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Morphological and Molecular Characterizations of Country Bean (*Lablab purpureus* L.) Genotypes for Drought Tolerance

Nazifa Tasnia[1*], Ruan Binte Haque[2], Mirza Mofazzal Islam[3], Md. Ashraful Haque[1*]

[1]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2]Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka, Bangladesh
[3]Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh
Email: *nazifa98bd@gmail.com, *ashraf_gpb2000@bau.edu.bd

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Abstract

Country bean, *Lablab purpureus* (L.) is considered one of the most important leguminous crops, but their cultivation under drought stress condition encounters challenges. In this study, an experiment has been conducted among 30 genotypes under drought condition to explore morphological diversity of qualitative and quantitative, biochemical, molecular analysis. The study identified significant variations in eight traits among the genotypes examined, with phenotypic variance exceeding genotypic variance, indicating both genetic and environmental influences. High heritability and genetic advance were observed in primary, secondary, and tertiary branch lengths, suggesting these traits are likely controlled by additive gene effects, making them effective targets for selection. Principal component analysis identified three components that made a substantial contribution, accounting for approximately 73.06% of the overall quantitative variations. Among the quantitative traits, the highest coefficient of variation (CV%) has been found in number of flowers (55.05%). While number of primary branches, primary branch length, number of secondary branches, secondary branch length, number of tertiary branches, tertiary branch length has individually more than 20% of CV%. The genotypes have been grouped into three clusters based on quantitative traits. Analysis of protein reveals that the genotypes of DS28 and DS29 have higher protein content than other genotypes. Dehydrogenase responsive genotypes have been found on DS28 and DS29 from the molecular analysis. The results suggest that the genotypes DS28 and DS29 could contribute as genetic resource of high protein content and DREB responsive, and the eight quantitative traits of 30 genotypes could be used for further breeding programme.

Keywords

Country Bean, Drought Tolerance, Morphological Diversity, High Heritability, Genetic Resource

1. Introduction

Lablab purpureus (L.), commonly known as the country bean, is a prominent species in the Fabaceae family, contributing significantly to the global consumption of grain legumes [1]. This species is an autodiploid, characterized by 11 chromosomal pairs (2n = 2x = 22) and a genome size of 473 Mb [2]. This legume stands out as one of the most versatile crops within the legume category, having been domesticated for various purposes, including its use as a pulse, vegetable (in the forms of green bean, pod, and leaf parts), green manure, and even as an ornamental crop [3]. Notably, the young and immature green pods of this crop are commonly prepared and consumed as vegetables. The pods are abundant in proteins, ranging from 22.4% to 31.3%, and contain 55% carbohydrates, making them an excellent and affordable alternative to costly animal protein sources [4]. This crop is cultivated in all 64 districts and is commonly found in homestead gardens across almost every village in Bangladesh. In terms of production, Bangladeshi farmers harvested approximately 169,735 metric tons of country beans from 61,628 acres of land [5].

Drought stress is increasingly jeopardizing global food production, with country bean-growing areas worldwide encountering significant issues due to dry conditions [6]. Developing cultivars with enhanced drought tolerance could mitigate this issue. Drought stress during the flowering and post-flowering stages can result in a 60% - 90% decrease in country bean yields [7] [8]. Drought stress leads to reduced osmotic pressure and disrupted water potential in plant cells, resulting in oxidative stress, cellular damage, and decreased rates of photosynthesis, carbon fixation, and primary metabolic processes. These effects impact both the survival and yield of the country bean [7]. Research involving 24 commercial cultivars of *Lablab purpureus* in Chile and Bolivia found that drought-tolerant genotypes exhibited greater flexibility in maintaining stomatal conductance, photosynthetic rates, abscisic acid synthesis, and resistance to photoinhibition, positively influencing yield [9].

The foremost challenge in the cultivation of country beans is the increased difficulty and decreased productivity under drought conditions. A promising approach to mitigating the adverse effects of drought is the strategic utilization of genetic diversity found within various bean genotypes. Genetic diversity provides a broad range of traits that can be leveraged to enhance drought tolerance, yield stability, and overall resilience of crops in fluctuating environmental conditions. By selecting and breeding genotypes with desirable traits, such as enhanced root systems, efficient water use, and heat tolerance, it is possible to improve the

adaptability and productivity of country beans in water-limited environments. Implementing a breeding program can lead to the development and enhancement of varieties that are well-suited to thrive under drought conditions. Increasing the protein content in country bean pods is vital due to their role as a significant protein source. Utilizing molecular analysis is an effective method for identifying genotypes associated with dehydrogenase response, facilitating the cultivation of varieties that can adopt in drought conditions.

This study involved the collection of thirty genotypes of country bean, which were cultivated under drought conditions to assess their morphological, biochemical, and molecular characteristics. Observations of both qualitative and quantitative traits revealed morphological diversity, indicating potential for further genetic enhancement to improve drought adaptation in country beans. Protein content in pods was evaluated from a random selection of plants, showing that all pods maintained a satisfactory protein level. Molecular analyses such as DNA extraction, PCR, and agarose gel electrophoresis were conducted to identify dehydrogenase responsive genes under drought stress, identifying two genotypes at 595 bp. This research can guide the development of country bean varieties that not only survive drought stress but also provide high yield and adequate nutrient content.

2. Materials and Methods

2.1. Experimental Site and Duration

The research was performed at the experimental farm of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Mymensingh, from August 2021 to March 2022. The experimental site had sandy loam soil with a pH ranging from 6.5 to 6.7. The region is characterized by a tropical climate with wet summers and dry winters. While the early stages of the experimental period experienced moderate rainfall and temperature levels, the study was specifically designed to simulate and analyze the impact of drought conditions on the plants. To ensure drought stress, controlled irrigation and water deficit treatments were employed during the critical growth stages of the plants, replicating the conditions needed to evaluate drought responses effectively.

