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RESEARCH

Unlocking the Genetic Potential of Soybean (*Glycine max* L.) Accessions for Enhanced Yield and Crude Protein

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Abstract

To elucidate improved germplasm for grain yield and seed protein content, ten soybean accessions were evaluated in a replicated randomized complete block design. This study reveals substantial phenotypic variation, with protein content spanning 34.48% to 42.10%. PK-7394 recorded the highest protein level (42.10%) but showed reduced grain yield, whereas Hardee produced a maximum yield of 3.89 t ha^{-1}. TGX-1990-114FN combined high protein content (40.98%) with competitive yield (3.85 t ha^{-1}), thereby corroborating its potential as a prime genetic resource. Correlational analyses revealed positive associations between grain yield and nodule count ($r = 0.55$) as well as seed diameter ($r = 0.16$). Protein content exhibited a high Shannon-Wiener diversity index (1.09), highlighting variability across accessions. By integrating high yield and enhanced protein content, TGX-1990-114FN emerged as an optimal genetic resource for breeding programs focused on developing high-yielding and nutritionally enriched soybean varieties. Such findings offer valuable insights into advancing agricultural productivity and addressing global food security challenges.

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Statement of Sustainability: In the race to ameliorate hidden hunger and fortify the underprivileged community, an example study of ten soybean accessions offers hope for food abundance. This study elucidates PK-7394 as a prime crude protein line (42.10%), invaluable for malnutrition, whilst Hardee led prime yield (3.89 t/ha), crucial for food security. In fact, TGX-1990-114FN, bot protein (40.98%), and superior yield (3.85 t/ha), harbinger ideal for breeding resilient, nutrient-rich crops. Further, stronger yields of robust root nodules and larger seeds highlight nature's correlated soil health and abundance. Unveiling this diversity in soybeans opens doors to sustainable farming as well as ground-up to co-create a hunger-free future, one seed at a time.

1. Introduction

Soybean (*Glycine max* (L.) Merrill), a member of Fabaceae, a cornerstone of global agriculture, is cultivated in almost all sorts of climates around the world (Fattah et al., 2024; Sharma et al., 2011). By 2030, soybean production is predicted to increase by 2.1 % per year to 359.7 million tons. Despite being the highest and cheapest protein sources compared to other protein-rich foods unlike ground evaluation of promising and elite germplasm is unprecedented (Kong et al., 2024). Furthermore, producing protein sources from plants can be a long-term solution for decreasing natural resource exploitation during cattle rearing for meat production (Lynch et al., 2018; Xiao et al., 2023). In Nepal, soybean comes in second, accounting for 7.7% (29061 metric tons (Mt)) of total legume production and occupying an average of 7.2% (23563 ha) of total legume land in Nepal dominated in the terai to the high hills, 200-2000 meters above sea level, either intercropped with maize in the uplands or on paddy bund in the lowlands (Gharti et al., 2014; Manandhar, 2021). High yield and commercial soybean production are required to meet future demand from a growing population and a variety of commercial purposes. Besides, soybeans possess a strong nutrition source, containing a crude protein ranges from 38 to 43% and 20% oil, unveiling an excellent plant-based nutrition (Kholmurodova et al., 2023). As compared to animal protein, the biological significance of soy protein is remarkable, with a rich composition of essential amino acids such as lysine and tryptophan, indispensable components of human health (Tan, 2023). Beyond their nutritional benefits, soybeans also play a pivotal role in diminishing the application of synthetic fertilizers. It fixes atmospheric nitrogen through their root nodules, a biochemical process that improves soil fertility. On average, soybeans are likely to fix a significant portion of their nitrogen needs through this process (Chatterjee et al., 2018). Studies disclose that between 40% to 70% of the nitrogen required by soybeans comes from biological nitrogen fixation, influenced by environmental conditions and the efficiency of symbiotic species (Ciampitti et al., 2021). Moreover, soybeans fit well in crop rotation systems, improving soil structure and breaking pest and disease cycles. Economically, they support farmer livelihoods globally and are increasingly promoted in climate-resilient farming systems due to their adaptability and low input requirements (Agriproject, 2025). Furthermore, genetic diversity among soybean accession holds untapped potential for key agronomic and biochemical traits. Consequently, understanding morphological variation is invaluable for selecting parental lines and preserving the crop genetic stocks. In fact, the expression of morphological above-ground tapestry as well as protein and oil content in soybean seeds apt to be influenced by often environmental factors, genetic factors, and their interaction, making it necessary to identify stable accession to these traits across different growing conditions despite, however investigation of potential of soybeans genotype only a few study has been conducted in legumes in order to analyze and select high yielding stable genotypes of morphometrics as well as protein content in Nepal (Basnet, 2024a; Ghimire et al., 2024; Basnet et al., 2024a). Due to this, a considerable gap remains between the current average yields and the theoretical potential of the crop in Nepal. Thus, we make a conjecture that there is no morphological variation and oil contents among the tested accessions is considered a null hypothesis.

