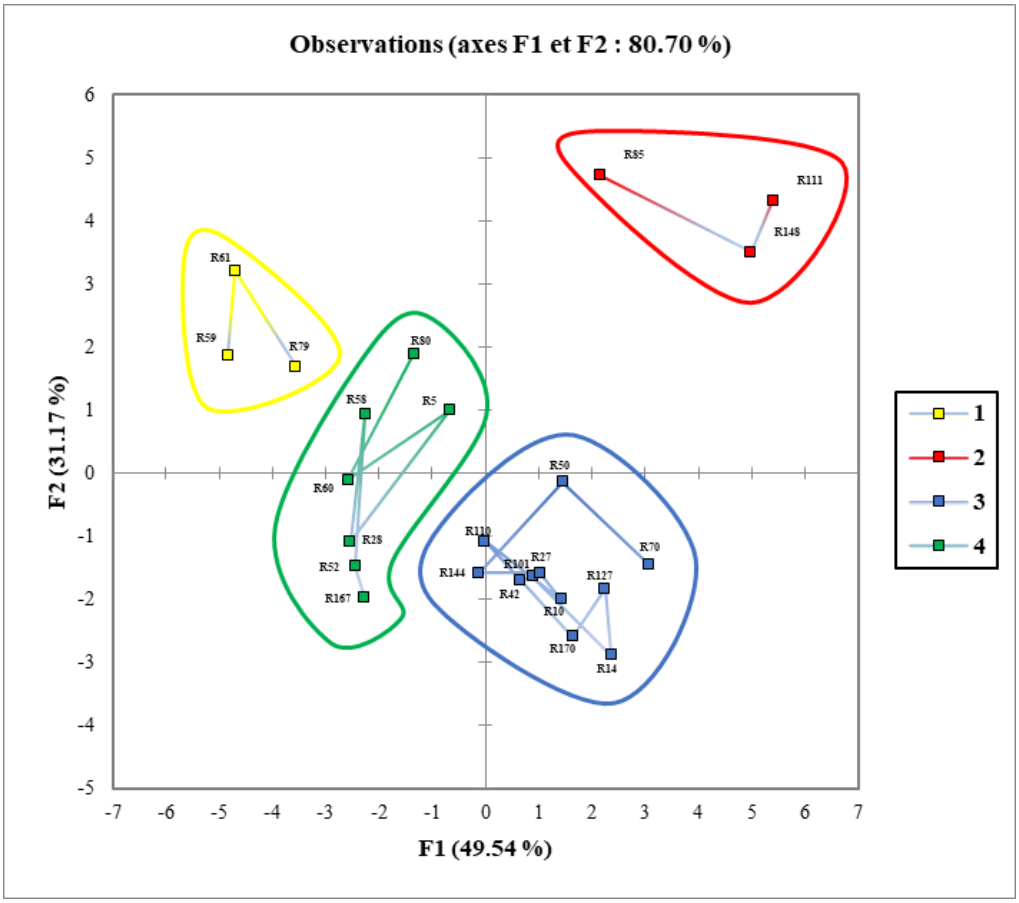
Variables	F1	F2	F3
Eigenvalue	2.770	2.119	1.547
Variability (%)	25.183	19.260	14.066
cumulative	25.183	44.443	58.510
Protein	0.007	0.213	0.114
Total soluble sugars	0.014	0.258	0.166
β-carotene	**0.754**	0.077	0.005
Lycopene	**0.742**	0.085	0.007
Ascorbic acid	0.124	**0.301**	0.146
Flavonoids	0.061	0.006	**0.445**
Polyphenols	0.078	0.041	**0.607**
Condensed tannins	**0.370**	0.332	0.023
Hydrolysable tannins	0.003	0.298	0.004
FRAP	**0.600**	0.045	0.020
DPPH	0.018	**0.461**	0.011

Table.8 Average performance of biochemical parameters for the ricebean collection

Class	1	2	3	4	R^2	Pr > F
Workforce	**3**	**3**	**7**	**11**		
Protein	21.427 a	14.755 a	22.849 a	22.753 a	0.173	0.273 ns
Total soluble sugars	5.670 a	5.723 a	5.837 a	7.062 a	0.345	0.034*
B-Carotene	16.550 a	15.284 b	15.112 b	14.639 b	0.416	0.012*
Lycopene	10.883 a	10.152 ab	10,069 ab	9.553 b	0.406	0.014*
Ascorbic acid	32.136 a	25.146 ab	11.257 b	22,623 ab	0.444	0.007*
Flavonoids	1.061 a	0.696 b	0.779 b	1.038 a	0.480	0.004*
Polyphenols	1.996 a	1.408 a	1.748 a	2.313 a	0.313	0.053 ns
Condensed tannins	0.708 b	0.566 b	1.277 a	1,070 ab	0.465	0.005*
Hydrolysable tannins	0.308 b	0.456 a	0.299 b	0.354 b	0.677	< 0.0001**
FRAP	3.396 b	23.529 a	22.026 a	24.135 a	0.717	< 0.0001**
DPPH	14.879 a	7.194 a	16.259 a	13.531 a	0.088	0.596 ns

*Means followed by the same letter in each group are not significantly different at the 5% level; ns = not significant (P>0.05); * : significant difference at 5% ;** : significant difference at 1%, F: Fisher's F, R^2 : coefficient of determination, ns = not significant (P>0.05); * : significant difference at 5%, ** : significant difference at 1%.*

Figure.2 Position of ricebean biochemical groups from CAH



Accessions that have shown a high capacity for β-carotene synthesis, could be exploited as a source of antioxidants to prevent chronic diseases and age-related macular degeneration (Fanciullino and Gautier, 2014). Legume seeds contain tannins, phenolic acids, anthocyanins and flavonoids, among others. The study found different levels of phenolic compounds (flavonoids, polyphenols, tannins) in the seeds. Variations in phenolic content in rice bean were previously reported by Katoch (2013).