2.2. Plant Materials

Table 1. List of country bean genotypes used for morphological and molecular analysis in the study.

Genotypes									
DS1	DS2	DS3	DS4	DS5	DS6	DS7	DS8	DS9	DS10
DS11	DS12	DS13	DS14	DS15	DS16	DS17	DS18	DS19	DS20
DS21	DS22	DS23	DS24	DS25	DS26	DS27	DS28	DS29	DS30

The experiment involved thirty genotypes of the country bean, *Lablab purpureus* (L.), as detailed in Table 1. The seeds for these genotypes were obtained from the Field Laboratory of the Department of Genetics and Plant Breeding at Bangladesh Agricultural University in Mymensingh. The genotypes selected for the study were those commonly found in local rural communities. The genotypes chosen for this research are widely cultivated varieties, esteemed for their high productivity by local farmers.

2.3. Experimental Design, Layout, and Setting the Experiment

The experiment followed a Randomized Complete Block Design (RCBD) with three replications. Seeds were sown on August 29, 2021. The plot for the experiment was prepared by digging pits approximately one week prior to sowing. Pits were spaced 2.0 meters apart between rows and within rows, and each pit was dug to a depth of 1.2 feet. Three to four seeds were sown in each pit. The seeds were planted at a depth of 2 to 3 cm into the soil.

2.4. Intercultural Operations

The seeds germinated within 4 - 6 days after sowing. After approximately one-month, extra seedlings were thinned out, leaving only one healthy plant per pit. Each young plant was supported with a single stick in its pit. Weeding was conducted twice: first at 25 days after sowing and again at 50 days after sowing. The harvesting of green edible pods started in the second week of January 2022 and continued until March 2022. The timing of pod harvesting varied among the genotypes, depending on their individual maturity stages.

2.5. Morphological Parameters Recorded

Data were collected on several parameters including plant height, number and length of primary, secondary, and tertiary branches, as well as the number of flowers.

2.6. Statistical Analysis

Multivariate analysis was conducted using XLSTAT software. PCA was used to identify groupings and determine the axes and attributes that significantly contributed to variance, based on the similarity matrix. Scatter plots were created using the first two principal components, as these accounted for the greatest variance. Agglomerative Hierarchical Clustering (AHC) was performed using Ward's minimal variance method and squared Euclidean distances to measure dissimilarity [10] [11]. Descriptive statistics like range, mean, standard deviation, and coefficient of variation (CV%) for various quantitative traits were calculated. A one-sample variance test was performed to evaluate the null hypothesis and determine the significance of the observed variation. Additionally, Pearson's correlation matrix was utilized to analyze the relationships between the variables [10]. RStudio software was utilized to compute the genotypic and phenotypic coefficients

of variation (GCV and PCV), environmental coefficient of variation (ECV), broad-sense heritability, genetic advance (GA), and genetic advance as a percentage of mean. Established methods were applied in RStudio to calculate these statistical parameters [12]-[14].

2.7. Biochemical Parameters Recorded

Protein content in the dry pods was measured from ten randomly chosen plants. On December 5, fresh pods were harvested from ten distinct genotypes, as detailed in Table 2. The seeds from each pod were oven-dried at 65˚C for 5 days to lower their moisture content. The crude protein content was determined using the Kjeldahl method, with the results multiplied by the 6.25 conversion factor and expressed as a percentage (%) based on AOAC standards [15].

Table 2. List of country bean genotypes used for biochemical analysis in the study.

Genotypes									
DS1	DS2	DS3	DS5	DS6	DS7	DS8	DS15	DS28	DS29

2.8. Molecular Analysis for Identification of Dehydrogenase Responsive Genes

The DNA was isolated from actively growing young fresh leaves (14 days old seedling) by DNeasy Plant Mini Kit method. A UV spectrophotometer was used to measure DNA concentration by reading the absorbance at wavelengths of 260 nm and 280 nm. The concentration was determined using the equation: Purity = absorbance at 260 nm/absorbance at 280 nm. The result of purity concentrations was recorded in this study (Table 3). DNA quality was checked by running the genomic DNA sample on 1.5% Agarose gel. Two set of SSR primers were used in the polymerase chain reaction (PCR). The names of the SSR primer were ROCII and SG6**.** The gene specific primers were designed by NCBI primer BLAST using country bean genome. PCR was an important step in molecular analysis. PCR cocktail was prepared for DNA marker and then 9 µl of the PCR cocktail was taken in the separate PCR tubes and 1 µl DNA extract of each sample was added in it (Table 4). The PCR tubes with the PCR reaction mixture were sealed and placed in a thermos cycler and the PCR reaction was started immediately (Table 5). For confirmation of the target gene, agarose gel electrophoresis was done after completing the PCR. The presence and absence of DNA band was considered for identifying the presence or absence of gene of interest.

3. Results and Discussion

3.1. Quantitative Traits

Significant variations have been observed among the *Lablab* germplasms in traits of plant height, number of primary branches, primary branch length, number of secondary branches, secondary branch length, number of tertiary branches,

tertiary branch length and number of flowers. Plant height ranged from 151 to 240 cm with the mean value of 180.63 cm, SD and CV are found to be 24.29 cm and 13.45%, respectively. DS20 exhibits the tallest plant height, while DS13 is recorded the lowest height. The average number of primary branches is 13.33 cm, with the ranged from 5 to 20 cm, SD and CV are found to be 3.84 cm and 28.83%, respectively.

Table 3. DNA extracted concentrations and purity from the genotypes.

