

Unlocking Nutritional Potential: First Profile of Ricebean (*Vigna umbellata*) Grown as Microgreens

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The Philippines is home to diverse plant species, yet many remain underexplored and underutilized. One of these is ricebean (*Vigna umbellata*), locally known as tapilan. It is commonly used as an animal feed, but it holds huge nutritional potential as its seeds are protein rich. Therefore, this study explored the potential of ricebean as microgreens, an emerging commodity in the Philippines. Microgreens is a commodity grown and harvested in just a short amount of time, typically ranging from 7-21 days. Most species are classified as microgreens with the presence of the first two true leaves. In this study, ricebean seeds were sorted based on their seed coat color, namely, yellow-green (RBYG), light greyed-orange (RBAO), and dark greyed-orange (RBRB). These morphotypes were grown in a two-layered microgreen tray with moist sterilized coco coir, maintained under ambient temperature ($27\pm 2^{\circ}\text{C}$), and subjected to white and red-blue light-emitting diodes (LED) lights. Following the extended BBCH scale for dicotyledon plants, harvesting was done prior to the first trifoliate leaf formation 5 days after sowing at BBCH 13. Among the three seed morphotypes, RBYG and RBAO exhibited an average of 98% germination with vigorous seedlings. Proximate analysis revealed that RBRB ($1.2100\pm 0.01\text{ g }100\text{g}^{-1}$) has the highest ash content followed by RBYG ($1.0933\pm 0.01\text{ g }100\text{g}^{-1}$) and RBAO ($1.0233\pm 0.03\text{ g }100\text{g}^{-1}$). In addition, RBYG ($0.8933\pm 0.09\text{ g }100\text{g}^{-1}$) and RBRB ($0.7900\pm 0.08\text{ g }100\text{g}^{-1}$) crude fat content is significantly higher than RBAO ($0.5667\pm 0.03\text{ g }100\text{g}^{-1}$). Overall, 100g of fresh ricebean microgreens contain $6.44\pm 0.60\text{ g}$ crude protein, $4.65\pm 0.59\text{ g}$ total carbohydrate, $0.76\pm 0.22\text{ g}$ crude fat, $0.99\pm 0.12\text{ g ash}^{-1}$, and $87.5\pm 0.50\%$ moisture content which are comparable to mungbean microgreens. Further validation with additional ricebean seedlots is needed to refine the findings of this exploratory study and establish guidelines for microgreens production at the household level.

Keywords: BBCH scale, microgreens, proximate composition, superfoods, *Vigna umbellata*

INTRODUCTION

With the increasing awareness of food and nutrition security, people have employed innovative production techniques which are suitable for a wide range of growing environments. Coming from a pandemic, we have experienced food shortage and insecurity, thus, hydroponic systems and soilless production systems using natural or artificial lighting are promising options to have continuous supply of clean and fresh vegetables at a household level. However, despite the technological innovations in growing crops, increasing the individual vegetable consumption rate remains a challenge especially in children and young adults (WHO 2018). In this context, as normal practice in delectable cuisines, adding garnishes like microgreens to make the dish more attractive, flavorful, and aromatic may encourage higher household consumption of nutrient-rich leafy vegetables.

The functional benefits of microgreens have drawn attention from nutrition and health institutions and have opened opportunities for new interest and research programs. Microgreens are the seedlings of edible plants harvested 7–21 days after germination or when the first true leaves start to emerge (Lee et al. 2004; Kou et al. 2013; Xiao et al. 2014a; Xiao et al. 2014b). Depending on the variety, microgreens contain considerably high levels of carotenoids, chlorophylls,

organic acids (Wojdylo et al. 2020) vitamin C, vitamin E, phenolic compounds, anthocyanins (Renna and Paradiso 2020) compared with its advanced growth and developmental stage. These phytochemicals possess antioxidant activity and play important physiological roles in the human body. The flavors, textures, colors, and scents may also vary depending on the growing condition like light intensity, temperature and growing medium (Koukounaras 2007; Xiao et al. 2012; Kou et al. 2014; Pinto et al. 2015; Bantis 2021).

