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Research Article

PHYTOCHEMICAL AND NUTRITIONAL VARIATION OF COUNTRY BEANS (*LABLAB PURPUREUS*) INVOLVING PARENTS AND HYBRIDS

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Abstract

The fifteen country bean genotypes (*Lablab purpureus*) were grown to assess the nutritional status and phytochemical analysis. Morphological variation among the genotypes was also evaluated. The experiments were set up using one-way randomized complete block design with three replications. These 15 genotypes grown in winter season from August 2022 to January 2023 were IPSA Sheem -1(P_1), Sikribi Sheem-1(P_2), Sikribi Sheem-2(P_3), BARI Sheem-1(P_4), Golgadda Sheem (P_5), and hybrids from $P_1\times P_2$, $P_1\times P_3$, $P_1\times P_4$, $P_1\times P_5$, $P_2\times P_3$, $P_2\times P_4$, $P_2\times P_5$, $P_3\times P_4$, $P_3\times P_5$, and $P_4\times P_5$. Nutritional parameters like crude protein (CP), antioxidant, dry matter (DM), and moisture content were studied. The highest CP (29.63%) was observed in IPSA Sheem-1 (P_1). We found the highest antioxidant (Free radical scavenging activity of DPPH) in hybrid from $P_4\times P_5$ (37.96%). $P_3\times P_5$ hybrid had the highest DM (13.11%) and the highest moisture content (%) value was in $P_4\times P_5$ (90.61%). In phytochemical analysis, the total phenolic content (TPC) and total flavonoid content (TFC) were studied. The highest TPC was (14.58 ug/mg) found in Golgadda Sheem (P_5). $P_1\times P_4$ hybrid had the highest TFC (4.25 ug/mg). Following morphological variation, the highest number (4.86) of seeds pod^{-1} was recorded in $P_1\times P_5$. Based on nutrients IPSA Sheem -1(P_1) was found to be more nutritious in the winter season compared to the other genotypes.

Introduction

The country bean, also known as "sheem," "lablab bean," and "hyacinth bean," is a versatile legume from the Papilionaceae subfamily of the Leguminosae family. It is a protein-rich vegetable crop with chromosomal numbers between 2n = 20 and 22 or 24 (Saravanan *et al.*, 2013). It is believed to have originated from Ethiopia in Africa or India (Maass, 2016; Sibiko *et al.*, 2013). In Bangladesh, country beans are cultivated in various regions, including Jashore, Dhaka, Noakhali, Cumilla, Sylhet, Pabna, Kishoregonj, Dinajpur, Tangail, and Chattogram (Hafeez *et al.*, 2023). The country bean is a photosensitive crop that is now widely cultivated throughout the year due to advanced lines developed by Bangladesh Agricultural Research Institute, Sylhet Agricultural University, BSMRAU, and BARDC (Biswas, 2016). It produces an average of 10- to 12-million tons of pods per hectare, with production increasing over the previous year. Country bean production increased over the previous year, reaching 228114.83 tons on an area of 25768.67 ha in the 2022–2023 growing season (BBS, 2023). Country bean is widely used in vegetable preparations in Bangladesh, India, and other countries, providing nutritious and delectable seeds to alleviate protein shortages (Morris, 2009).