2. Materials and Methods

2.1. Experimental Site, Soil Sampling, and Weather Data

Conducted at the Agronomy Farm of Agriculture and Forestry University (AFU) in Rampur, Chitwan, Nepal, from July to December 2021. Situated at an altitude of 179 meters above sea level (masl), the experimental site had been previously planted with soybeans during the preceding year. Soil samples were meticulously collected from this plot in July 2021 prior to initiating trials; these samples were then properly composited, ground, sieved (2 mm), and analyzed for fertility status in AFU's laboratory facilities (Figures 1 and 2; Table 1).

Table 1. Physical and chemical properties of soil at experimental site.

Property	Value	Extraction Method
Soil pH	5.8-acidic	Digital pH Meter
Soil organic matter	0.87-low	(1-S/B)0.6810 = 3.42 because this study has 11.6 and 23.4 values of S and B (Walkley and Black method)
Nitrogen	0.12-low	Micro-K-Jeldal Method
Phosphorous	10.5 ppm(medium)	Modified Olsen's Bicarbonate Method
Potassium	9.5 ppm(low)	Flame photometer method

2.2. Germplasm Information

From a consignment of fifteen soybean accessions (six released and nine pipeline varieties) provided by the National Grain Legumes Research Program, NARC Nepal in 2021, ten were selected for further study based on their high protein content. These ten accessions (comprising two released and eight pipeline varieties) were subsequently evaluated during the 2021 growing season and provide a complete list of these selected accessions.

Table 1. List of the soybean accessions used in the experiment.

Accession Name	Status of Accession	Origin
LS-77-16-16	Pipeline	Nigeria
TGX-1990-106 FN	Pipeline	Nigeria
Puja (Check)	Released	India
PK-7394	Pipeline	India
TGX-1990-114 FN	Pipeline	Nigeria
TGX-1990-45F	Pipeline	Nigeria
IARS-87-1	Pipeline	India
HARDEE	Released	USA
AGS-376	Pipeline	Taiwan
TGX-1989-48-FN	Pipeline	Nigeria