Microgreens are convenient and cost-effective to grow in any location. Ideally, microgreens are suited for indoor production and are part of the global movement towards controlled environmental agriculture (CEA) (Riggio et al. 2019). CEA was developed as a response to food insecurity, reduction in arable lands, and increasing world population. Paraschivu et al. (2021) noted that in the era of the COVID-19 pandemic, farmers must adjust food production to the new socio-economic conditions and highlighted microgreens as a potential profitable business.

Ricebean [*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi], locally known as tapilan, is an underutilized indigenous crop with promising benefits. Bepary et al. (2017) found out that the primary nutrients present in

the ricebean seed varieties ranged from 54.21–60.49% carbohydrates, 15.64–21.60% protein, 1.22–2.3% fat, 5.53–6.56% crude fiber, and 3.34–3.8% ash. In the Philippines, farmers utilize ricebean as a green manure, reduce weeds, and as boundary, barrier or support crop (Centre for Agriculture and Bioscience International 2019). The seeds are mainly used as animal feed but are also utilized as food in the form of vegetables, pulse, and flour (Ju 2004).

Unfortunately, despite the gaining popularity of microgreens and potential health benefits of ricebean, microgreen farming in the Philippines is still at infancy. In addition, despite the wide diversity of functional indigenous food plants, most of the commercial microgreens in the country are imported high-value seed crops. This study explored the potential of ricebean as microgreens by characterizing its seeds, documenting its ontogenetic development, identifying optimal cultivation methods, and unlocking its nutritional value through proximate composition profiling.

MATERIALS AND METHODS

Plant Material

A total of 25 kg ricebean seed samples were obtained from a local market in Sudipen, La Union, Philippines in September 2021. The seeds were packed in transparent plastic cellophane bag and were kept under ambient room condition. The seller assured that the samples are locally produced, of good quality, could be readily cooked as vegetable, and could be used as planting material for seed production or other purposes.

Seed Morphometric Characterization

The seeds were subjected to morphological characterization involving the assessment of attributes such as seed coat color, presence of seed coat pattern, shape, luster, hilum appearance, as well as measurements encompassing length (L), width (W), thickness (T), and 100-seed weight, following established standard procedures as outlined by Alercia (2011). Differences in seed morphology were the basis of separating the treatments for this study. Additionally, calculations were performed to determine the seed perimeter (P) using Equation 1 and the seed area (A) utilizing Equation 2. The assessment of seed shape was conducted by considering the eccentricity index (EI) as per the methodologies described by Balkaya and Odabas (2002), and Iwata et al. (2010). Furthermore, the flatness index (FI) was determined employing Equation 4, drawing from the work of Cerda and Fayos (2002), and Cervantes et al. (2016). Lastly, the circularity index (CI) was calculated using Equation 5, with reference to the studies conducted by Rovner and Gyulai (2007) and Cervantes et al. (2016).

$$P = 2L + 2W \text{ (Eq. 1)}$$

$$A = L \times W \text{ (Eq. 2)}$$

$$EI = \frac{L}{W} \text{ (Eq. 3)}$$

$$FI = \frac{L + W}{2H} \text{ (Eq. 4)}$$

$$CI = \frac{4\pi \times \text{area}}{\text{perimeter}^2} \text{ (Eq. 5)}$$

Microgreen Establishment and Cultivation

The setup of was established at the researcher's household at Brgy. Evangelista Tayug, Pangasinan. The experiment lasted from October 2021 to January 2022. A germination test was conducted to determine the germination rate and seedling vigor. A total of 100 seeds per seed lot were planted on a growing tray with moistened paper towel. The set-up was maintained under ambient room conditions with an average air temperature of $27 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5\%$. The number of germinated seeds was counted at 3 days after sowing, when the length of the radicle and plumule is twice the length of the seed (BBCH 08). The percent germination of each morphotype was calculated.