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Vegetables, rich in carbohydrates, proteins, vitamins, minerals, fiber, and energy, are essential for healthy growth and phytonutrients. Proper vegetable diet reduces risk factors for chronic diseases (Das *et al.*, 2023). In developing countries like Bangladesh, legumes, especially pulses and beans, are important and reasonably priced sources of protein. Green pods of the Lablab bean are high in protein, antioxidants, minerals, and vitamins (Rehman *et al.*, 2001, Bello-Pérez *et al.*, 2007). The country bean has gained popularity recently and is now produced on a wide basis. However, supply is limited in winter due to photo and/or thermo-sensitive behavior. Genetic manipulation has produced photo and/or thermo insensitive cultivars, providing year-round flowering and fruiting properties (Shaahu *et al.*, 2015). According to Rai *et al.* (2014), country bean pod includes protein (102-635.6 mg), sugar (0.188-1.11 mg), chlorophyll (0.121- 0.716 mg), phenol (1.7-9.67 mg), proline (0.02-7.06 g), and carotenoids (0.04-0.231 mg). Environmental factors influence pod and seed setting, flower abscission, and flower abortion in common bean, limiting year-round pod producing potential due to poor off-season retention. Photo-insensitive country bean varieties have been developed by the Horticulture Research Center, BSMRAU, Department of Horticulture, and SAU to bypass seasonal restrictions and increase market availability by 9-10 months (Mortuza *et al.*, 2009). Bangladesh is a promising location for bean cultivation, but its vegetable production has struggled to keep up with demand due to long-term "lean periods" from August-October. With rising demand for high-value vegetables, there is potential to expand winter bean production and increase farmers' revenue. Further information on the nutritional content of country bean genotypes in Bangladesh is needed (Uddin *et al.*, 2007; Sennhenn *et al.*, 2017). Several studies on country bean have been undertaken in the past. However, there is very little information on the nutritional content of country bean genotypes available in Bangladesh. Further information on the morphological behavior of popular winter country bean genotypes is needed. The current study was planned to determine the morphological behavior, nutritional status and phytochemical analysis of country bean.

Materials and Methods

Collection of Samples

A species of *Lablab purpureus* of 15 genotypes (5 parents and 10 F_1 generations) samples were collected from the field of the Department of Horticulture at the Sylhet Agricultural University. The samples were taken in a plastic bag and transferred into Biotechnology and Genetic Engineering Laboratory, Faculty of Biotechnology and Genetic Engineering, Sylhet Agricultural University, Sylhet-3100. The pod samples of 15 genotypes are shown in Figure 1 & 2.

Figure 1. Selected pod samples from 15 genotypes

Figure 2. Pods of country bean in horticulture field.

***Extraction of pods* (*Lablab purpureus*)**

Sliced plants were extracted using distilled water from a local supplier, washed under running water, dried in an oven at 45°C and ground using an electronic blender before extraction.

Extraction of pod samples with soaking method

0.01 g powdered pod samples were soaked in distilled water, mixed, and left for 72 hours at room temperature. Whatman no. 1 filter paper was used to filter the liquid phase, which was then used for anti-activity tests.

Phytochemical screening of the pod samples

Determination of total phenol contents

The total phenolic content (TPC) was determined using gallic acid as a standard. Reagents were prepared by preparing Folin-ciocalteu reagent (FCR), 7.5% sodium carbonate solution (Na_2CO_3), and a blank solution. Standard gallic acid was prepared by dissolving 10 mg in 70 ml distilled water and adding distilled water to make a final volume of 100 ml. Serial dilutions were performed to prepare concentrations of 12.5, 25, 50, 75, and 100 µg/ml. The reaction mixture was mixed with 2.5 ml FCR reagent, 0.5ml extract, or a different concentration of standard, and 2.5 ml 7.5% Na_2CO_3. The absorbance was recorded at 760 nm against the reagent blank, and the total phenolic compounds were determined using a reference curve with gallic acid (Keskin *et al.*, 2012).

Determination of total flavonoid contents

This experiment preparation of a test for quercetin, a chemical compound. The reagents used include 10% aluminum chloride, 1M potassium acetate, and a blank. The blank was prepared with aluminum chloride solution, potassium acetate solution, and distilled water. The standard quercetin was prepared by dissolved 10 mg in 100 ml methanol and serially diluted to different concentrations (12.5, 25, 50, 75, and 100 µg/ml). The experimental procedure involved adding 1.5 ml methanol to 0.1 ml extract or standard, 0.2 ml of aluminum chloride solution, 0.2 ml of potassium acetate solution, and 5.6 ml of distilled water. The solutions were filtered through Whatman no-1 filter paper and incubated at room temperature. The absorbance was measured at 420 nm against the suitable blank (Csepregi *et al.,* 2013).

