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Research Article

PHYTOCHEMICAL PROPERTIES, YIELD AND YIELD ATTRIBUTES OF PROMISING COUNTRY BEAN GENOTYPES GROWN IN ACIDIC SOIL CONDITION DURING WINTER

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Abstract

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An experiment with three promising country bean genotypes- BARI Sheem-1, Sikribi Sheem-1 and Goalgadda was conducted at the experimental field and laboratory of Horticulture Department, Sylhet Agricultural University, from November 2022 to March 2023. The objective was to observe their growth, green pod and seed yield, nutrition and phytochemical properties in acidic soil condition of Sylhet. The RCB (Randomized Complete Block) design was used with three replications. Days to first flower was found earlier for Sikribi Sheem-1 (48.50 days) and BARI Sheem-1 (51.75 days). Sikribi Sheem-1 recorded the earliest harvest at 71.5 days. The highest number of green pods plant⁻¹ was in Sikribi Sheem-1 (582.5), while Goalgadda had the lowest (329.45). Goalgadda also had the largest fruit size with pod length (13.95 cm) and breadth (3.37 cm), leading to the heaviest individual fruit weight (10.14 g). Despite having the fewest pods, Goalgadda matched the pod yield of the other varieties due to its heavier fruit. Sikribi Sheem-1 had the highest number of dried pods (477.75), seed yield plant (347.50 g), and seed yield ha (2.37 ton). Goalgadda had the heaviest 100-seed weight (50.52 g). In green pods, Goalgadda had the lowest fibre (0.25%) and crude fibre (6.30%), indicating it is more palatable. Goalgadda also had the highest ash content (6.73%), while Sikribi Sheem-1 had the highest crude protein (26.51%) and BARI Sheem-1 had the most Vitamin C (13.21 g100g⁻¹). Goalgadda exhibited the highest phytochemical properties with total phenolic content (14.60 µgmg⁻¹) and antioxidant activity (31.12%). The flavonoid content was significantly higher in BARI Sheem-1 (3.36 μgmg⁻¹) and Goalgadda (3.11 μgmg⁻¹) compared to Sikribi Sheem-1 (2.22 μgmg⁻¹). All three varieties are nutrient-rich and suitable for cultivation in acidic soils for pod production, with Sikribi Sheem-1 being the best for seed production.

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Introduction

Country bean (*Lablab purpureus* L. Sweet), local people called "Sheem", the most valuable, nutritious, and protein-rich leguminous vegetable is grown extensively during the winter months in Bangladesh. This crop is believed to be cultivated in India first and then spread to all over the world (Sibiko *et al.*, 2013). In the world, country bean is called by different other names such as Field bean, Hyacinth bean, Kikuyu bean, Lablab bean, Indian bean, and Dolichos bean. This is considered one of the leading income-generating crops in Bangladesh and has grown in significant acreage after brinjal and tomato (Khan *et al.*, 2020). Currently, the country bean is successfully cultivated to meet the national demand in Cumilla, Noakhali, Sylhet, Dhaka, Kishoregonj, Tangail, Jasohore, Dinajpur, and Chattogram districts with an increasing trend in the production in other regions of the country (Singh and Gupta, 2019). Besides, every homestead in rural areas normally grows at least a bush of country bean to meet the family requirement.

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Country bean plays a big dietary role to all classes of people in Bangladesh by supplying proteins, carbohydrates, fibre, vitamins and different phytochemicals (Saikia *et al.*, 1999). The immature pods and seeds both are eaten either boiled or used to make other delicious curries. Besides, ripe seeds are often used to make a special kind of soup called "dhal" in Bangladesh (Sultana, 2001). The sun-dried mature seeds are sometimes preserved for use later as vegetables. The 100 g of edible green pods of the country bean contain around 110 mg calcium, 4.7 mg iron, 35 mg vitamin C, 2.4 mg vitamin A and 4.2 g protein (Anonymous, 2013). Approximately 4.5% protein content of green pod and 25% protein content of dry seed has made this crop a highly demanding vegetable irrespective of both rich and poor (Ema *et al.*, 2022). Moreover, it also contains significant amount of riboflavin, thiamin, niacin, iron, and vitamin C (Rehana, 2006).