The recommended number of seeds and seed weight were determined using Equation 6 and Equation 7. In Equation 6, the seed area, initially measured in mm^2 , was converted to cm^2 to ensure consistent units for the calculations. Meanwhile, in Equation 7, the average seed weight was derived from the 100-seed weight by dividing the total weight (in grams) by 100 to estimate the weight of a single seed.

$$\text{number of seeds} = \frac{\text{tray area}}{\text{seed area}} \text{ (Eq. 6)}$$

$$\text{weight of recommended number of seeds} = \text{number of seeds} \times \text{average seed weight} \text{ (Eq. 7)}$$

A total of 70 g seeds per seed morphotype were planted onto food grade polypropylene microgreen trays with drainage holes measuring 17 x 17 cm containing sterilized soilless potting mix (coco peat, pumice rocks, carbonized rice hull, vermicast and lime). Each tray was placed on top of a drip tray filled with tap water. The set-up was maintained indoors with an average air temperature of $27 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5\%$ throughout the growing period. At 5 days after sowing, the set-up was exposed to white and red-blue (400-840 nm) light-emitting diodes (LED) lights.

Ontogenetic Development Using BBCH Scale

Cultivation methods must also be tied to phenological observations to establish management practices and proper time of harvest. In line with this, the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale was established to provide a uniform standard to describe phenological events and document plant phenological phenomena (Zhao et al. 2019). The ontogenetic development of ricebean from intact seed to full expansion of primary leaves was recorded following the BBCH scale (Federal Biological Research Centre for Agriculture and Forestry 2001; Biodiversity International 2007). The primary and secondary growth stages were described based on the actual observation.

Proximate Analysis of Freshly Harvested Ricebean Microgreens

The proximate composition of freshly harvested microgreens including the moisture content, ash (minerals), crude fat, crude protein, total carbohydrates, and were assessed following the procedure published by the Association of Official Analytical Chemists (AOAC 2016).

Table 1. Seed length, width, thickness, perimeter, and area of three (3) ricebean morphotypes.

Morphotype	Length (mm)	Width (mm)	Thickness (mm)	Perimeter (mm)	Area (mm ²)
RBYG	7.31 ±0.24	4.35 ±0.22	3.14 ±0.16 ^b	23.30 ±0.84	31.92 ±2.41
RBAO	7.49 ±0.25	4.49 ±0.12	3.46 ±0.11 ^a	23.98 ±0.60	33.71 ±1.58
RBRB	7.70 ±0.31	4.46 ±0.12	3.48 ±0.14 ^a	24.32 ±0.83	34.50 ±2.29

Value = Mean ± SD (n = 20). The values with the same superscript in a column are not significantly different at $p \leq 0.05$. Abbreviations: RBYG=yellow green; RBAO= light greyed-orange; RBRB=dark greyed-orange.

Data Analysis

The quantitative data pertaining to seed characterization, along with the outcomes of proximate analysis, were subjected to analysis of variance (ANOVA) by the F test. Prior to conducting ANOVA, a meticulous examination of the data was performed to ensure compliance with statistical assumptions. This involved the application of Levene's test to assess normality and the Shapiro-Wilk test to evaluate homogeneity of variances, as elucidated by Garson (2012). Subsequently, the means were compared employing the Tukey's test, with a significance level of 5%, as executed using Statistical Tool for Agricultural Research version 2.0.1.

RESULTS AND DISCUSSIONS

Morphometric Traits of Ricebean Seeds

Using the RHS Color Chart Edition VI, the three ricebean seed morphotypes are easily distinguished based on the seed coat color: a) RBYG - yellow-green (152B-D), b) RBAO – light greyed-orange (163A-B), and c) RBRB – dark greyed-orange (175A-C) (Figure 1). Notably, there are no variations in terms of the hilum structure, seed coat luster, and seed coat pattern between and among the morphotypes.

Table 1 displays the seed size of the three seed morphotypes based on length, width, thickness, perimeter, and area. Based on the average eccentricity index (1.697 mm), flatness index (1.7833), and circularity value (0.73), the ricebean seed shape is between sphere and spindle which is commonly referred as kidney or bean shape (Cerdeira & Fayos 2002; Balkaya & Odabas 2002; Iwata et al. 2010; Cervantes et al. 2016) (Table 2). The recorded 100-seed weight (n=9) is 8.01 g.