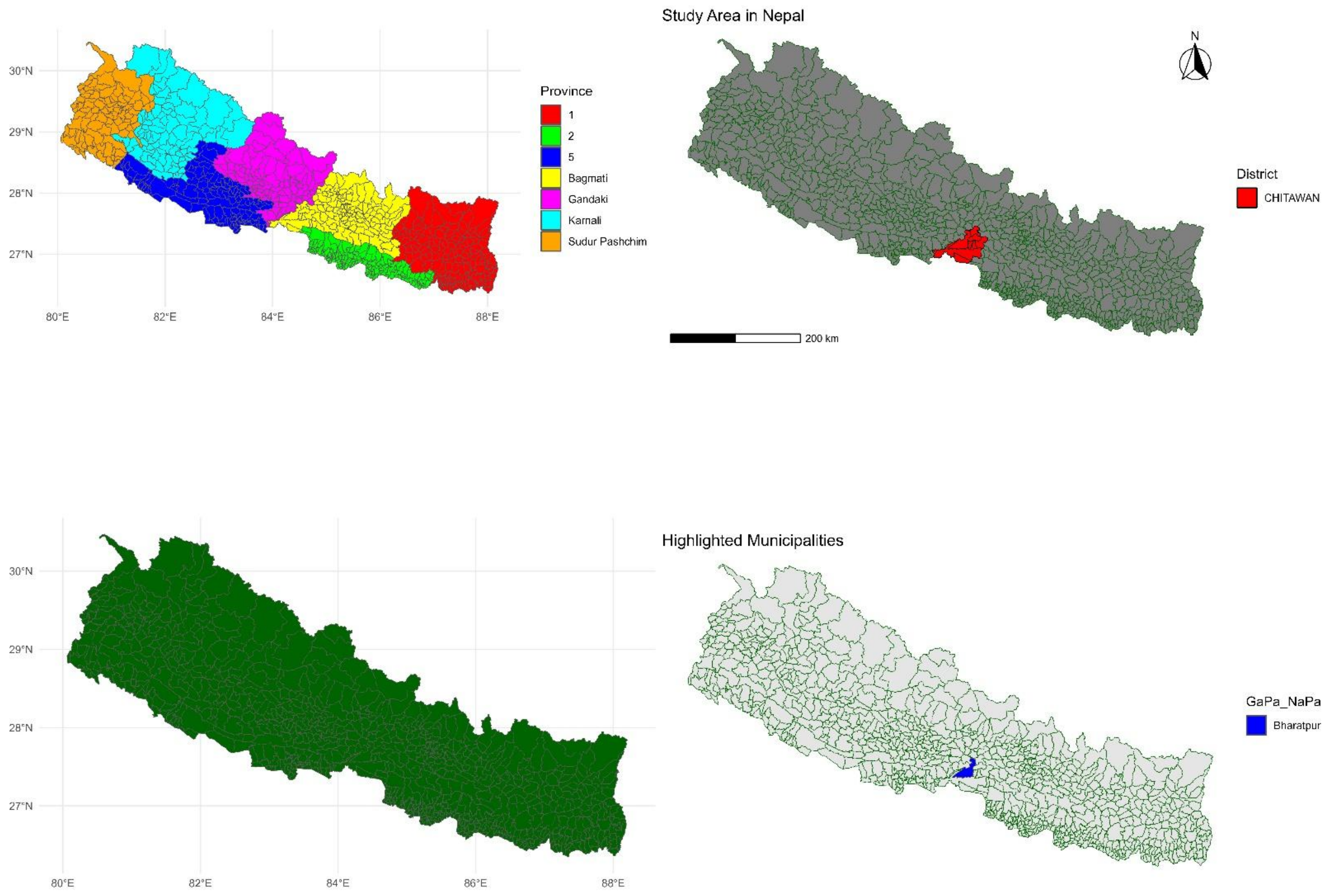

Figure 1. Illustrating the map of the studied area of Chitwan, Nepal.

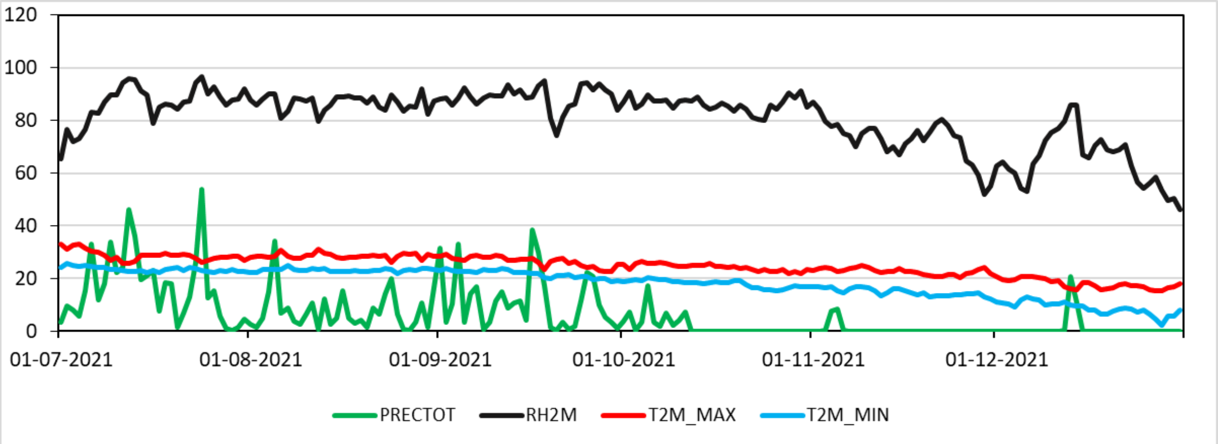

Figure 2. Graphics depicting the weather pattern of the studied area: RRETOT: Precipitation total, RH2M: Relative humidity 2M above the surface, T2 max: Temperature maximum 2 m above surface, and T2min: Temperature minimum 2 m below surface.

2.3. Experimental Design and Cultural Practices

A replicated (n=3) randomized complete block design was employed. Plots (8 m², 4 m x 2 m) were manually sown (2 seeds/spot, 50 cm x 10 cm row/plant spacing, 4-5 cm depth). A basal fertilizer application (20:40:20 kg N:P_2O_5: K_2O ha^{-1}, Urea/DAP/MOP) was incorporated pre-plant; no subsequent fertilization occurred. Moreover, thinning at 20 days post-planting established a final density of 160 plants/plot (≈10 cm intra-row spacing). Manual weeding (hoeing) was performed at 22 and 42 days after sowing. A prophylactic insecticide spray (chlorpyriphos 1 mL L^{-1} + cypermethrin 1.5 mL L^{-1}) targeted common soybean pests. Rain-fed conditions precluded irrigation. Harvesting occurred at >90% pod discoloration (brown/black). At last, three days of sun-drying preceded manual threshing and winnowing. Cleaned seed from each plot was bagged separately.