Different types of beans are available in the warmer zones of America, Africa, Australia, and Asia. (Khalil, 2000). In Bangladesh, different genotypes of country beans are grown by the farmers of which heritable and non-heritible characteristics are varied greatly (Islam *et al.*, 2002 and 2011). Rahman *et al.*, (1985) reported that the cultivated country bean genotypes are morphologically different from each other. The growth related characteristics such as leaf number, brunch number, plant height and yield related characteristics such as pods number, both the green pod and seed yield are varied from one genotype to another.

Sylhet is a special agricultural zone with uneven topography and mostly acidic soil in Bangladesh. Different country bean genotypes are grown in this region, especially during winter. Beside commercial cultivation, at least a bush of country bean is common in every household. A cultivar named Goalgadda is very popular among the growers of this region. The field performance of Goalgadda cultivar along with other popular varieties in terms of growth, green pod yield, seed yield and other qualitative parameters such as protein, fibre, ash, phytochemicals and various essential elements were not investigated in the near past. Sylhet Agricultural University (SAU) and Bangladesh Agricultural Research Institute (BARI) have developed some high yielding varieties of country bean which can be tested with the local variety Goalgadda for quantitative and qualitative potential under Sylhet conditions. Therefore, this study was conducted to observe the plant growth, green pod and seed yield, nutrition and phytochemical properties of three promising country bean genotypes such as BARI Sheem-1, Sikribi Sheem-1 and Goalgadda under acidic soil condition.

Materials and Methods

The experiment was conducted at the experimental field as well as laboratory of Horticulture Department, Sylhet Agricultural University (SAU), Sylhet, during November, 2022 to March, 2023. According to UNDP and FAO (1988), the experimental site falls under the Eastern Surma-Kusiyara Flood Plain (Agro-ecological Zone-20) and lies between 23°57′ to 25°13′ and 90°56′ to 92°21′ North latitude and East longitude, respectively. The clay loam type soil of this region is mainly characterized by low pH level of around 4.83 (Saha et al., 2016). Three popular country bean genotypes namely BARI Sheem-1, Sikribi Sheem-1 and Goalgadda were considered in this study for assessing plant growth, green pod, seed production potentiality, different nutritional and phytochemical properties. The experiment was conducted following RCB (Randomized Complete Block) design with three replications. The land was fertilized as per recommendations by Rashid (1999). About 15 days before seed sowing, full dose of recommended fertilizer except half of the muriate of potash and full dose of urea was applied in the field. The remaining muriate of potash and urea fertilizer were applied in three equal installments as side dressing after 16, 32 and 48 days after seed sowing. Unit plot size was 3.0 m × 2.0 m in which plants were spaced at 1.0 × 1.0 m distance between plant to plant and row to row, respectively. In each pit, 3 seeds were sown and after germination only one healthy plant was kept. Bamboo made trellis was provided in each plot to creep the plants and weeding, irrigation etc. were done based on requirement. To protect the plants from insect infestation, insecticide (Imitaf @0.1%) was sprayed twice at 25 and 40 days after sowing. Data on different parameters were collected as follows-

Phenological parameters

Data on different phenological parameters such as height of plant at 35 DAS (Days after sowing), branch number at 35 DAS, SPAD value at 35 DAS, Days to first flower and first harvest were collected.

Pod yield and Yield attributes

Data on pods number plant⁻¹, pod length (cm), pod width (cm), individual pod weight (g) and pod yield plant⁻¹ (kg) as well as ha⁻¹ (tons) were collected.

Seed yield and Yield attributes

Data on days to harvest of dried pod, pods number plant⁻¹, seeds pod⁻¹, seed yield plant⁻¹ (g) as well as ha¹ (tons) and 100 seed weight (g) were collected.

Nutritional Properties of green pod

Data on different nutritional parameters of green pods were taken based on following procedures-

a. Fibre content: About 100 g fresh pod of each variety in three replications was chopped into small pieces with sharp knife and cooked in water for 20 minutes. The cooked pod pieces were then meshed with the hand and passed through a 0.25 mesh size sieve to collect the fibre. The fibre was then dried at room condition for a day and weight was taken with help of an electric balance. The percentage of fibre content was calculated with Eq. 1.