Seed Germination

In RBYG and RBAO seed morphotypes have an average of 98% germination 2 days after sowing (DAS) with vigorous seedlings; whereas, RBRB seeds had poor germination (0%) and produced abnormal seedlings. This observation was also recorded in cowpea (Gaafar et al. 2016), common bean (Sadohara et al. 2022), and adzuki bean (Chu et al. 2021). Seed coat structure and its color are important traits for legume species not only to determine the quality and commercial values of seeds but also to reveal seed germination parameters for agricultural applications (Tiryaki et al. 2016). For instance, in adzuki bean, seed coat color is linked to the anthocyanin and flavonoid metabolism pathways (Chu et al. 2021). This was also recognized by Guo et al. (2022) as they characterized flavonoids as germination inhibitors in Chinese pistache seeds.



Figure 1. The three (3) ricebean morphotypes: yellow-green (RBYG), light greyed-orange (RBAO), and dark greyed-orange (RBRB).

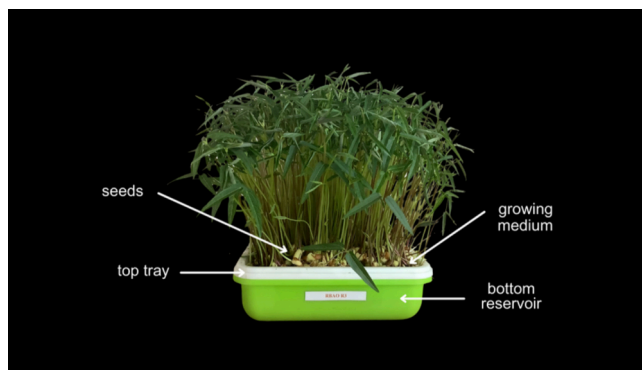


Figure 2. Cultivation setup of ricebean microgreens.

Table 2. Seed shape indices of the three (3) different ricebean morphotypes.

Seed lot	Eccentricity Index (EI)	Flatness Index (FI)	Circularity Index (CI)
RBYG	1.69 ±0.06	1.86 ±0.06 ^a	0.73 ±0.01
RBAO	1.67 ±0.06	1.74 ±0.06 ^b	0.73 ±0.01
RBRB	1.73 ±0.04	1.75 ±0.04 ^b	0.73 ±0.00

Value = Mean ± SD (n = 20). The values with the same superscript in a column are not significantly different at $p \leq 0.05$. Abbreviations: RBYG=yellow green; RBAO= light greyed-orange; RBRB=dark greyed-orange.

Table 3. Recommended seeding density for commercially available microgreen trays.

Microgreen Tray Sizes	Recommended Number of Seeds	Recommended Amount (g)
17 x17 cm	850-900	70-75
25 x25 cm	1950-2000	155-160
24x 32 cm	2400-2450	195-200
65 x70 cm	3850-3900	315-320

Table 4. Ontogenetic development of ricebean microgreens based on the BBCH scale as observed from the experiment.

Principal Growth Stage	BBCH Code	Stage Description	Period
			Hours after sowing
Germination	0	Dry seed	0
	3	Seed imbibition	4
	5	Radicle emergence	8
	7	Radicle elongation; epicotyl emergence	20-32
	8	Epicotyl elongation; apical hook formation with distinct purple pigmentation	48-52
	9	Germination completed	60
			Days after sowing
Leaf development	10	Primary leaf emergence; epicotyl continues to elongate forming a more visible apical hook with distinct purple pigmentation	4
	11	Primary leaves increase in size; epicotyl continues to elongate with visible apical hook	5
	12	Primary leaves increase in size, V-shaped; epicotyl continues to elongate in upright position	5
	13	Primary leaves unfold showing a pair of lanceolate simple leaves in opposite position; epicotyl continues to elongate in upright position with distinct purple pigmentation at the basal region	6