2.4. Nitrogen and Protein Content Evaluation

Utilizing the Kjeldahl method, protein content quantification was facilitated at the Animal Nutrition Laboratory in Rampur Chitwan, Nepal. This analytical process entailed employing 250g seed samples as the matrix for analysis and involved meticulously weighing two-gram aliquots from powdered samples for digestion in concentrated sulfuric acid (H_2SO_4), catalyzed by a Kjeldahl tablet until complete organic matter breakdown was visually discernible through clarity of digestate. Subsequently, ammonia liberated upon addition of sodium hydroxide solution underwent steam distillation into standardized 0.1N HCl augmented with methyl red indicator; unreacted HCl being subsequently titrated against standardized 2M NaOH solution marked endpoint via transition from red to yellow coloration. Concurrently, reagent blanks were processed to account for background nitrogen contributions thereby ensuring accuracy and precision in protein determination. Based on Guo et al. (2022a) and Kholmurodova et al. (2023) to following formula was used to calculate the protein content:

$$\text{Protein } (\%, \text{dry basic}) = \text{Nitrogen } (\%, \text{dry basic}) \times 6.25$$

2.5. Shannon Index

Shannon Weiner index (H') was calculated using the formula below:

$$(H') = -\sum_{i=1}^{R} pi\, ln\, pi$$

Where, *pi* is the proportion of the traits, i.e., *pi* = population bearing given trait / total population.

2.6. Statistical Analysis

All data was entered, processed, and computed into Microsoft Excel 2016. R studio version 4.1.1 was used for data analysis. The analysis of variance (ANOVA) procedure described by (Gomez, 2012) for a randomized complete block design study was used to statistically examine all of the data collected. Fisher's least significant difference (LSD) procedure was used to separate the treatment means for the significant difference at the 0.05 probability level (Abdi and Williams, 2010).

3. Results and Discussion

3.1. Agronomic Performances

An analysis of variance (ANOVA) revealed significant differences (P≤0.001) among soybean accessions in several key traits, including days to flowering, maturity, plant height, nodes per plant, pods per plant, seeds per pod, seed diameter, grain yield, and seed protein content (Figures 3-5). Notably, TGX-1989-45FN exhibited significantly longer days to flowering at 59 days compared to LS-77-16-16 which demonstrated the shortest duration at 40 days. Similarly, TGX-1989-48FN was found to mature significantly later at 132 days whereas LS-77-16-16 matured earlier at 98 days. Nodules varied significantly as well; TGX-1989-48-FN had the highest count with 66 nodules per plant compared to LS-77-16-16 which recorded only 53 nodules. Plant height also showed considerable variation: AGS376 and TGX1990–114FN were notably taller at 106 cm and 92 cm respectively compared to LS–77–16–16 which stood at just 55 cm. Furthermore, significant disparities were observed in other traits such as node number where TGX–1989–48-FN recorded a higher count of nodes (22 nodes) than others like LS–77–16–16 (14 nodes). Additionally, branches/plant was found significantly higher in AGS376 with eight branches compared to IARS-87−1 which had only three branches—the minimum observed among all accessions. This results further indicated that Hardee produced a significantly higher number of pods per plant with an average of seventy-five pods while LS17−1616 recorded a lower figure with twenty-eight pods per plant. Moreover, there was notable variation in hundred seed weight; TGX1990−106FN had a significantly higher hundred seed weight measuring fourteen grams whereas LS-77-16-16 registered the lowest value at ten grams.

Significant differences were also noted in seed diameter: IARS87−1 displayed a larger diameter measuring five point four four millimeters while LS771616 showed the smallest diameter measuring four point three seven millimeters.

In terms of grain yield Hardee and TGX1990114FN achieved notably high yields reaching three point eight nine tons/hectare and three point eight five tons/hectare respectively whereas the lowest yield was observed for LS771616 recording one point thirty-four tons/hectare. Lastly protein content varied substantially across accessions; PK7394 exhibited the highest protein content reaching 42.1% while TGX1990106FN recorded the lowest value standing at 34.48%. The identification of superior cultivars through the study and manipulation of germplasm is a major goal of plant breeding (Singh et al., 2023). According to (Chiemeke et al., 2024), large levels of diversity were found in various soybean morphological traits, including yield attributes and seed yield. Days to flowering, days to maturity, plant height, nodes per plant, no. of pods per plant, no. of seeds per pod, seed diameter, grain yield, and seed protein content were all shown to be significantly different between soybean genotypes (P <0.001).

This finding was comparable to that of the mungbean study (Basnet, 2024b; Basnet et al., 2024b), which found significant differences in plant height, days to blooming, days to maturity, no. of pods per plant, and hundred seed weight among soybean genotypes. Based on the researchers (Ghimire et al., 2024; Anand et al., 2024) soybean genotypes differed significantly in plant height, days to maturity, seed weight, and grain yield. Days to flowering, days to maturity, plant height, no. of pods per plant, 100 seed weight, and grain yield were all observed to have a wide range of variation by Chakelie et al. (2024). The genetic make-up of different germplasms could explain the diversity in plant height among them. Thus the number of grains per area and the grain mass determine the soybean grain yield, and both yield components are influenced by environmental conditions, management practices, and cultivars. The amount of protein in soybean grains can also differ depending on the environment and genotype (Nair et al., 2023). Seed protein levels differed significantly between genotypes in our study.