Genotypes	Purity	Concentration/ng/µl sample type
DS1	2.18	15 ds DNA
DS2	1.94	35.6 ds DNA
DS3	2.64	85.5 ds DNA
DS4	2.64	33.8 ds DNA
DS5	2.19	126.4 ds DNA
DS6	2.25	15.4 ds DNA
DS7	2.01	79.8 ds DNA
DS8	2.21	41.4 ds DNA
DS9	2.66	20.8 ds DNA
DS10	2.69	21.1 ds DNA
DS11	1.93	48.2 ds DNA
DS12	2.24	115.3 ds DNA
DS13	2.25	85.0 ds DNA
DS14	2.13	74.2 ds DNA
DS15	2.22	11.9 ds DNA
DS16	2.05	14.5 ds DNA
DS17	2.11	11.5 ds DNA
DS18	2.37	9.6 ds DNA
DS19	2.56	9.2 ds DNA
DS20	2.15	11.3 ds DNA
DS21	2.35	11.8 ds DNA
DS22	2.72	15.2 ds DNA
DS23	2.17	12.5 ds DNA
DS24	1.89	10.7 ds DNA
DS25	2.12	14.9 ds DNA
DS26	2.63	9.4 ds DNA
DS27	2.07	11.4 ds DNA
DS28	2.09	31.2 ds DNA
DS29	2.05	20.5 ds DNA
DS30	2.48	21.8 ds DNA

Table 4. Components of PCR cocktail.

Sl. No.	Component	Quantity (for single reaction)
1	Master mixture	5 µl
2	ddH_2O	2 µl
3	Primer forward	1 µl
4	Primer Reverse	1 µl
	Total	9 µl

Table 5. Steps of polymerase chain reaction (PCR).

Steps	Temperature (˚C)	Duration
Initial denaturation	94	2 min
Denaturation	94	1 min
Annealing	55	1 min
Extension	72	2 min
Final extension	72	5 min
Store	4	

DS4 and DS9 are observed to be the highest number of primary branches, while DS10 exhibits the lowest. The average length of primary branches ranged from 77 to 167 cm, with a mean of 121.80 cm, a coefficient of variation (CV) is 26.46%, and a standard deviation (SD) is 32.23 cm. DS5 is recorded the highest primary branch, whereas DS24 is the shortest.

The number of secondary branches varied between 2 and 12 cm, with an average of 5.40 cm. The standard deviation (SD) was 2.50 cm, and the coefficient of variation (CV) was 46.29%. DS20 exhibited the highest number of secondary branches, whereas DS2 had the fewest. Secondary branch lengths ranged from 17 to 148 cm, with an average length of 77.67 cm. The standard deviation was 32.44 cm, and the coefficient of variation was 41.77%. DS24 had the longest secondary branches, whereas DS3 had the shortest. The number of tertiary branches averaged 2.40 cm, ranging from 1 to 4 cm. The standard deviation was 0.93 cm, and the coefficient of variation was 38.84%. The genotypes DS4, DS8, and DS14 had the highest number of primary branches, whereas DS7, DS10, DS13, DS17, DS23, and DS29 had the lowest. Tertiary branch lengths ranged from 6 to 30 cm, with an average length of 13.90 cm. The standard deviation was 5.26 cm, and the coefficient of variation was 37.85%. DS20 had the longest tertiary branches, whereas DS7 had the shortest. The number of flowers averaged 2.47 cm, ranging from 1 to 6 cm. The standard deviation was 1.36 cm, and the coefficient of variation was 55.05%. DS25 had the highest number of flowers, whereas DS1, DS3, DS8, DS9, DS11, DS14, DS19, and DS28 had the fewest (**Table 6**). The observed variations stem from the different genotypes, offering substantial potential for yield enhancement. High coefficients of variation (CV > 20%) in traits like the number

and length of branches and the number of flowers indicate considerable variability, which influences plant yield. The high CV values highlight traits with substantial variability, which can be harnessed for further enhancement. The extensive diversity in traits offers ample opportunities to select genotypes suited to specific breeding program needs [16].

Table 6. The descriptive statistics and analysis of quantitative morphological traits of *Lablab* germplasms.

Variables	Mean	Max	Min	SD	CV (%)	Standard error of mean	p-value* (Two-tailed)
Plant Height (cm)	180.63	240	151	24.29	13.45	4.436	0.886
Number of Primary Branches	13.33	20	5	3.84	28.84	0.702	0.825
Primary Branch length (cm)	121.80	167	77	32.23	26.46	5.885	0.757
Number of Secondary Branches	5.40	12	2	2.50	46.29	0.456	0.905
Secondary Branch length (cm)	77.67	148	17	32.44	41.77	5.923	0.993
Number of Tertiary Branches	2.40	4	1	0.93	38.84	0.170	0.905
Tertiary Branch length (cm)	13.90	30	6	5.26	37.85	0.960	0.991
Number of Flowers	2.47	6	1	1.36	55.05	0.248	0.528

Traits having more than >20.00% higher CV value are highlighted with orange colour. * = significance at alpha 0.05 of one-sample variance z-test.

3.2. Analysis of Genetic Parameters, Heritability, and Genetic Advance

Successful crop improvement programs rely on a breeder's capacity to identify and harness genetic variability and highly heritable traits, once environmental influences on phenotypic variation are accounted for [17]. Understanding both the phenotypic (PCV) and genotypic coefficients of variation (GCV) is crucial for breeders to estimate heritability and predict the potential for genetic advance (GA) through trait selection.

In this study, the phenotypic and genotypic coefficients of variation, as well as heritability, were estimated for eight traits within the collected germplasms. The phenotypic coefficient of variation (PCV) exceeded the genotypic coefficient of variation (GCV) for all traits (**Table 7**). This suggests that the observed variation is influenced by both genetic factors and environmental effects on these traits. Traits such as primary branch length (26.11, 23.65), number of secondary branches (42.23, 26.90), secondary branch length (42.13, 42.10), number of tertiary branches (37.99, 27.54), and tertiary branch length (35.79, 33.53) exhibited high phenotypic and genotypic coefficients of variation.