Fibre (%) =
$$\frac{\text{Fresh weight of Fibre}}{\text{Fresh weight of pod}} \times 100 \text{ (Eq. 1)}$$

b. Dry matter (%): The dry matter content was calculated by using Eq. 2. About 200 g of fresh pod sample per varieties in three replications were placed in oven to dry them at 72 °C for 60 hours to obtain the dry weight.

Dry matter (%) =
$$\frac{\text{dry weight of pod}}{\text{fresh weight of pod}} \times 100 \text{ (Eq. 2)}$$

c. Ash content (%): For determination of ash, about 50 g fresh country bean pod sample was weighted (X) and taken in a crucible cup. The sample with crucible cup was weighted again (X1). Pre-ashing of the sample was done by placing crucible cup containing pod sample on a heater and then ignited at 600° C for 5 hours in a muffle furnace. The crucible cup containing sample was then kept in desiccators for cooling and final weight was taken (X2). The ash content was determined using following equation (Eq. 3).

Ash (%) =
$$\frac{X1-X2}{X} \times 100$$
 (Eq. 3) (Eq. 3)

d. Crude fibre (%): About 0.5 g finely grinded sample (W) was taken in a conical flask and boiled for 30 minutes after adding 100 ml H₂SO₄ (1.25%) in the flask. Then the content of the conical flask was filtered with linen cloth and washed with hot water to make the residue acid free. Washed residue was backed again into a conical flask containing 100 ml NaOH solution (1.25%) and boiled exactly for 30 minutes. The content was again filtered and washed with hot water until the content was alkali free. After washing with acetone, the content was dried with 105° C overnight and dry weight was taken (W1). Transferred the content to a muffle furnace at 525° C to ash the material and after cooling weight was taken (W2). The crude fibre content was determined using Eq. 4.

Crude fibre (%) =
$$\frac{W1-W2}{W} \times 100$$
 (Eq. 4)

e. Crude Protein (%): Crude protein was analyzed by following Mortuza et al., (2009) with little modifications. About 0.5 g grinded sample was taken in a Kjendhal digestion flask. Kjendhal catalyst powder and 25 ml H₂SO₄ were then added. After that, the flask was heated and allowed to digest for 1 hour, until the content in the Kjendhal flask turned greenish colored. The solution was then cooled for 1 hour. Blank digestion was also carried out alongside. Add 100 ml of 40% NaOH to the solution and the flask was then transferred to the distillation set for heating. To trap the evolved NH₃, 10 ml of 2% boric acid solution with 3 drops of indicator (mixture two parts of 2% methyl red solution with one part of 0.2% methylene blue solution) were added. The solution was then titrated with standard HCl until pink color was developed. Same procedure was also followed for blank sample. The nitrogen percentage was calculated using Eq. 5.

Nitrogen (%) =
$$\frac{\text{(Sample titre-Blank titre)} \times \text{Molarity of HCl} \times 14 \text{ (Atomic mass of N)}}{\text{Weight of sample taken} \times 1000} \times 100 \text{ (Eq. 5)}$$

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Then, the protein percentage was calculated using following equation (Eq. 6).

Protein (%) = Nitrogen (%)
$$\times$$
 6.25 (Conversion factor) (Eq. 6)

f. Vitamin C: Vitamin C content of green pod was measured by following Salkic *et al.*, (2009). About 1.0 g green pod sample was homogenized in 10 ml Na₂C₂O₄ (0.056 M) solution for 2 minutes and the homogenate was filtered by using filter paper (Whatman No. 1). Then, around 0.5 ml filtrate was diluted with 5.0 ml 0.056M Na₂C₂O₄ solution and reading at 266 nm was taken using UV-Visible spectrophotometer (Model- UV-1900i, Shimadzu, Japan) where Na₂C₂O₄ (0.056 M) used for blank sample. Calibration curve using L-ascobic acid was used as reference.