This researcher cites reasons for genotypic and environmental differences, such as location, exposure to ultraviolet B, diseases and pests.

Seeds from accession RB28 (3.43 mg EAG/g) showed high levels of polyphenols. A comparable concentration of phenolic compounds was reported by Katoch (2013) with seed levels of 2.16 and 3.40 mg EAG/g DM. Variations in total flavonoid content in the seeds were also close (0.20 ± 0.01 mg EQ/g and 0.77 ± 0.16 mg EQ/g) to that observed by Bhagyawant *et al.,* (2019) on rice bean seed extracts. Bouaziz *et al.,* (2005) assert that its compounds are secondary plant metabolites and act as natural antioxidants.

Epidemiological studies by Bakoyiannis *et al.,* (2019) and Ginwala *et al.,* (2019) report that consumption of foods rich in phenolic antioxidants protects against chronic non-communicable human diseases such as cancer, aging, diabetes and cardiovascular disease.

Tannins have the property of precipitating proteins and establishing more complex bonds with starch, cellulose and minerals, thus affecting their digestibility. Condensed seed tannins ranged from 0.28% to 0.58%, while hydrolysable seed tannins were 1.70% to 2.99%. Earlier studies by Katoch (2013) and Awasthi *et al.,* (2011) also reported similar tannin contents in rice bean seeds. However, even though tannins are known as "anti-nutritional" factors; some studies have shown that with their ability to bind to proteins, tannins can be used to remove certain toxins from the gut. In addition, tannins can inhibit the growth of bacteria responsible for tooth decay, making them useful for oral health. Tannins have a positive effect on protection against parasitic invasion, as well as reducing microbial activity. Accessions with a high phenolic compound content (RB111, RB167, RB10, RB42) could be a preferred source of phenolic compounds for people suffering from toothache and cardiovascular disease, thus helping to achieve Sustainable Development Goals (SDGs) 3, namely good health and well-being.

Seed antioxidant activities assessed by DPPH and FRAP methods generally showed significant variation in the different activities. The DPPH antioxidant activities of seeds are different from those reported by Bhagyawant *et al.,* (2019) on rice bean accessions. These authors measured DPPH activity ranging from 9.36% to 18.57%. The difference in antioxidant activity potential was explained by the presence of condensed tannins and phytic acid. In addition, environmental conditions such as soil type, harvest time, growth temperature and post-harvest management affect antioxidant capacity and phenolic content of plants (Alirezalu *et al.,* 2018; Gholizadeh-Moghadam *et al.,* 2019). Ricebean accessions rich in antioxidant activity could prove beneficial in the treatment of various cancer diseases, diabetes or all respiratory diseases (Cai *et al.,* 2004). PCA and CAH are statistical methods widely used to simplify the complexity of high-dimensional data. CAH classified rice bean accessions into four groups. Analysis of the mean values in different groups showed that none contained all the necessary traits that could be selected and used in the breeding program. Accessions in groups III and IV contained high levels of protein, sugars, phenolic and antioxidant activity. Accessions from these groups could be used as a source for designing protein-rich foods, suitable for use as protein supplements, and useful for combating protein-energy malnutrition in developing countries. Group I accessions contain high levels of pigments and ascorbic acid, while group II contains higher levels of hydrolysable tannins. Accessions from both groups would be suitable for people suffering from cardiovascular disease and diabetes. The results will benefit breeders in identifying potential parental lines for developing rice bean varieties. Thanks to the various hybridizations, it will be possible to obtain varieties with improved nutritional value, which will be beneficial for the treatment of human and animal diseases, while combating malnutrition.

The results of the present study on rice beans revealed good biochemical properties, with great variability between accessions in macromolecule composition, pigments, ascorbic acid, analysis of phenolic compound composition and assessment of antioxidant activity. Of the 24 accessions, accessions RB27, RB10 revealed appreciable levels of macromolecules (proteins and sugars), pigments and vitamin C. Accessions RB59,

RB60 showed appreciable levels of phenolic compounds and flavonoids, but low levels of tannins.

Accession RB127 showed high values for FRAP and DPPH. Accession RB79 stood out from the others, presenting a very advantageous average biochemical composition of the parameters analyzed.

Interesting correlation coefficients between parameters were observed, especially between lycopene and B-carotene. Quantified pigments (B-carotene, lycopene), condensed tannins, FRAP ferric ion reducing activity, ascorbic acid, DPPH radical scavenging capacity, flavonoids and polyphenols contributed more to the variability observed in the accessions, as revealed by the three PCA components. CAH revealed that many nutrient-rich accessions have more than one trait with high nutritional value, which can be found in all four distinct groups. These accessions can be used as direct parents in rice bean breeding programs to produce rice bean varieties that meet specific nutritional and human health needs.

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Author Contributions

Coulibaly Zinmanké: Conceived the original idea and designed the model and wrote the manuscript; Barro Antoine: Supervised the work and participated in the analysis and correction of the manuscript; Dabiré Amana Mètuor: Contributed to the drafting of the manuscript; Sama Hemayoro: Contributed to biochemical analyses; Nanama Tuwendsida Joseph: Contributed to the drafting of the manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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