It suggests that there are both genetic diversity and environmental influence

contributing to the trait's variability. Moderate-high PCV (26.85) and moderate GCV (16.07) are recorded for number of primary branches. The low GCV of plant height (7.79) and number of flowers (8.12) suggests that influence of the environment on the expression of this characters (Table 7). Hence, relying solely on the phenotypic expression of this trait for selection would be ineffective in enhancing crop improvement.

Table 7. Estimation of genetic parameters in eight characters of thirty genotypes of *Lablab* germplasms.

Traits	GV	PV	EV	GCV	PCV	ECV	Heritability (broad sense)	GA	GA (% mean)
PH	190.92	452.19	261.27	7.79	11.99	9.11	42.22%	18.49	10.43
NPB	4.41	12.31	7.89	16.07	26.85	21.51	35.84%	2.59	19.83
PBL	837.76	1020.93	183.16	23.65	26.11	11.06	82.06%	54.01	44.14
NSB	1.94	4.78	2.84	26.90	42.23	32.55	40.59%	1.83	35.31
SBL	1049.75	1051.35	1.61	42.10	42.13	1.65	99.85%	66.69	86.66
NTB	0.59	1.11	0.53	27.54	37.99	26.17	52.55%	1.14	41.12
TBL	21.03	23.96	21.03	33.53	35.79	12.51	87.78%	8.85	64.72
NF	o.05	1.41	1.46	8.12	42.20	42.97	3.70%	0.037	3.22

The quantitative morphological traits are plant height (*PH*), *number of primary branches* (*NPB*), *primary branch length* (*PBL*), *number of secondary branches* (*NSB*), *secondary branch length* (*SBL*), *number of tertiary branches* (*NTB*), *tertiary branch length* (*TBL*) *and number of flowers* (*NF*).

GV: *Genotypic Variance*, *PV*: *Phenotypic Variance*, *EV*: *Environmental Variance*, *GCV*: *Genotypic Coefficient of Variance*, *PCV*: *Phenotypic Coefficient of Variance*, *ECV*: *Environmental Coefficient of Variance*, *GA*: *Genetic Advance*, *GA* (% *mean*): *Genetic Advance as percentage of mean*.

High heritability combined with a high genetic advance as a percentage of the mean suggests that additive gene effects may be influencing this trait, which can be advantageous for plant breeders during selection. High heritability coupled with high genetic advance as percent of mean has been observed in following characters, primary branch length (82.06, 44.14), secondary branch length (99.85, 86.66), tertiary branch length (87.78, 64.72) (Table 7). High values for heritability and genetic advance as a percent of the mean for seed yield indicate that this trait is likely influenced by additive gene effects, making selection for improvement highly effective. Selected varieties of cowpea exhibited a considerable degree of heritability in relation to yield [18]. High heritability combined with moderate genetic advance could be attributed to moderate phenotypic standard deviation values. Since heritability is high for these traits and the selection differential remains constant [19]. Here, moderate heritability, and genetic advance as percent of the mean has been observed in plant height (42.22, 10.43), number of primary

branches (35.84, 19.83), number of secondary branches (40.59, 35.31), number of tertiary branches (52.55, 41.12).

3.3. Analysis of Correlation Matrix

Genotypic correlations for various trait combinations in the *Lablab* germplasms are displayed in Table 8. Several quantitative traits exhibit significant correlations, either positive or negative. Plant height (PH) shows a strong positive and significant correlation with the number of primary branches (NPB). It also shows positive but non-significant correlations with the number of secondary branches (NSB), the number of tertiary branches (NTB), and the length of tertiary branches (TBL). Conversely, plant height (PH) has negative and non-significant correlations with primary branch length (PBL), secondary branch length (SBL), and the number of flowers (NF). This pattern suggests that selecting for taller plants (PH) may effectively enhance the number of primary branches in *Lablab* germplasms.

Table 8. Correlation matrix among the quantitative morphological characters of *Lablab* germplasm.

Variables	PH	NPB	PBL	NSB	SBL	NTB	TBL	FN
PH	1							
NPB	0.48**							
PBL	−0.13	0.04						
NSB	0.19	0.41*	0.09					
SBL	−0.16	−0.17	−0.34	0.38*				
NTB	0.34	0.69**	0.05	0.39*	0.09			
TBL	0.11	−0.05	0.03	0.65**	0.52**	−0.04		
NF	−0.25	−0.36*	−0.14	−0.13	0.49**	−0.23	0.20	1

**Indicate highly significant results ($P < 0.05$ and $P < 0.02$); *Indicate significant results ($P < 0.05$ and $P < 0.02$). The quantitative morphological traits are plant height (PH), number of primary branches (NPB), primary branch length (PBL), number of secondary branches (NSB), secondary branch length (SBL), number of tertiary branches (NTB), tertiary branch length (TBL) and number of flowers (NF).

The number of primary branches (NPB) exhibits a strong positive and significant correlation with the number of tertiary branches (NTB). It shows a significant negative correlation with the number of flowers (NF) and a significant positive correlation with the number of secondary branches (NSB). Additionally, NPB has positive but non-significant correlations with primary branch length (PBL), while it shows negative and non-significant correlations with secondary branch length (SBL) and tertiary branch length (TBL).

Primary branch length (PBL) demonstrates positive but non-significant correlations with the number of secondary branches (NSB), the number of tertiary branches (NTB), and tertiary branch length (TBL). In contrast, it has negative and non-significant correlations with secondary branch length (SBL) and the number

of flowers (NF).

The number of secondary branches (NSB) shows a strong positive and significant correlation with tertiary branch length (TBL). It also exhibits a positive and significant correlation with secondary branch length (SBL) and the number of tertiary branches (NTB). NSB has a negative and non-significant correlation with the number of flowers (NF).