Phytochemical parameters of green pod

Data on different phytochemical properties of green pod were observed on the basis of following protocols:

1. Antioxidant activity (%): Determination of antioxidant by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was done by following Brand et al., (1995). By dissolving 4 mg of DPPH in 100 ml of 95% methanol, DPPH solution was made and stored in dark condition. Plant extract was developed by mixing 5 mg of dried powder of green pod (oven dried for 60 hours at 72°C and powdered with an electric grinder machine) with 5 ml methanol. The control solution used for comparison was mixture of methanol and DPPH solution in 1:3 ratio. To determine the antioxidant activity, plant extract (1ml) was dilute with DPPH solution (3 ml) and the resulting mixture was kept in complete darkness for 30 minutes. The absorbance was recorded at 517 nm wavelength using UV-Visible spectrophotometer (Model- UV-1900i, Shimadzu, Japan). The following equation was used to compute the percentage of antioxidants (Eq. 7):

Antioxidant (%) =
$$\frac{\text{Control reaction absorbance} - \text{Testing specimen absorbance}}{\text{Control reaction absorbance}} \times 100 \text{ (Eq. 7)}$$

2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC): About 1 g fresh pod sample was blended with 80% ethanol (6 ml) and resulting mixture was centrifuged for 20 minutes (8000 rpm). To determine the TPC content, 1 ml previously prepared mixture was diluted with 0.75 ml, 0.25 ml and 1.0 ml of Folin-Ciocalteu reagent, Na₂CO₃ (7.5%), and distilled water in a test tube, respectively. Before taking the absorbance at 765 nm wavelength using UV-Visible spectrophotometer (Model- UV-1900i, Shimadzu, Japan), the dilution was incubated for 90 minutes in a water bath at 30°C. The TPC content was determined using standard curve of gallic acid (Debnath et al., 2018).

TFC was quantified by following Wolfe et al., (2003) with little changes. To determine the TFC content, 0.4 ml previously prepared pod mixture was poured into a 10 ml tube containing 2 ml water (distilled). Then, 0.12 ml of NaNO₂ (5%) solution was added to the tube and kept the tube with mixture at room temperature (5 minutes). After that, 0.24 ml AlCl3 was introduced in the tube and waited for 6 minutes. Before diluting the mixture with distilled water (0.44 ml), 0.8 ml of 1 mol/L NaOH was added. After thoroughly mixing the solution, the absorbance at 765 nm wavelength using UV-Visible spectrophotometer (Model- UV-1900i, Shimadzu, Japan) was taken. The result was obtained as mg of rutin g⁻¹ fresh weight.

Statistical analysis

One-way analysis of variance (ANOVA) was done using RStudio (Version 4.0.3). All the data remained untransformed. Differences between mean values were checked by using Tukey HSD (Honest Significant Difference) test. Significant differences were considered at p<0.05.

Results and Discussion

Phenological parameters of three country bean varieties such as BARI Sheem-1, Sikribi Sheem-1 and Goalgadda are presented in table 1. Plant height and chlorophyll content of leaf (measured by SPAD meter) at 35 DAS showed no significant differences among the country bean varieties. The maximum branch number plant⁻¹ at 35 DAS were counted from BARI Sheem-1 and Sikribi Sheem-1 in comparison to Goalgadda. Similar variation branching habit was previously observed by Singh (1989). Earliest flowering was observed in BARI Sheem-1 and Sikribi Sheem-1. Purseglove (1977) observed

variations in days to first flowering of hyacinth bean and reported that some varieties can produce flower at about 42 DAS. The number of days to first harvest significantly differed among the varieties, where the minimum days required for Sikribi Sheem-1. Variations in days to first harvest was previously reported by Akter *et al.*, (2017) while studied with five different country bean genotypes.