Nutritional attributes

Determination of antioxidant activity

This study prepared a 0.004% (w/v) DPPH solution by dissolving 4 mg DPPH in 95% methanol. Standard ascorbic acid was prepared by dissolved 10 mg in distilled water to make 0.1 mg/ml concentration. Plant extract was prepared by dissolved 1 ml of each sample's crude extract in 9 ml methanol to make 0.5 mg/ml concentration. A mixed solution of 1 ml methanol and 3 ml DPPH was used as a control. The free radical scavenging activity of the extracts and ascorbic acid was measured at 517 nm using the stable radical DPPH with slight modifications (Susanti *et al.,* 2007). The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

Percent of inhibition = [(A_{DPPH} –A_{sample})/ A_{DPPH}] ×10, [Were, A_{DPPH} = Absorbance of DPPH**,** A_{sample} = Absorbance of sample (extract/ascorbic acid)]

Determination of dry matter (DM%)

Fresh pods weight and then it dried weight were recorded. After that, DM was determined by using the following formula:

$$DM\ (\%) = (\text{Weight of the dry sample/Weight of sample}) \times 100$$

Determination of moisture content (%)

To calculate a percentage of moisture content (MC), subtract the dry weight from the wet weight, divided the result by the wet weight, then multiply by 100. MC was determined by using the following formula: MC% = (w-d/w) × 100 [Here, MC =moisture content (%), w = weight while wet, d =weight while dry].

Crude protein analysis (CP%)

According to Mortuza *et al.*, 2009, prepared a process for the preparation of a standardized HCL (0.2N) solution. The process involved preparing chemicals such as a catalyst, concentrated H_2SO_4, 4% boric acid solution, mixed indicator, NaOH 40% solution, and HCI 0.2N. A sample was taken on nitrogen-free paper and placed on a Kjeldahl flask. The flask was placed on a digestion chamber heater, heated to 200%, and the solution became clear. The flask was then cooled and transferred to a wooden rack. A conical flask was prepared for stem distillation, and 4% boric acid solution was added to trap the developed NH_3. The delivery tube was fixed to the flask, and the distillation chamber was turned on. The liquid sample was prepared for distillation, and a standardized HCL (0.2N) solution was titrated on the distillate. The nitrogen proportion (N_2%) was calculated. The process involved several steps to ensure accurate results. CP was determined by using the following formula:

$$CP = \% \text{ of } N_2 \times 6.25$$

Statistical analysis

Results of the tests of these experiments were corrected and compared by using Tukey Pairwise Comparisons method in Minitab-19 software and followed the one-way ANOVA.

Results and Discussion

Pod color

Pod color varied among the 15 genotypes (Figure 1 & table 1). Green pods were found in $P_4 \times P_5$, and Sikribi sheem-1. Purplish Green were found in $P_1 \times P_2$, $P_1 \times P_4$, $P_2 \times P_4$, $P_3 \times P_5$, IPSA sheem-1, and Goalgadda sheem. In F1 generation from $P_1 \times P_3$, $P_2 \times P_5$ genotypes were found Deep Green. Light Green were found in $P_1 \times P_5$, $P_2 \times P_3$, $P_3 \times P_4$, Sikribi sheem-2 and BARI sheem-1.

No. of seeds pod^{-1}

No significant variation was observed among the genotypes at number of seeds pods^{-1}. No. of seeds varied from 3.47 to 4.86 (Table 1).