Secondary branch length (SBL) has strong positive and significant correlations with tertiary branch length (TBL) and the number of flowers (NF). It shows a positive but non-significant correlation with the number of tertiary branches (NTB). The number of tertiary branches (NTB) has negative and non-significant correlations with both tertiary branch length (TBL) and the number of flowers (NF). Lastly, tertiary branch length (TBL) exhibits a positive but non-significant correlation with the number of flowers (NF).

3.4. Principal Component Analysis (PCA) Analysis of Quantitative Morphological Characters

Principal Component Analysis (PCA) was conducted on 8 quantitative morphological traits to identify the main factors or components that significantly influence the overall indicators. The eigenvalues, the percentage of contribution to variability, and the cumulative contribution rates of the first three principal components (F1, F2, F3) are depicted in Figure 1. The PCA results show that these three components collectively explain approximately 75% of the total variation in quantitative traits. These components were selected for further analysis (Table 9, Figure 1). The first principal component (F1) explains the largest portion of the variance at 31.18%, followed by the second component (F2) with 27.08%, and the third component (F3) which accounts for 14.79% of the total morphological variation (Table 9).

Table 9. Eigenvalues, proportion of variability and cumulative rate of the 8 quantitative morphological characters of *Lablab* germplasm contributed to the first three components (PCs).

Component	F1	F2	F3
Plant Height (cm)	0.639	−0.078	−0.313
Number of primary branches	0.872	−0.136	−0.133
Primary branch length (cm)	0.105	−0.248	0.872
Number of secondary branches	0.627	0.613	0.307
Secondary branch length (cm)	−0.093	0.881	−0.213
Number of tertiary branches	0.787	0.027	−0.170
Tertiary branch length (cm)	0.171	0.812	0.330
Number of flowers	−0.514	0.519	−0.173
Eigenvalues	2.49	2.17	1.18
Variability rate (%)	31.18	27.08	14.79
Cumulative (%)	31.18	58.27	73.06

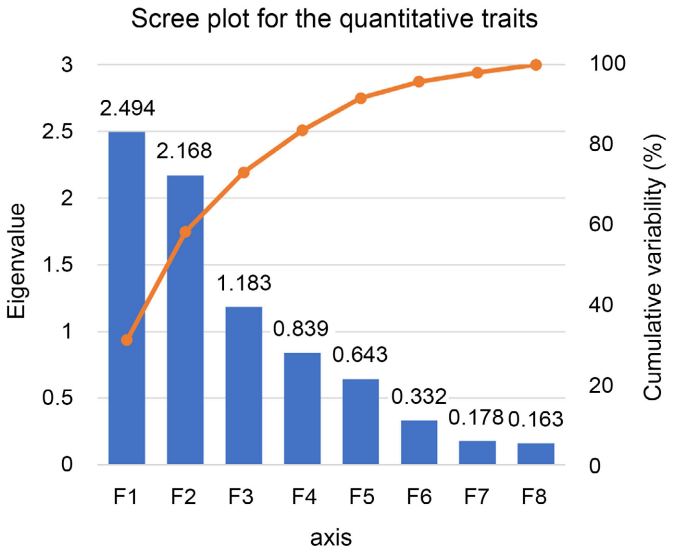

Figure 1. The scree plot illustrates the eigenvalues of various principal components (represented by the bars) and the cumulative percentage of variability (depicted by the line) as revealed by the PCA analysis of the *Lablab* germplasms based on quantitative morphological traits.

The Principal Component Analysis (PCA) of the quantitative traits shows that the plant height (PH), number of primary branches (NPB), primary branch length (PBL), number of tertiary branches (NTB), number of secondary branches (NSB), and tertiary branch length (TBL) are significantly represented in the first principal component (F1). This component has positive contributions from all variables except for secondary branch length and number of flowers, which have negligible contributions. The second principal component (F2) is positively influenced by the number of secondary branches, secondary branch length, number of tertiary branches, tertiary branch length, and number of flowers, but negatively influenced by plant height, number of primary branches, and primary branch length. The third principal component (F3) shows positive contributions from primary branch length, the number of secondary branches, and tertiary branch length (**Table 9**).

Together, the first two components (F1 and F2) account for 58.27% of the total variation in quantitative morphological traits. **Figure 2** illustrates the contributions and directions of the various traits within these principal components. It also presents the genotypic distribution of *Lablab* germplasms. Genotypes located close to each other in the plot show similarities across the eight assessed variables, indicating a lack of significant diversity as they cluster within the same region. Notably, genotypes such as DS25, DS20, DS5, DS12, DS10, DS13, and DS23 are positioned farther from the others along F1 and F2, suggesting that crosses between these genotypes might yield better segregants.

PCA is a valuable tool for understanding the relationships between variables, simplifying data collection, and interpreting complex datasets [20]. The analysis of the eight quantitative traits reveals that the first three components contribute 73.06% of the total variation, aligning with previous findings where the first six

components of the *Lablab* germplasms core collection explained 73.74% of the total variation [21]. Similarly, in the field pea crop, the first three principal components account for 75% of the total variation [22].