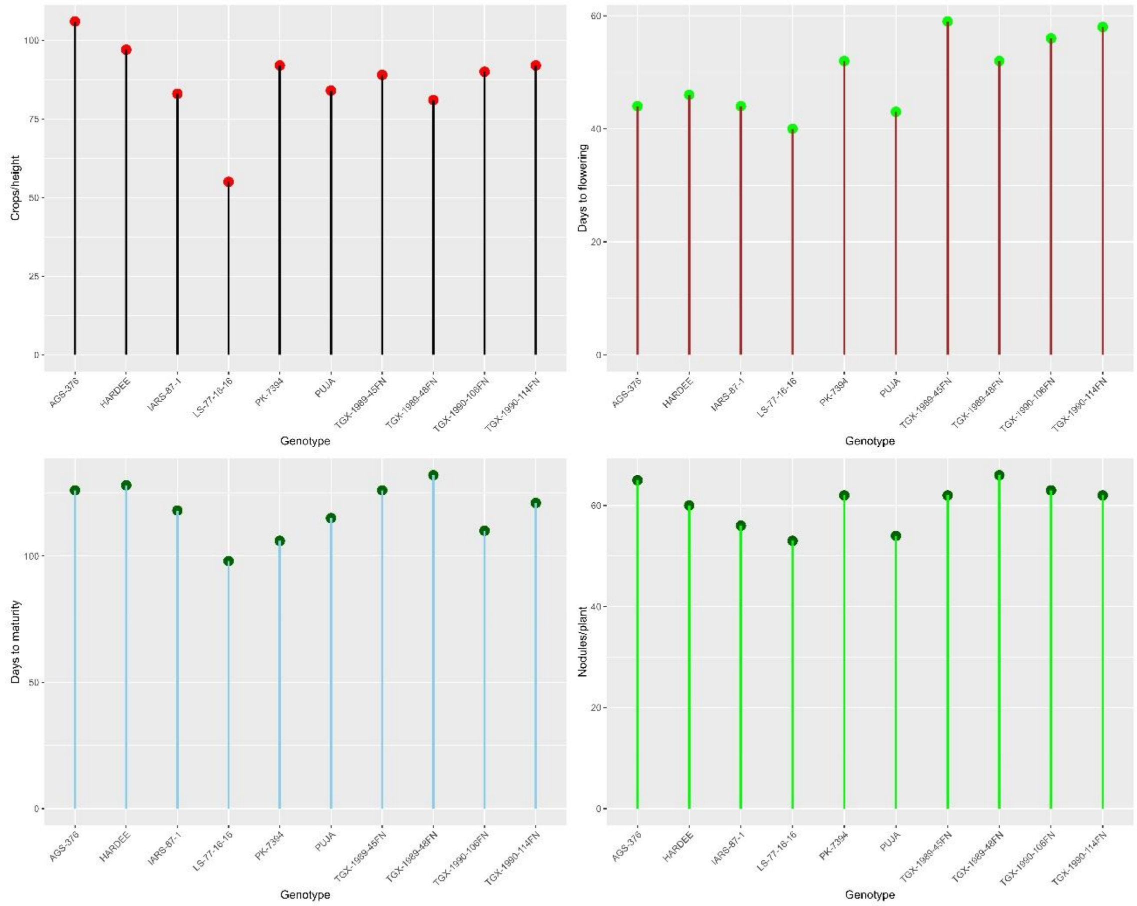

Figure 3. Depicting days to flowering, maturity, nodules/plant, and plant height.