Table 1. Morphological variation of country bean genotypes

Genotypes	Pod color	No of seeds pod^{-1}
$P_1 \times P_2$	Purplish Green	4.00
$P_1 \times P_3$	Deep Green (Purple Border)	3.98
$P_1 \times P_4$	Purplish Green	4.16
$P_1 \times P_5$	Light Green (Purple Border)	4.86
$P_2 \times P_3$	Light Green	3.87
$P_2 \times P_4$	Purplish Green	4.36
$P_2 \times P_5$	Deep Green (Purple Border)	4.41
$P_3 \times P_4$	Light Green	3.65
$P_3 \times P_5$	Purplish Green	3.47
$P_4 \times P_5$	Green	3.47
IPSA Sheem 1(P_1)	Purplish Green	3.76
Sikribi Sheem-1(P_2)	Green	4.38
Sikribi Sheem-2(P_3)	Light Green	3.92
BARI Sheem-1(P_4)	Light Green	3.52
Golgadda Sheem (P_5)	Purplish Green (Purple Border)	3.89

Phytochemical constituents of selected variant of country bean (Lablab purpureus) involving hybrids and parents

Phytochemical analysis of 5 parents and 10 1st Generation (F_1) of these parents indicates the presence of phenolic and flavonoids content and these results are shown in the Table 2.

Table 2. Phytochemical Screening of 15 genotypes of *Lablab purpureus* (aqueous Extract)

Genotypes	**Phytochemical tests**	
	Phenolic content	**Flavonoid content**
$P_1 \times P_2$	+++	+++
$P_1 \times P_3$	+	+
$P_1 \times P_4$	+++	+++
$P_1 \times P_5$	+++	+++
$P_2 \times P_3$	+++	++
$P_2 \times P_4$	++	+++
$P_2 \times P_5$	+	+++
$P_3 \times P_4$	++	+
$P_3 \times P_5$	+	++
$P_4 \times P_5$	++	+++
IPSA Sheem 1(P_1)	+++	++
Sikribi Sheem-1(P_2)	+	++
Sikribi Sheem-2(P_3)	++	+
BARI Sheem-1(P_4)	++	+++
Golgadda Sheem(P_5)	+++	+++

Interpretation:
+++= High Concentration (Phenolic Content >=12, Flavonoid Content >=3)
++= Moderate Concentration (Phenolic Content <12-10, Flavonoid Content <3-2)
+ = Low concentration **(**Phenolic Content <10, Flavonoid Content <2)

Determination of total phenolic and flavonoid content

The total phenolic and flavonoid contents in aqueous extracts of 15 genotypes of Lablab purpureus were assessed, revealing notable variations. Among the genotypes, the aqueous extract of P_5 exhibited the highest total phenolic content, measured at 14.58 ± 0.48 µg/mg GAE, surpassing the others. Other genotypes also contained significant amounts of phenolic compounds presented in Table 3. Similarly, the total flavonoid content varied across the genotypes. The aqueous extract of the hybrid $P_1 \times P_4$ demonstrated the highest flavonoid content, recorded at 4.25 ± 0.22 µg/mg QE. Other genotypes also showed considerable flavonoid levels, details of which are also included in Table 3. These findings highlight the diversity in phenolic and flavonoid profiles among the genotypes studied.

Table 3. Results of total phenolic and flavonoid content for aqueous extraction of 15 genotypes of *Lablab purpureus*