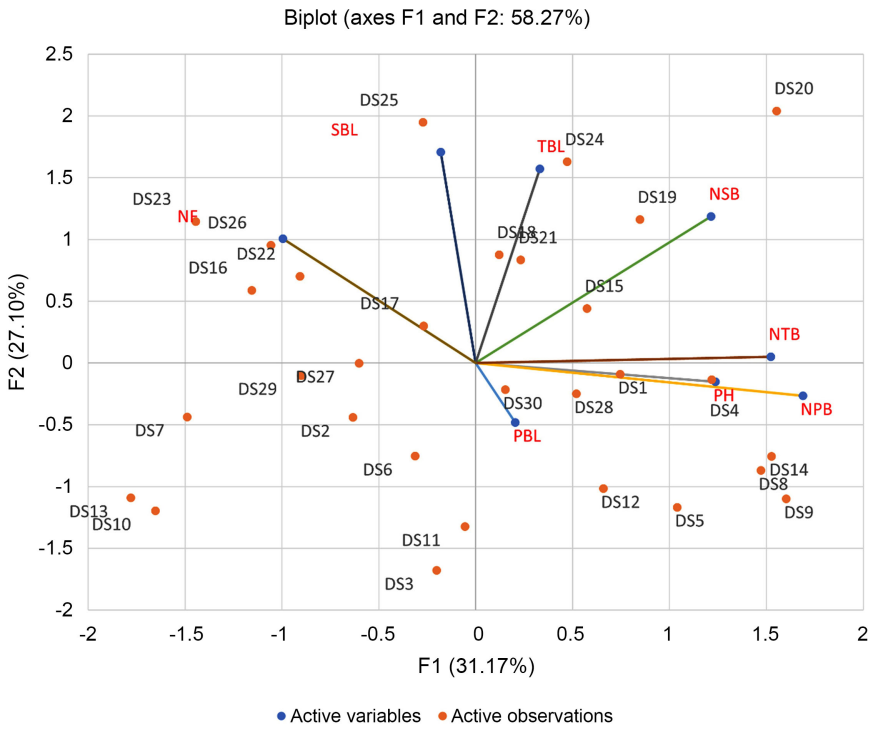

Figure 2. The biplot of 30 *Lablab* germplasms for principal components one (F1) and two (F2) for the quantitative traits. The lines represent the contribution, both in magnitude and direction, of the eight quantitative morphological traits within the principal components F1 and F2.

3.5. Cluster Analysis

The agglomerative hierarchical clustering (AHC) analysis was performed using the Ward method. The resulting dendrogram, shown in **Figure 3**, illustrates the grouping of the 30 *Lablab* germplasms based on their quantitative morphological traits. These germplasms were categorized into 3 distinct clusters. Specifically, 12, 6, and 12 germplasms were grouped into Clusters I, II, and III, respectively (**Table 10**, **Figure 3**). Clusters I and III are the largest, each containing 12 genotypes.

Further analysis of the mean performance for different quantitative traits within each cluster, as compared to the overall mean of the 30 germplasms, is summarized in **Table 11**. Cluster I, which includes 12 germplasms, is characterized by higher mean values for the number of flowers compared to Cluster III and the overall mean for the 30 germplasms (**Table 11**). Cluster II, which consists of 6 germplasms, exhibits higher values for primary branch length, the number of secondary branches, secondary branch length, tertiary branch length, and the number of flowers. Cluster III, also comprising 12 germplasms, shows higher mean values for traits such as plant height, the number of primary branches, and the number of tertiary branches compared to the other clusters and the overall mean

for the 30 germplasms evaluated (Table 11).

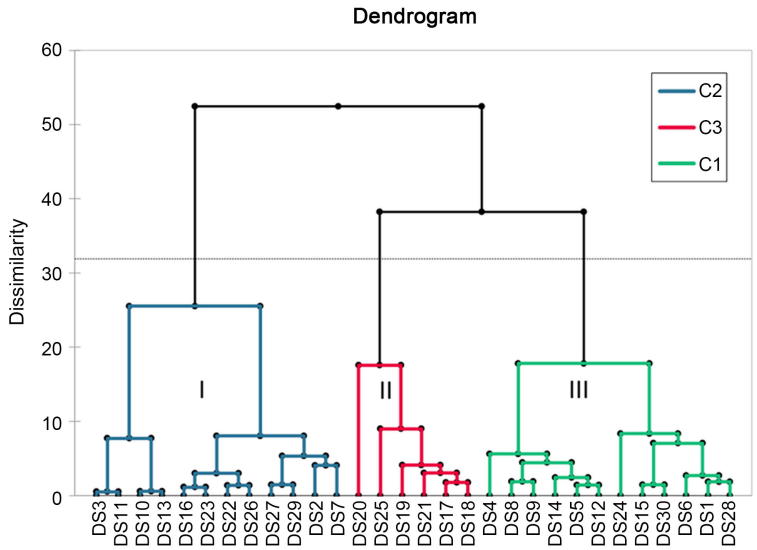

Figure 3. Agglomerative hierarchical clustering (AHC) dendrogram analysis using Euclidean distance into different clusters by the Ward method [9] for quantitative morphological traits of 30 *Lablab* germplasms.

Table 10. Groups of 30 genotypes according to cluster analysis.

Cluster number	Number of genotypes	Genotypes
I	12	DS3, DS11, DS10, DS13, DS16, DS23, DS22, DS26, DS27, DS29, DS2, DS7
II	6	DS20, DS25, DS19, DS21, DS17, DS18
III	12	DS4, DS8, DS9, DS14, DS5, DS12, DS24, DS15, DS30, DS6, DS1, DS28

Table 11. The mean performance of different 8 quantitative traits in each cluster along with mean of the evaluated 30 germplasm.

Traits	Mean performance of different clusters based on 8 quantitative traits			
	Cluster I	Cluster II	Cluster III	For 30 germplasm
PH	168.42	181.17	192.58	180.63
NPB	9.25	13.17	16.83	13.33
PBL	116.67	147.67	114	121.80
NSB	3.67	8.33	5.75	5.40
SBL	74.17	100.17	69.92	77.67
NTB	1.67	2.33	3.67	2.40
TBL	12.42	21.33	11.67	13.90
NF	2.83	2.83	1.92	2.47

Cluster analysis organizes objects based on high levels of similarity, which is beneficial for identifying diverse allele sets in future heterotic breeding programs [23]. None of the clusters encompassed all the desired traits for breeding purposes;

rather, desirable traits were distributed across different clusters. These traits significantly contribute to the genetic diversity observed in country bean germplasms [24]. The dendrogram indicates that germplasms with similar morphological characteristics are often grouped within the same cluster. This observation aligns with findings in Soybean and other crops [25]-[30]. Crossbreeding between highly divergent clusters is recommended to develop genotypes with preferred traits, aiding breeders in selecting parent lines for hybrid varieties and leveraging heterosis in subsequent generations.