Table 1. Phenological parameters of country bean genotypes

Varieties	Plant height at 35 DAS (cm)	Branches plant ⁻¹ at 35 DAS	SPAD Value at 35 DAS	Days to first flower	Days to first harvest
BARI Sheem-1	184.93	6.65a	45.02	51.75b	80.50a
Sikribi Sheem-1	193.12	5.98ab	44.03	48.50b	71.50 b
Goalgadda	183.20	5. 42b	43.93	57.5 a	80.00a
P-value	0.18	0.04	0.65	<0.001	<0.001

Yield and yield attributes of three country bean genotypes are presented in table 2. Variations were found among the varieties for all measured parameters except pod yield plant⁻¹ and pod yield ha⁻¹. Sikribi Sheem-1 yielded the highest pod number plant⁻¹ (582.50) followed by BARI Sheem-1 (528.75) and Goalgadda (329.45). Akter *et al.*, (2017) harvested different number of pods plant⁻¹ of five different country bean genotypes grown in acidic soil condition of Sylhet. Individual pod weight of Goalgadda was significantly higher than two other varieties since pod length and pod width of Goalgadda was the highest among the varieties studied in this experiment. Similar result in terms of individual pod weight, pod length and width was previously observed by Akter *et al.*, (2017). Sultana (2001) found significant variations in pod size of different country bean genotypes. Pod length and width of the present study are in the same range of the experiment conducted with 249 genotypes in Australia by Pengelly and Maass (2001).

Table 2. Yield and yield contributing attributes of country bean pod

Varieties	No. of pods plant ⁻¹	Individual pod wt. (g)	Pod yield plant ⁻¹ (kg)	Pod length (cm)	Pod width (cm)	Pod yield (ton ha ¹)
BARI Sheem-1	528.75b	6.69b	3.68	10.53b	2.29c	24.90
Sikribi Sheem-1	582.50a	6. 48b	3.78	10.59b	3.04b	25.61
Goalgadda	329. 45 c	10.14a	3.33	13.95a	3.30a	22.55
P-value	<0.001	< 0.001	0.11	< 0.001	< 0.001	0.11

Effect of three country bean varieties on seed yield and yield contributing attributes are presented in table 3. Variations in respect to seed yield and yield attributes were observed except number of seeds per pod among the genotypes. The minimum number of days to first harvest of dried pod was required in Sikribi Sheem-1. Sikribi Sheem-1 yielded the maximum number of dried pod per plant (477.75), while the minimum number of dried pod was harvested from Goalgadda (255.25). Number of dried pods varied greatly among country bean genotypes reported by Akter et al. (2017). The 100 seed weight of Goalgadda (50. 42 g) was comparatively higher since the seed size of Goalgadda was a little larger than that of the two other varieties. The highest seed yield plant and seed yield ha was recorded in Sikribi Sheem-1 (347.50 g and 2.37 ton, respectively). This may be due to the highest number of pods plant of Sikribi Sheem-1. The number of dried pod is the main contributing factor to the per unit seed yield of country bean is reported by Akter *et al.*, (2017).

Table 3. Seed yield and yield attributes of country bean genotypes

Varieties	Days to harvest of dried pod	No. of pod plant ⁻¹	No. of seed pod ⁻¹	Seed yield plant ⁻¹ (g)	100-seed weight (g)	Seed yield (ton ha ⁻¹)
BARI Sheem-1	102.50ab	417.70b	4.50	315.40b	38.26b	2.12b
Sikribi Sheem-1	98.25b	477.75a	4.84	347.50a	36.98b	2.37a
Goalgadda	104.75a	255.25c	5.07	291.95b	50. 42a	1.98b
P-value	0.04	<0.001	0.14	< 0.001	< 0.001	<0.001

Nutritional properties of the green pod of three country bean varieties are presented in table 4. In the green pod, fibre content varied from 0.25 to 0.36% with the highest in Sikribi Sheem-1 and lowest in Goalgadda variety. The minimum fibre content of Goalgadda variety might be more useful for better edibility. The crude fibre content of Goalgadda variety (6.30%) was also measured the lowest among the studied three varieties. In two other varieties such as BARI Sheem-1 and Sikribi Sheem-1, the crude fibre content were recorded 7.72% and 10.44%, respectively. The crude fibre percentage of country bean varieties in this study were much lower than the average 28% crude fibre in dry matter of country bean, which is a major challenge for growing this crop as a forage in the tropical region. High temperatures in tropical region results in decreased amount of soluble carbohydrate causing increased fibre content and decreased digestibility (Norton and Poppi, 1995). The highest percentage of dry matter of BARI Sheem-1 (11.08%) was estimated significantly higher than the Sikribi Sheem-1 (9.85%). The highest ash content was present Goalgadda variety (6.73%) and the lowest was in BARI Sheem-1 (6.35%). Crude protein content of Sikribi Sheem-1 (28.51%) was quantified the highest than two other varieties. Variations in protein content among 21 pole type Indian Lablab bean genotypes was observed by Rai *et al.*, (2014). The maximum Vitamin C content was found in BARI Sheem-1 (13.21 g/100g). Vitamin C is an essential dietary component in human diet and they are unable to synthesize it endogenously (Li and Schellhorn, 2007).