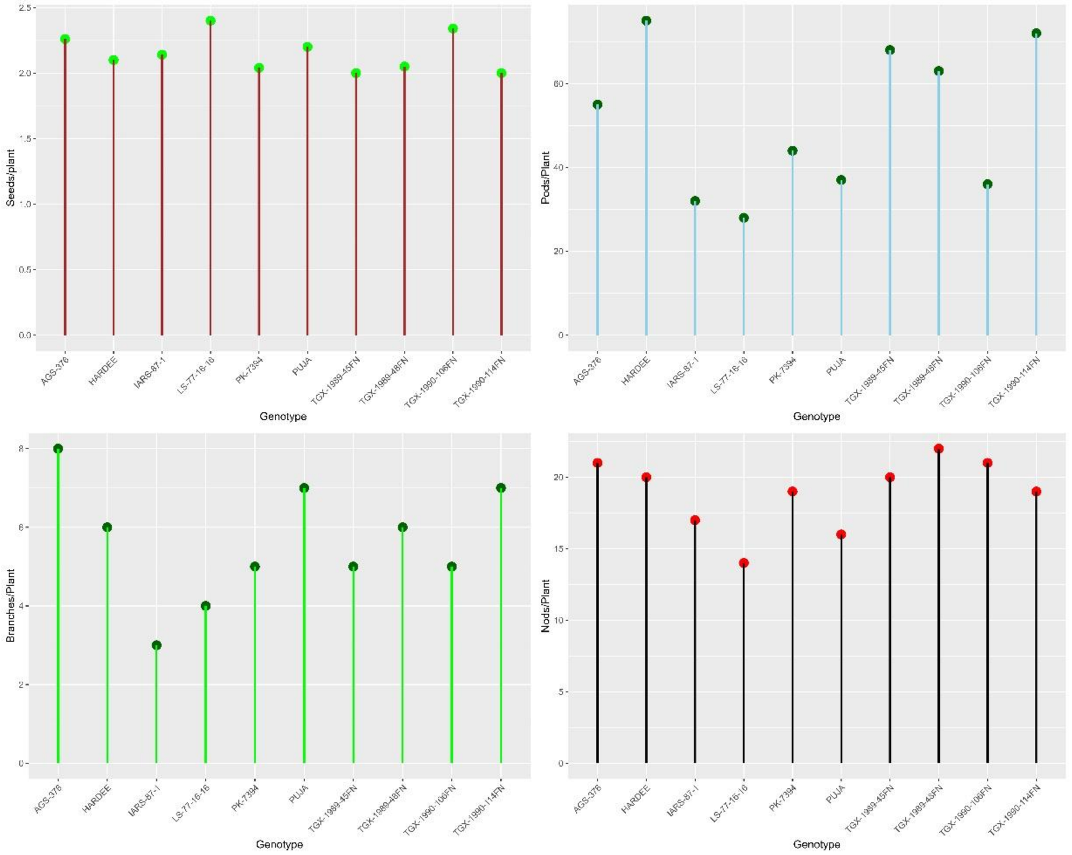

Figure 4. Illustrations of seeds/plant, pods/plant, branches/plant, and nodes/plant.

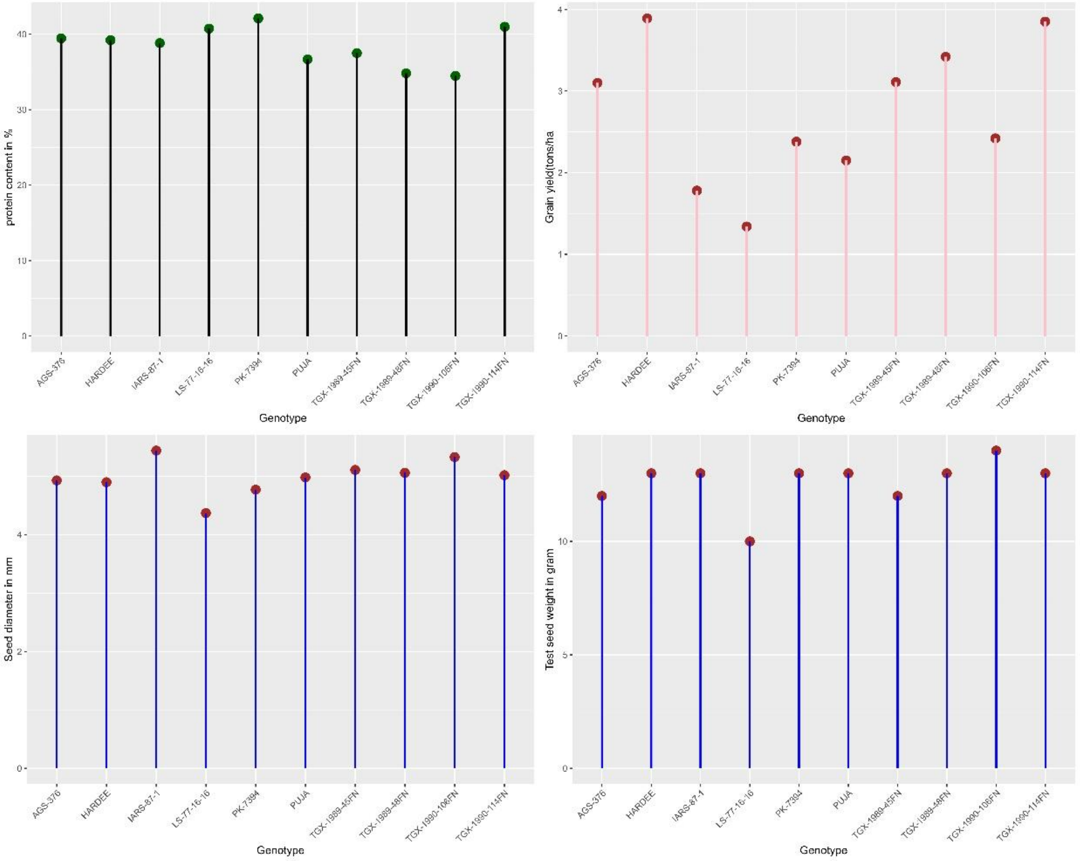

Figure 5. Illustration of grain yield/ha, seed diameter, total protein %, and test weight of studied germplasm.