3.6. Qualitative Traits

The diversity of flower colors among the *Lablab* germplasms is illustrated in **Figure 4**. The flower colors, specifically the wing and petal hues, exhibit six types of variation. The predominant colors are white, purple, and light purple. The *Lablab* germplasms studied show six distinct flower color variations: white, light purple, light violet, pink, purple, and very light violet. These colorful flower traits are important for attracting pollinators, which play a crucial role in cross-pollination [31] [32]. Additionally, the pigments responsible for these colors are part of a diverse group of compounds that provide defensive benefits to plants, including protection against UV radiation, diseases, and insect pests [33].

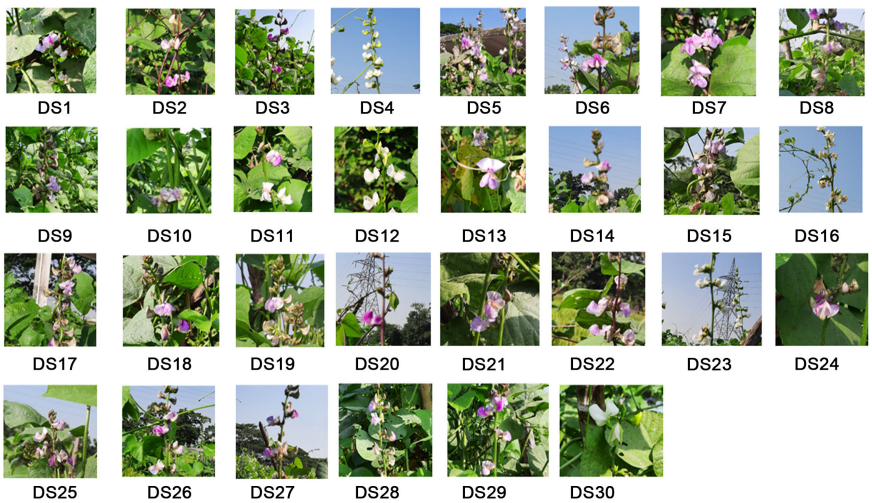

Figure 4. Flower colour diversity among the 30 Lablab germplasm from Bangladesh.

3.7. Protein Content

The protein content across different *Lablab* germplasms ranges from 33.60% to 45.67%. The highest protein content is observed in genotype DS28, with 45.67%, while the lowest is found in DS3, at 33.60% (**Figure 5**). The dry seeds of country beans contain five protein fractions: globulin-1 (phaseolin), globulin-2, albumin, prolamine, and alkali-soluble proteins [34]. The protein content in country beans can vary due to environmental factors and the interaction between the plant's genetics and its growing location [35]. Furthermore, the protein content is slightly altered when beans are cooked [36].

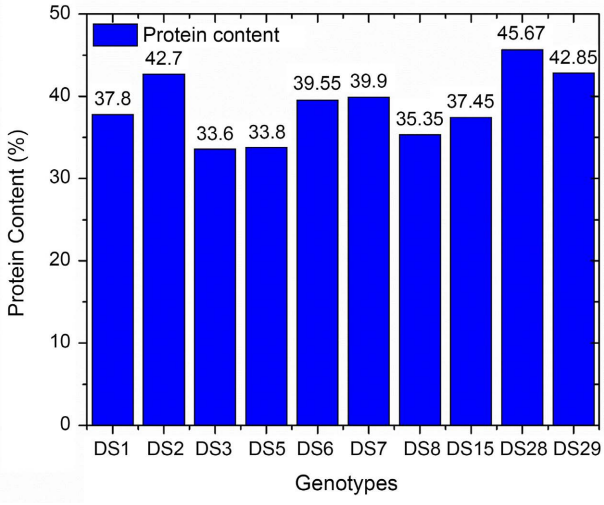

Figure 5. Crude protein in dry bean pods of ten randomly selected genotypes.

3.8. Molecular Analysis

Following the DNA extraction from leaf samples, agarose gel electrophoresis was performed to verify the presence of DNA in the samples. DNA was successfully detected in all thirty samples (**Figure 6(a)**, **Figure 6(b)**). The primers ROCII and SG6 effectively identified 2 out of the 30 genotypes, producing distinct bands at approximately 595 bp, which confirmed the presence of the targeted gene (**Figure 6(c)**, **Figure 6(d)**). The distinct banding patterns for all thirty genotypes are detailed in **Table 12**. The ROCII and SG6 markers are associated with the bc3 gene. These SSR markers, linked to the bc3 gene, are utilized to identify genomic regions that influence dehydrogenase responsiveness. This information can be applied in marker-assisted selection to enhance the genetic traits of the country bean crop, specifically selecting lines with alleles for DREB responsive traits.