Table 4. Nutritional properties of country bean pods

Varieties	Fibre content (%)	Dry matter (%)	Ash content (%)	Crude fibre (%)	Crude Protein (%)	Vit C (mg 100g ⁻¹)
BARI Sheem-1	0.30ab	11.08a	6.35c	7.72b	22.15b	13.21a
Sikribi Sheem-1	0.36a	9.85c	6.62b	10.44a	28.51a	12.37b
Goalgadda	0.25b	10.30b	6.73a	6.30c	22.4b	12.33b
P-value	0.01	< 0.001	<0.001	< 0.001	<0.001	0.001

The phytochemical properties and antioxidant activity shows significant variation among the three country bean varieties. The total phenol content of Goalgadda (14.60 μgmg⁻¹) was measured the highest, significantly differed with BARI Sheem-1 (10.99 μgmg⁻¹ dry weight) and Sikribi Sheem-1 (8.52 μgmg⁻¹). Total flavonoid content of BARI Sheem-1 (3.36 μgmg⁻¹) and Goalgadda (3.11 μgmg⁻¹) were measured significantly higher than the Sikribi Sheem-1 (2.22 μgmg⁻¹). Goalgadda showed the highest antioxidant (31.12%) than two other varieties such as BARI Sheem-1 and Sikribi Sheem-1. Different experimental results suggest that various polyphenolic compounds such as flavonoids and phenolic content which are found in plant species have multiple biological effects, including antioxidant activity (Vinson *et al.*, 1995). Grassmann *et al.*, (2002) reported that, several biological activities (antiviral, antioxidant, diuretic, anti-rheumatic etc) have relation with phenols. The phenolic compounds perform diverse mechanism in the body. They block, interfere or suppress the activities of enzymes involved in reactive oxygen species generation, quenching free radicals, chelating transition metals to render inactive species (Wong, 2006). In agricultural point of view, TPC have received due attention principally for their relation with defense responses to diseases, since phenol oxidases are oxidized them easily which yield quinines that are highly reactive and toxic to pathogens (Sonali *et al.*, 2015).

Table 5. Phytochemical properties and antioxidant activity in country bean pods

Varieties	Total Phenolic Content (μgmg ⁻¹ dry weight)	Total Flavonoid content (μgmg ⁻¹ dry weight)	Antioxidant (%)
BARI Sheem-1	10.99b	3.36a	29.85b
Sikribi Sheem-1	8.52c	2.22b	28.90b
Goalgadda	14.60a	3.11a	31.12a
P-value	0.001	<0.001	0.002

In conclusion, it was observed from the present study that Sikribi Sheem-1 was the earliest in flowering and harvesting. Although Goalgadda produced the minimum number of pods plant⁻¹, due to its weighty fruit, it attributed to the same pod yield as of two other varieties. Seed production potentiality of Sikribi Sheem-1 was the highest among the varieties. The

lowest fibre and crude fibre content of Goalgadda suggested the increased digestibility of this variety. All studied varieties are the potential source of natural antioxidants, TPC and TFC.

Conclusion

In conclusion, it was observed from the present study that Sikribi Sheem-1 was the earliest in flowering and harvesting. Although Goalgadda produced the minimum number of pods plant-1, due to its weighty fruit, it attributed to the same pod yield as of two other varieties. Seed production potentiality of Sikribi Sheem-1 was the highest among the varieties. The lowest fibre and crude fibre content of Goalgadda suggested the increased digestibility of this variety. All studied varieties are the potential source of natural antioxidants, TPC and TFC.

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