3.2. Shannon Weaver index

Error! Reference source not found. specifies 11 distinct quantitative traits, each accompanied by its respective descriptor states, valid percentage distributions, and Shannon Diversity Index (SDI) values. The traits encompass a broad spectrum of agricultural parameters, such as "Days to Flowering," "Days to Maturity," "Number of Nodules per Plant," "Plant Height," "Seed Diameter," and "Protein Percentage." For each trait, multiple descriptor states (e.g., "Early," "Medium," "Long" for "Days to Flowering") are delineated, with the valid percentage reflecting the proportional distribution of these states within the studied population. The SDI, an indicator of trait variability, varies across the traits, with those exhibiting higher indices—such as "Grain Yield" (SDI = 1.06) and "Protein Percentage" (SDI = 1.09)—demonstrating greater diversity in descriptor states, while traits like "Seed Diameter" (SDI = 0.32) display comparatively lower diversity. This detailed categorization and the associated SDI values provide a comprehensive understanding of the degree of variation within each trait.

3.3. Correlation Analysis

Grain yield was positively correlated with nodules (r= 0.55) and seed diameter (r= 0.16). Yield and protein content showed a negative correlation (r= -0.06) (**Error! Reference source not found.**). There was a non-significant negative correlation between protein content and grain yield found in our study which might be due to the influence of environmental factors during the research period. A decrease in seed protein content associated with deficit stress late in the season may have been caused by a concurrent increase in temperature or a decrease in nitrogen supply. Correlation analysis gives crucial information on the relationships between major agronomic variables (Zuffo et al., 2020). Protein content was found to be significantly and negatively correlated to seed diameter, no. of nodules per plant, and grain yield, implying that finding a soybean accession with both higher grain yield and higher protein content would be difficult. A group of researchers found that seed size and protein content have negative correlations (-0.24 and -0.29) (Kholmurodova et al., 2023b). Nodule number was positively correlated with grain yield and protein content. It might be due to the nitrogen fixation process with the help of nodules present in the roots of the soybean plant. Due to the nitrogen fixation process, nitrogen content in the soil may increase and ultimately increase in yield. In our study, we found a non-significant negative correlation between protein content and grain yield, which could be attributable to the influence of environmental conditions during the study period. Similar, findings were reported to seed protein content and grain yield was negative (Guo et al., 2022b). The negative correlation between protein content and yields suggested that selecting high protein content reduces grain yields (Assefa et al., 2018).