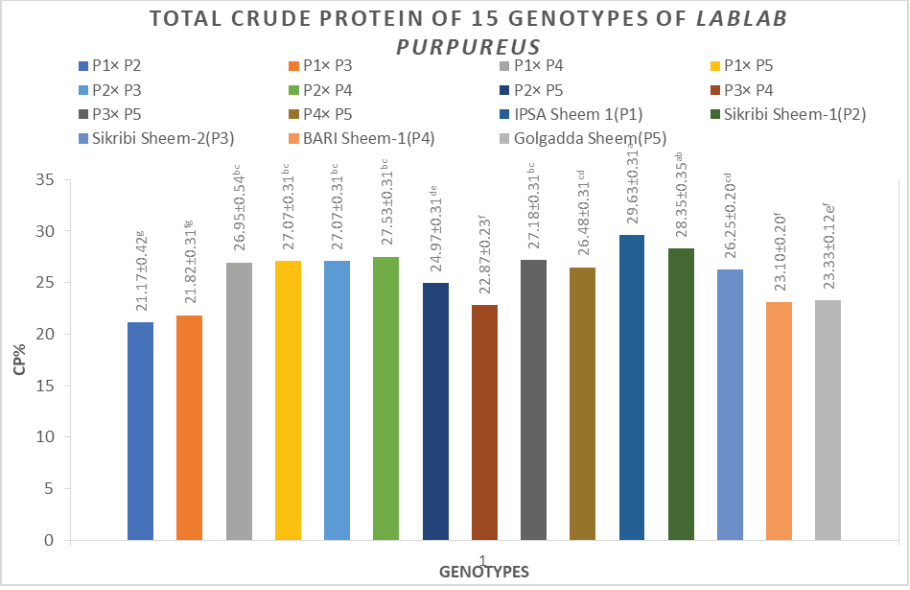
Genotypes	Mean Conc. (ug/mg QE) of Dried Extract (±SEM) Flavonoid	Mean Conc. (ug/mg GAE) of Dried Extract (±SEM) Phenolic
P1× P2	3.13±0.38^{abcd}	13.27±0.94^{abc}
P1× P3	1.13±0.00^{f}	9.54±0.09^{cde}
P1× P4	4.25±0.22^{a}	13.71±0.19^{ab}
P1× P5	4.02±0.22^{ab}	12.51±0.06^{abcd}
P2× P3	2.47±0.38^{cdef}	12.79±0.27^{abc}
P2× P4	3.13±0.38^{abcd}	10.84±0.04^{abcde}
P2× P5	3.13±0.00^{abcd}	8.67±0.22^{de}
P3× P4	1.80±0.39^{def}	10.30±0.16^{bcde}
P3× P5	2.69±0.22^{bcde}	7.91±0.16^{e}
P4× P5	3.36±0.59^{abc}	11.49±0.47^{abcde}
IPSA Sheem-1(P_1)	2.25±0.22^{cdef}	13.20±0.36^{abc}
Sikribi Sheem-1(P_2)	2.25±0.22^{cdef}	8.56±2.60^{de}
Sikribi Sheem-2(P_3)	1.58±0.22^{ef}	11.60±0.47^{abcde}
BARI Sheem-1(P_4)	3.35±0.22^{abc}	10.95±0.53^{abcde}
Golgadda Sheem (P_5)	3.13±0.00^{abcd}	14.58±0.48^{a}

This means having the same letter(s) in a column does not differ significantly

Nutritional value estimation of selected variant of country bean (Lablab purpureus) involving hybrids and parents

Determination of total crude protein

The total crude protein of 15 genotypes of *Lablab purpureus* were determined by Kjeldahl method which value shown in Figure 3. Crude Protein content varied significantly among the genotypes. Maximum CP% was found in IPSA Sheem-1 than others and it was about 29.63±0.31 and the least CP% in P_1× P_2 and it was about 21.17±0.42.