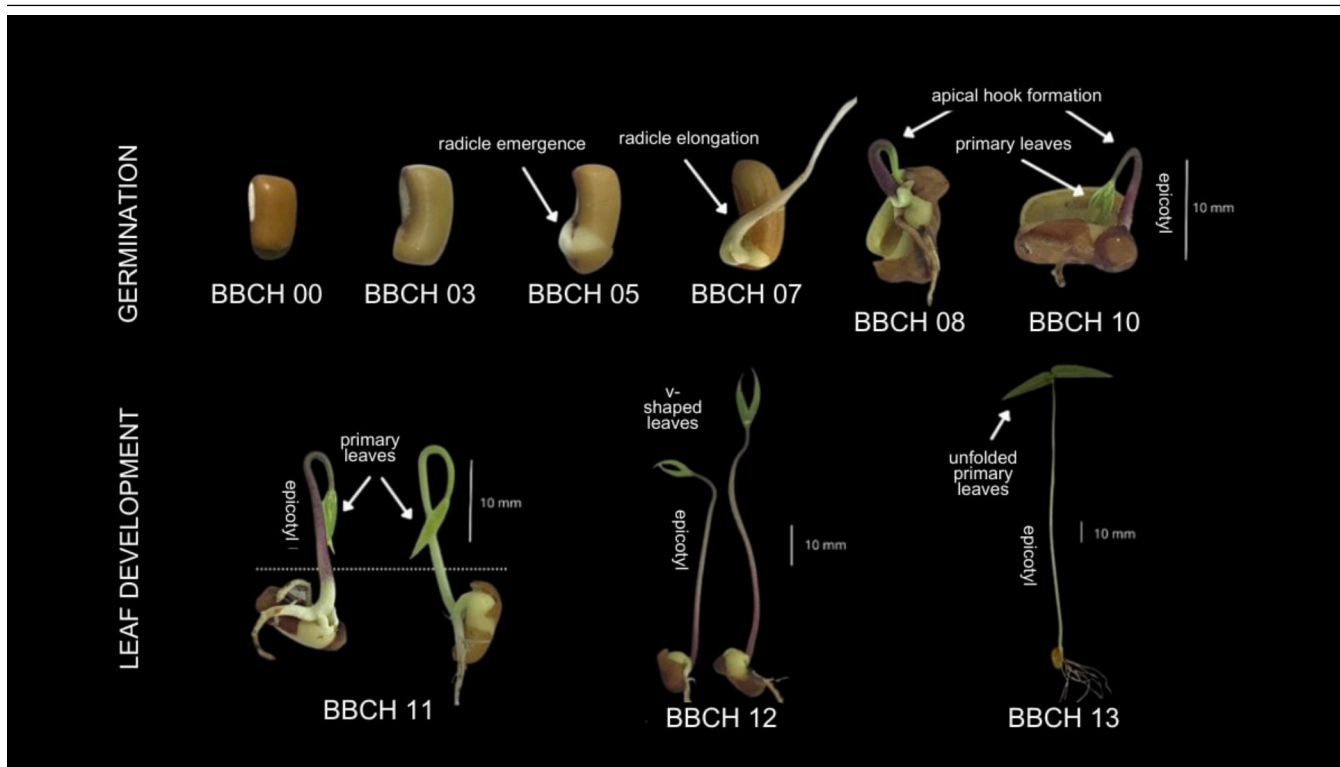


Figure 3. Diagrammatic presentation of ontogenetic development of ricebean microgreens based on the BBCH scale.

Cultivation Methods

The results of seed morphometry were used to calculate the recommended seeding rate for four (4) different types of growing trays (Table 3).

Microgreen trays with a top tray and a bottom reservoir must be used for microgreen production (Figure 2). The sterilized soilless growing medium, along with the ricebean seeds, should be placed on the top tray. The ricebean seed should not be buried deeply in the growing medium, but rather lightly pressed on top of it.

At 5 days after sowing, or when the roots have begun to grow below the top tray, fill the bottom reservoir with water. The microgreen setup could be placed in an area with sufficient sunlight or expose them under white or red-blue light-emitting diode (LED) lights.