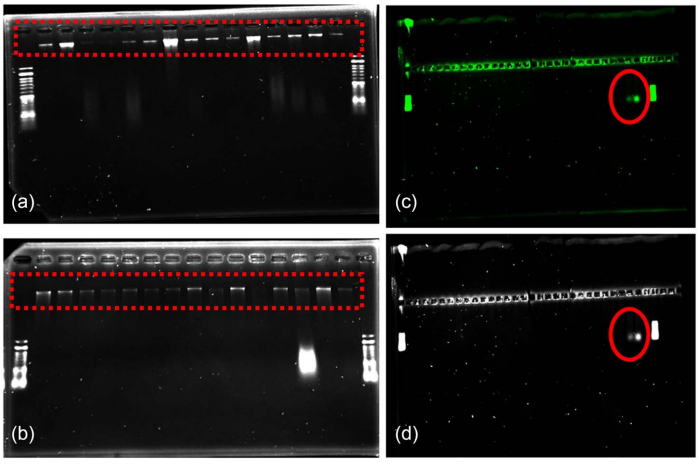

Figure 6. (a) DNA banding pattern of 1st 15 genotypes of Country Bean after gel electrophoresis (b) DNA banding pattern of another 15 genotypes of Country Bean after gel electrophoresis. (c) and (d) The two images show the banding pattern after gel electrophoresis at 28 and 29 number genotypes using SSR primers. The name of the SSR primers is SG6 and ROCII.

Table 12. Different banding pattern of 30 genotypes.

Genotypes	Banding pattern		Resistant	Susceptible
	Presence (P)	Absence (A)	(R)	(S)
DS1	-	A	-	S
DS2	-	A	-	S
DS3	-	A	-	S
DS4	-	A	-	S
DS5	-	A	-	S
DS6	-	A	-	S
DS7	-	A	-	S
DS8	-	A	-	S
DS9	-	A	-	S
DS10	-	A	-	S
DS11	-	A	-	S
DS12	-	A	-	S
DS13	-	A	-	S
DS14	-	A	-	S
DS15	-	A	-	S
DS16	-	A	-	S
DS17	-	A	-	S
DS18	-	A	-	S
DS19	-	A	-	S
DS20	-	A	-	S
DS21	-	A	-	S
DS22	-	A	-	S
DS23	-	A	-	S
DS24	-	A	-	S
DS25	-	A	-	S
DS26	-	A	-	S
DS27	-	A	-	S
DS28	P	-	R	-
DS29	P	-	R	-
DS30	-	A	-	S

4. Discussion

The results of this study illustrate the significant impact of environmental conditions, particularly local weather patterns, on the phenotypic traits of *Lablab* genotypes. Mymensingh's climate, characterized by wet summers and dry winters, had a noticeable influence on traits such as plant height, branching, and flowering

patterns. The moderate rainfall observed during the early stages of the experimental period likely promoted uniform vegetative growth, as seen in the consistent plant height among the genotypes. However, the controlled drought conditions imposed during critical growth phases led to a marked variation in branching and pod-related traits, highlighting the influence of water availability on these phenotypic characteristics.

Phenotypic plasticity was evident in the way different genotypes adjusted to the imposed drought stress. For instance, genotypes that exhibited a greater number of primary and tertiary branches suggest an adaptive mechanism to optimize reproductive success under limited water conditions. This finding aligns with previous studies in other crops, such as field peas and soybeans, where drought stress led to similar morphological adaptations, including alterations in branching and flowering behavior. The high genotypic coefficients of variation observed in traits like branch length emphasize the potential for selecting genotypes with favorable adaptive traits for drought tolerance.

Several factors may influence the successful improvement of country bean crops, especially under drought conditions. These factors include the availability of genetic diversity, effective marker-assisted selection, and the ability to leverage phenotypic traits with high heritability. The genetic diversity observed in this study provides a valuable resource for breeding programs. By focusing on traits such as primary branch length and tertiary branch number, which exhibited high variability and heritability, breeders can make informed selections to develop more drought-tolerant varieties [37].

Moreover, successful crop improvement depends on the integration of advanced molecular techniques with traditional breeding practices. Marker-assisted selection (MAS), using SSR markers like ROCII and SG6, can expedite the breeding process by enabling the identification of genotypes with desirable traits, such as dehydrogenase responsiveness for enhanced drought tolerance. Examples from other crops demonstrate the effectiveness of this approach. For instance, in rice, the use of MAS to incorporate drought-resistance genes has resulted in the development of varieties like "Sahbhagi Dhan", which has been widely adopted in drought-prone regions of India [38]. Similarly, in maize, the Water Efficient Maize for Africa (WEMA) initiative has successfully introduced drought-tolerant varieties that have improved crop yields in regions facing severe water scarcity [39].

The combination of genetic diversity, strategic selection of high-heritability traits, and the use of molecular breeding techniques are critical factors for successful crop improvement. The insights gained from this study can guide breeding programs aimed at developing country bean varieties capable of thriving under drought stress, ultimately contributing to enhanced food security and sustainable agriculture.

5. Conclusion

This study, conducted under drought conditions, aimed to analyze morphological

diversity in both qualitative and quantitative traits, measure protein content in pods, and identify dehydrogenase-responsive genotypes among 30 different *Lablab* genotypes. The morphological analysis revealed significant variation across eight morphological traits among the 30 genotypes. These genotypes could be utilized as valuable genetic resources for further improvement of various traits in the country bean. Notably, traits such as primary branch length, the number of secondary branches, secondary branch length, the number of tertiary branches, and tertiary branch length exhibited high genotypic coefficients of variation. Consequently, these traits are considered favorable targets for enhancement through effective phenotypic selection. Most traits showed positive correlations with each other, suggesting that selecting for highly heritable and easily measurable phenotypic traits could facilitate the simultaneous improvement of these traits. Cluster analysis, incorporating all eight traits from the 30 genotypes, grouped the genotypes into three main clusters. This clustering was influenced not only by geographical origin but also by the quantitative traits. The statistical analyses indicate that the eight traits across the 30 genotypes could be used to develop superior varieties capable of thriving under drought stress. Biochemical and molecular analyses identified genotypes DS28 and DS29 as having higher protein content and being dehydrogenase responsive. These findings highlight DS28 and DS29 as valuable sources of protein and genetic resources, particularly useful for breeding programs aimed at improving drought tolerance.

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Data Availability

The data that support the findings of this study are available in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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