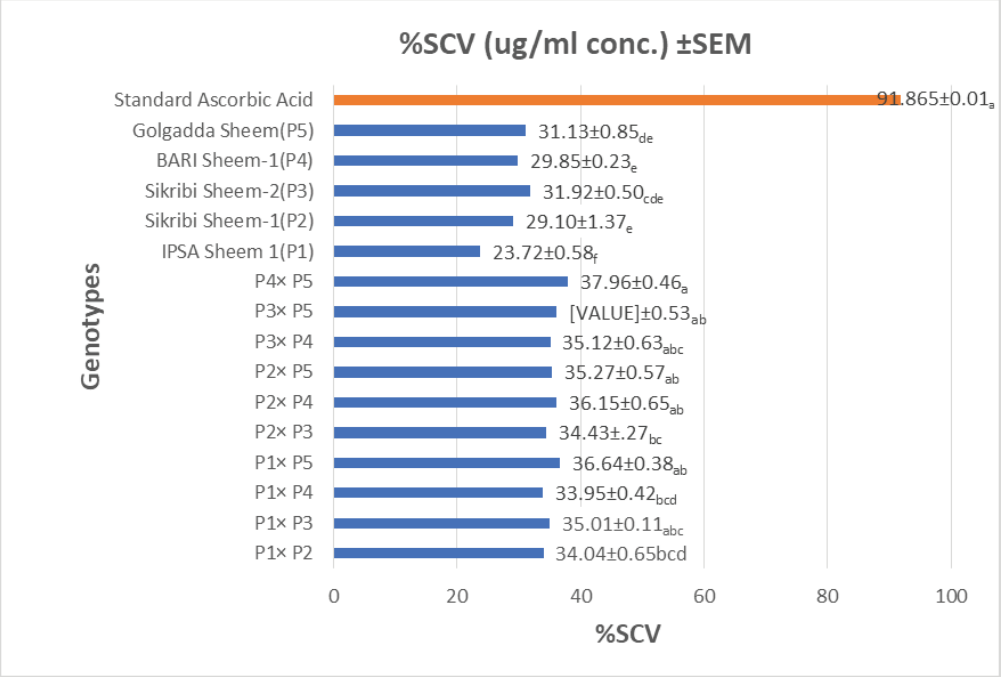


This means having the same letter(s) in a column does not differ significantly.

Figure 3. Total crude protein of 15 genotypes of *Lablab purpureus*

Determination of antioxidant

Compared to standard ascorbic acid, free radical scavenging activity (%SCV) among the 15 genotypes of *Lablab purpureus* indicate highest value in P_4× P_5 (37.96±0.46) and the lowest in IPSA Sheem -1 (23.72±0.58).



This means having the same letter(s) in a column does not differ significantly.

Figure 4. Comparison of free radical scavenging activity (%SCV) among 15 genotypes *Lablab purpureus* and standard ascorbic acid.

Determination of DM% and moisture content of 15 genotypes of Lablab purpureus

Nutritive analysis of 15 genotypes of *Lablab purpureus* is reveled highest DM% and lowest Moisture content (%) value in P_3× P_5 and lowest DM% and highest Moisture content (%) value in P_4× P_5 that shown in Table 4.

Table 4. DM% and Moisture Content of 15 genotypes of *Lablab purpureus*

Genotypes	DM% (±SEM)	Moisture Content (%)
P_1× P_2	10.72±0.006^{h}	89.28
P_1× P_3	11.59±0.006^{c}	88.41
P_1× P_4	10.21±0.006^{k}	89.79
P_1× P_5	10.98±0.006^{f}	89.02
P_2× P_3	11.90±0.006^{b}	88.10
P_2× P_4	10.85±0.006^{g}	89.15
P_2× P_5	10.62±0.006i	89.38
P_3× P_4	11.51±0.006^{d}	88.49
P_3× P_5	13.11±0.006^{a}	86.89
P_4× P_5	9.39±0.006^{l}	90.61
IPSA Sheem -1 (P_1)	11.43±0.006^{e}	88.57
Sikribi Sheem-1(P_2)	10.58±0.006^{j}	89.42
Sikribi Sheem-2 (P_3)	11.90±0.006^{b}	88.10
BARI Sheem-1(P_4)	10.84±0.006^{g}	89.16
Golgadda Sheem (P_5)	11.40±0.006^{e}	88.60

This means having the same letter(s) in a column does not differ significantly.

Morphology properties

We have studied and observed four different pod colors green, purple green, light green, and deep green among 15 genotypes. In the case of pod characteristics, this experiment showed similar findings as some of other researchers (Akter *et al.,* 2017). Three different pod colors were observed among the genotypes. Out of 44 country bean accessions, Islam (2008) found green, 41 light green, purple margin, and purple pods. In this experiment, the number of seeds per pod was much higher in some genotypes. The local variety $P_1\times P_5$ produced the maximum number of seeds per pod which may be an inherent character of this genotype whereas the lowest in $P_3\times P_5$ and $P_4\times P_5$. Studies by earlier workers (Singh *et al.,* 2004; Mohan *et al.,* 2009 and Shankar *et al.,* 2011) revealed the existence of wide variation among the genotypes in length, width, thickness, and weight of pod, pod length, and width ratio, and even the number of seeds per pod. The results of the present study are in good agreement with the earlier observations.