Ricebean Microgreens Ontogenetic Development

The growth and development of ricebean starting from intact seed to microgreen harvesting was illustrated using the BBCH scale. The microgreen development covers the two principal growth stages which are the germination (BBCH 00-08) and leaf development (BBCH 10-12) (Table 4).

Table 5. The proximate composition (g 100 g⁻¹ ± SD) of freshly harvested microgreens of the three ricebean seed morphotypes.

Morphotype	Ash (g 100g ⁻¹)	CF (g 100g ⁻¹)	CP (g 100g ⁻¹)	TC (g 100g ⁻¹)	MC (%)
RBYG	1.0933 ±0.01 ^b	0.8933 ±0.09 ^a	6.6733 ±0.37	4.4733 ±0.41	86.8667 ±0.27
RBAO	1.0233 ±0.03 ^c	0.5667 ±0.03 ^b	6.2100 ±0.37	4.8300 ±0.41	87.3700 ±0.60
RBRB	1.2100 ±0.01 ^a	0.7900 ±0.08 ^a	6.3500 ±0.27	4.6800 ±0.23	86.9700 ±0.38

Value=Mean ±SD (n=3). The values with the same superscript in a column not significantly different at $p \leq 0.05$. Abbreviations: RBYG: yellow green, RBAO: light greyed-orange, RBRB: dark greyed-orange; CF: crude fat, CP: crude protein, TC: total carbohydrate, MC: moisture content

Table 6. Comparison of proximate composition of ricebean with other Fabaceae microgreens.

Microgreens	A (g 100g ⁻¹)	CF (g 100g ⁻¹)	CP (g 100g ⁻¹)	TC (g 100g ⁻¹)	MC (%)
Ricebean	0.99±0.12	0.76±0.22	6.44±0.60	4.65±0.59	87.50±0.50
Mung Bean*	0.64±0.04	0.36±0.01	4.55±0.05	7.16±0.02	87.29±2.28
Fenugreek*	0.50±0.00	0.18±0.00	4.03±0.06	5.12±0.02	90.17±1.22
Green Pea*	0.36±0.02	0.15±0.00	3.73±0.02	3.39±0.04	92.37±2.38
Lentil*	0.61±0.00	0.43±0.02	6.47±0.11	5.92±0.01	86.57±1.12

(*) Based on Kowitcharoen et al., (2021). Abbreviations: A: ash, CF: crude fat, CP: crude protein, TC: total carbohydrates, MC: moisture content; (°) Based on the average of all seed morphotypes in the study.

Under ambient conditions, the ricebean seeds require 2-3 days to germinate that is when the length of the radicle and plumule is twice the length of the seed (BBCH 08), as seen in Figure 3. The complete exposure of the primary leaves marks the onset of leaf development (BBCH 10) and subsequent hypocotyl elongation until the full expansion of the primary leaves (BBCH 13) exhibiting a cryptocotylar hypogeal type of germination, wherein the cotyledons remain just below the ground surface with a very short hypocotyl. At 3 days after germination (BBCH 13), the microgreens are ready for harvesting and are characterized by having an average plant height of 16.42 cm and a pair of green lanceolate leaves of about 26 mm in length and 6 mm wide; the stem is somewhat translucent with distinct purple pigmentation at the base.

Harvesting

For ricebean microgreens, the following harvesting techniques are being recommended: 1) harvest at BBCH 13, when the primary leaves are already fully expanded, 2) cut the seedling at about 1-1.5 cm above the surface of the growing media, or depending the desired length, by batches using a pair of clean sharp scissors or kitchen knife; harvesting by group will minimize mechanical damage and facilitate proper handling during packaging, and 3) harvest early in the morning when transpiration rate is relatively lower to have the best quality of produce (Kyriacou et al. 2016).

Proximate Composition of Freshly Harvested Ricebean Microgreens

Generally, the ricebean microgreens contain high crude protein (5.84-7.0433 g 100 g⁻¹) followed by total carbohydrate (4.0633-5.24 g 100 g⁻¹), crude fat (0.5367-0.9833 g 100 g⁻¹) and ash (0.9933-1.22 g 100 g⁻¹) with moisture content ranging from 87-88% (Table 5).