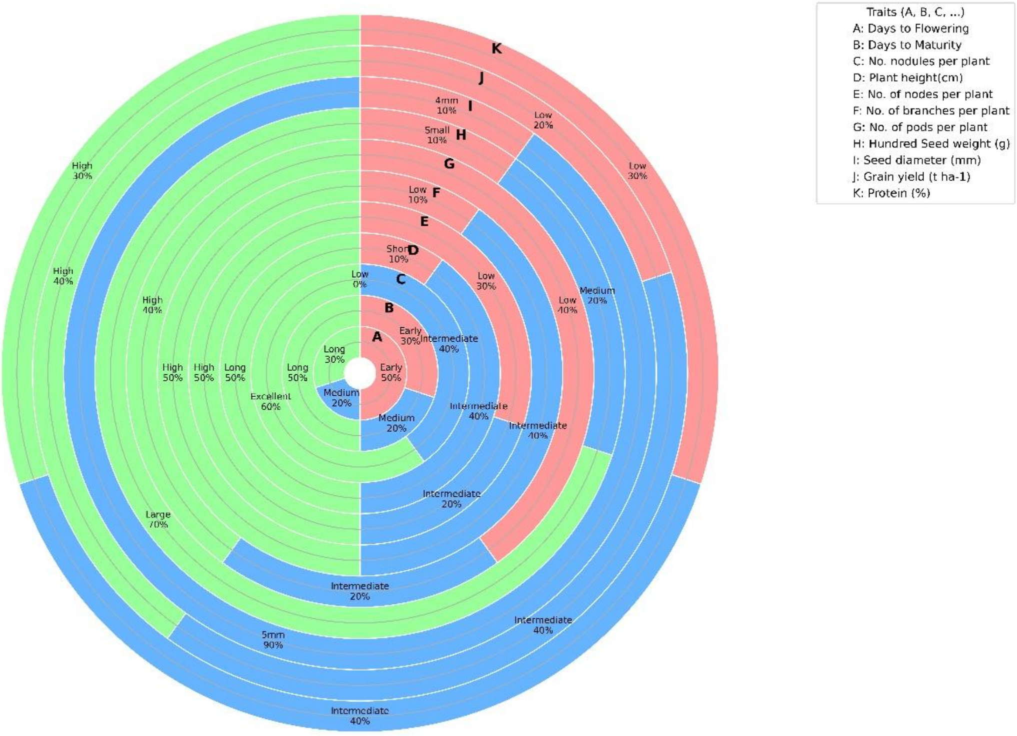

Figure 6. Descriptor states and Shannon–Weaver index of quantitative traits of soybean accessions.

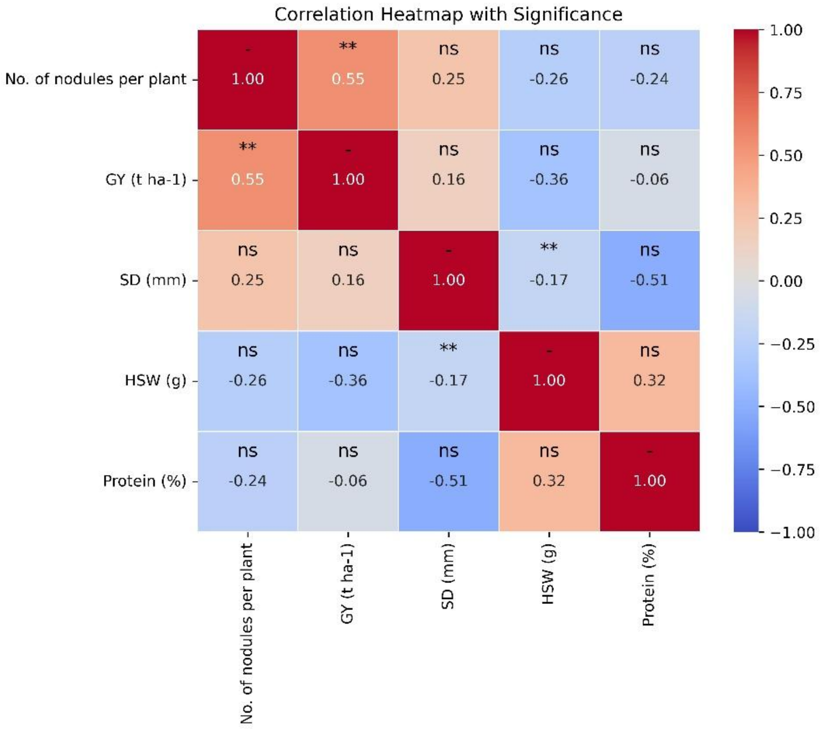

Figure 7. Correlation heatmap illustrating the correlation among the variables.

4. Conclusion

In conclusion, high protein content and grain yield are desirable traits for soybean breeding programs because they assist fulfill the food and feed ingredients for livestock, poultry, and other industries, and they provide more than 70% of the protein consumed by humans. PK-7394, TGX-1990-114FN, and TGX-1990-106FN were shown to have significantly increased protein content in this study. As revealed by correlation, the highest yield determinative traits were pods per plant branches per plant, nodes per plant, plant height, and days of maturity, and so simultaneous selection for these traits could boost soybean grain yield. The trait protein content showed the most diversity. As a result, accessions with high protein content could be useful germplasm resources in plant breeding programs. As a result, TGX-1990-114FN outperformed the other accessions in terms of protein and grain yield, and this accession might be employed in breeding programs to increase protein content.

Authors contribution: Conceptualization: Santosh Bhandari, Sagar Lamsal; Investigation: Santosh Bhandari, Sagar Lamsal; Methodology: Santosh Bhandari, Sagar Lamsal; Formal analysis: Santosh Bhandari, Sagar Lamsal; Writing—original draft preparation: Santosh Bhandari, Sagar Lamsal; Writing—review and editing: Santosh Bhandari, Sagar Lamsal, Krishna Hari Dhakal, Madhav Prasad Pandey, Roshan Ghimire; Visualization: Santosh Bhandari, Sagar Lamsal; Software: Santosh Bhandari, Sagar Lamsal; Funding acquisition: Santosh Bhandari, Sagar Lamsal; Supervision: Santosh Bhandari, Sagar Lamsal, Krishna Hari Dhakal, Madhav Prasad Pandey, Roshan Ghimire. All authors have read and agreed to the published version of the manuscript.

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Institutional/Ethical Approval: Not applicable.

Data Availability/Sharing: The datasets used and analyzed during the current study will be made available from the corresponding author upon a reasonable request.

Supplementary Information Availability: Not applicable.

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