Phytochemical properties of country bean genotypes

Due to their potent phytochemical compounds, recently they are getting a lot of attention. The quantity and quality of phenols present in vegetables may be significantly influenced by genotype, environment, type of soil, and growing conditions. The total phenolic content of P_5 genotype was the highest among other genotypes which was 14.58±0.48 ug/mg. On the other hand, $P_3\times P_5$ showed the lowest TPC which was 7.91±0.16 ug/mg. Earlier, Vadodariya *et al.* (2022) reported phenol content in the range of 2.46–2.61 g/100 g on a DW (dry weight) basis, and Rai *et al.* (2014) reported the same in the range of 1.70–9.67 mg/g. The results of the present study are higher than the earlier ones. Flavonoids, a subclass of polyphenols or phenolic found mostly in fruits and vegetables, are beneficial for human health due to their antibacterial, antiviral, anti-inflammatory, and antioxidant properties (Panche *et al.,* 2016). The Flavonoid content of the genotypes was statistically different. The flavonoid content of the studied genotypes was found highest in the $P_1\times P_4$ genotype (4.25 ± 0.22^{a} ug/mg) followed by the lowest in $P_1\times P_3$ (1.130 ± 0.00^{f} ug/mg). The results of the present study were higher than that of Saini, Singh, Dubey, and Srivastava (2016) who reported it to be 0.65–1.54 mg/100 g. This higher value might be due to differences in the genotypic constitution and agroecological conditions.

Nutritional properties of country bean genotypes

Country bean genotypes were shown variations in nutritional properties. Sarma *et al.* (2010) experimented with 9 landraces of country beans and found crude protein ranged from 16.44-21.47 g per 100 g of tender pods. The protein content of the present experiment was higher than their result. This may be because of the genetic character of the country's bean genotypes. Davari *et al.* (2018) also found similar results for seed protein content. IPSA Sheem-1 had the maximum concentration of crude protein (29.63 ± 0.31^{a} %) whereas the minimum in $P_1\times P_2$ (21.17 ± 0.42^{g} %). The protein content of this sample was however comparatively lower than the recommended 23-56 g/100 g human daily protein requirement at National Research Council (1989). Antioxidant activity is an excellent example of the functional benefit that plant extracts can deliver. Since fresh green pods of Dolichos bean are consumed as vegetables, so the antioxidant activity in the present study has been estimated with dried samples. Free radical scavenging activity (%SCV) is utmost in the $P_4\times P_5$ genotype (37.96 ± 0.46^{a} ug/ml conc.) alongside bottom-most in IPSA Sheem-1 (23.72 ± 0.58^{f} ug/ml conc.). Since the present study was done and earlier reported ones were on DW basis, so comparison with the earlier works could not be done. However, Maheshu, Priyadarsini, and Sasikumar (2013) reported the antioxidant activity of Dolichos bean in the range of 2.5–4.9 µ g/mL on DW basis. According to Al-Snafi *et al.* (2017), the dry matter was determined to be about 11.8% whereas we have found the maximum and minimum dry matter content revealed $P_3\times P_5$ (13.11 ± 0.006^{a} %) and $P_4\times P_5$ (9.39 ± 0.006^{l} %). In this study, we recorded the highest moisture content value in $P_4\times P_5$ (90.61%) and the lowest value in $P_3\times P_5$ (86.89%). The Moisture content was determined to be 88.2%, which agrees with data reported previously by Sulaiman *et al.* (2018).

Conclusion

The study reveals significant variation in morphological parameters among genotypes, with IPSA Sheem-1(P_1) having the highest protein, followed by Sikribi Sheem-1(P_2), and exhibiting similar phenolic content to Golgadda Sheem (P_5). These beans, a proteinaceous vegetable, have potential for improved genotypes through breeding and biotechnological methods due to high antioxidant activity and flavonoid components.

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Conflict of Interest: There no conflict of Interest.

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