To compare the three morphotypes, RBRB (1.2100 g 100g⁻¹) has the highest ash content followed by RBYG (1.0933 g 100g⁻¹) and RBAO (1.0233 g 100g⁻¹) indicating that RBRB may contain higher mineral content (Arockianathan et al. 2019) and lipids including carotenoids, tocopherol, sterol and similar compounds (Khan et al. 2013). Though, the three morphotypes

examined did not show significant difference in terms of in crude protein, total carbohydrate and moisture content, RBYG (0.8933 g 100 g⁻¹) and RBRB (0.7900 g 100 g⁻¹) crude fat content is significantly higher than RBAO (0.5667 g 100 g⁻¹). Further studies are needed to investigate the variations in ash and crude fat content between and among the seed morphotypes tested.

Interestingly, ricebean microgreens' average crude protein content (6.44165 g 100g⁻¹) is about 60% higher than ricebean grains with 2.5975 g 100g⁻¹ (Bhagyawant et al. 2019). The relatively high crude protein content is attributed to the synthesis of enzyme proteins or a compositional change following the degradation of other constituents during germination. This was also observed in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) wherein the carotenoid profile of the microgreens was higher than in the intact seeds (Niroula et al. 2019). Considering this, consuming ricebean as microgreens is more beneficial in terms of its protein content. It is also notable that although RBAO has the lowest ash, crude fat, and crude protein it contains the highest amount of total carbohydrates. Furthermore, the moisture content may vary depending on the number of days after sowing and growing conditions.

The mean of ricebean microgreens' proximate composition was also calculated and compared to other leguminous microgreens, as displayed in Table 6. However, the closest comparison to ricebean is mungbean microgreens since it is an accessible alternative. To compare, ricebean and mungbean microgreens have 34.39% difference in terms of the crude protein content. This indicates that ricebean is also a competitive choice for its nutritional value and could be a viable substitute for mungbean microgreens.

CONCLUSION

Despite having similar size, weight, and shape, ricebean seeds displayed differences in germination and proximate composition based on the seed coat

color namely yellow-green (RHS 152B-D; RBYG), light greyed-orange (RHS 163A-B; RBAO), and dark greyed-orange (RHS 175A-C; RBRB). The 100-seed weight and shape indices values were used to establish recommended seeding densities for given microgreen trays available in the market. In addition, the newly described ontogenetic developmental stages of ricebean from intact seed to unfolding of primary leaves following the BBCH scale would be used as standard reference for microgreen characterization and evaluation and development of further cultivation techniques.

Ricebean microgreens are also potential source of phytonutrients such as crude protein (5.84-7.0433 g 100g⁻¹), total carbohydrate (4.0633-5.24 g 100g⁻¹), crude fat (0.5367-0.9833 g 100g⁻¹), and ash (0.9933-1.22 g 100g⁻¹) with a moisture content of 87-88%. The three morphotypes have comparable levels of crude protein and total carbohydrates; however, RBYG and RBRB are considered superior to RBAO in terms of ash content and crude fat. Despite the variability in morphotypes, which could be attributed to genetic factors, the above findings suggest that ricebean is a competitive choice for its nutritional value and could be a viable substitute for commercial microgreens. The labeling of microgreens as "superfoods" is also appealing to children and younger generations who want to enjoy the freshness, crunchiness, and richness of these miniature leafy greens.

RECOMMENDATION

The results of this exploratory study need further validation using more seedlot of ricebean to formulate guidelines for microgreens production at household level. To promote the development of microgreen industry, further studies on phytochemical and molecular aspects of using native and underutilized vegetable species in the Philippines must be conducted. More importantly, evaluation and selection of desirable morphotypes for microgreen production would open opportunities not only for research and academic institutions but also for farmer-growers, garden hobbyists, and entrepreneurs who would venture in the ricebean microgreen industry.

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