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RESEARCH ARTICLE

Evaluation of genotypic variations in the protein content of soybean through near infrared spectroscopy

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Abstract

Demand for soybean seeds with increased protein content is high in the international market. In this context, breeding soybean varieties targeting high protein content is the need of the day. In this paper, elite soybean germplasm accessions were screened for protein content. Eighty-eight soybean germplasm was evaluated for protein content through near infrared spectroscopy. A prediction model to determine the protein content of soybean germplasm through a non-destructive method was developed to calibrate the NIR. The protein content of 39 lines was analyzed in wet lab conditions and used to calibrate in NIRs between 1400 and 2400 nm at 2 nm intervals. High determination coefficient and low values of root mean square error (2.585) and standard error of prediction (2.832) confirmed the model's utility for predicting the protein content of unknown samples. Accordingly, the protein content of 49 germplasm lines revealed that the genotypes LU96, TNAU20056, and SL525 depicted high values of 56.66, 55.51, and 54.48% protein content, respectively, but with fewer yields. The genotype RKS45 recorded a protein content of 45.33% with a single plant yield of 34.09 g, which can be further utilized for hybridization and selection. Thus, a non-significant correlation was observed between the protein content and single plant yield, suggesting that an increase in protein content will not directly influence the yield parameters. This paper provides a simple methodology to accurately determine the protein in a large set of samples in a short time, which helps in speed breeding programs.

Keywords

germplasm; soybean; near infrared analysis; protein content; yield

Introduction

Pulses act as an important vegetable protein source for mankind and animals. Among the pulses, soybean is the crop that acted as a chief protein source identified in the Asian continent from ancient times (1). Soybean, called "yellow meat of the field", is considered a potential weapon against global hunger because of its high nutritional content (2). Protein contains amino acids essential for human beings of all ages. Soybeans are the only vegetable protein with all eight essential amino acids (3, 4). Although proteins from animals are good sources, their production enhances environmental issues, and their long-term consumption increases disease risk (5, 6). Soybean was believed to have many antinutritional properties, but later

isoflavones were found to have profound roles in the prevention of diseases including reduced cholesterol, prevention of prostate and breast cancer, prevention of bone loss, and alleviating menopausal symptoms (7). The global area of soybean during 2022 is 121 million ha, with a production contribution of 129 million tonnes (8). Brazil occupies the first rank in soybean cultivation, followed by the USA, Argentina, China, and India (9).

The quality of the grains is an important parameter for cultivation and also for commercial value. The amount of protein, starch, and hardness of a grain determines its price in many different countries. Various techniques exist for assessing these quality characteristics, including the use of infrared technology and chemical composition to identify various constituents in grains, including those found in online systems inside the plantation (10). Infrared spectroscopy is pointed to as a fast and reliable method of examining the safety and quality of food (11). Several researchers have detailed the ability of that technique to classify products in terms of physical, chemical, and sensorial properties. To determine whether a product strictly complies with the label or with current laws, mid-infrared (MIR) and near-infrared (NIR) spectroscopic techniques have been used in authentication processes (12). Karl Norris was a pioneer in the analytical development of NIR techniques, and since the early 1960s, NIR technology has been linked to the analysis of grains and their derivatives. NIRS produces accurate results and is less expensive when adequately calibrated with reliable data than conventional or wet chemistry methods and composition measurement methods, such as those currently utilized by the American Oil Chemists' Society (AOCS). A wide range of grains and oil seeds has been analyzed by NIRS techniques with varying degrees of success. Early research on soybeans demonstrated that protein, oil, and moisture could be measured with dispersive/filter-based near infrared (NIR) instruments (13).

Soybean seeds possess a high protein level of approximately 40% and an oil content of approximately 20% (14). The approximate content of soybean protein is 35 to 40%, lipid content is 20%, dietary fibre is 9%, and moisture content is 8.5% (15). USDA germplasm collection was reported to have protein content ranging from 28–55% (16). In soybean, protein and oil content are inversely related in a proportion of 2:1 due to carbon flux regulation (17). To obtain new genetic combinations, the first screening of germplasm for protein content is required in soybeans. Hence, the Bradford method was deployed for determining the protein content of a few germplasms to calibrate and validate the near infrared analyzer. Many researchers have utilized near infrared analyzers, *viz*., *Brassica* sp. (18), wheat (19), sunflower (20), maize (21), and foxtail millet (22) for analyzing protein content.

Hence, in this study, an easy and accurate method has been standardized to estimate the protein content of the existing soybean genotypes. The samples were analyzed using the Bradford method of protein estimation, and 39 samples were utilized for calibration of the near infrared analyzer. Further, a total of 88 samples were analyzed to classify the germplasm based on protein content. This work provides an outlook on the protein content in the germplasm, which can be exploited in future breeding programs.

Materials and Methods

Collection of genotypes

Around 400 soybean genotypes are being maintained in the gene bank of the Department of Plant Genetic Resources, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Among the genotypes, a set of 135 genotypes from genetic resources were characterized based on morphometric analysis and DUS characterization (23). However, protein content has not been estimated in these lines, which is highly essential to identifying high expression lines. A total of 88 soybean genotype seeds were used in this study. Seeds of 88 soybean germplasm accessions were raised in the field for multiplication. The genotypes were sourced from various regions, *viz.,* CLARK from the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Canada, CSB 0806 from China, and genotypes from India. The varieties NRC 132, NRC 142, and NRC 147 from the Indian Institute of Soybean Research, India, and the varieties *viz*., MACS 1281, MACS 1460, and MACS NRC 1667 from the Maharashtra Association for the Cultivation of Science, Agharakar Research Institute, Pune, Maharashtra, and the variety CO (Soy) 3 from the Ramaiah gene bank, TNAU, Coimbatore, were also included in the study.

Analysis of protein content using near infra-red analyser

The protein content of 39 genotypes out of 88 genotypes was analyzed using the Bradford method (24). The grounded seeds were kept inside the rotating cup of the near-infrared analyzer. It was ensured that grounded seeds were uniformly spread without gaps. The near-infrared analyser spectrophotometer wavelength was set 1400 to 2400 nm with a resolution of 2 nm in ratio mode. The scanning was done in remote mode. The protein content of each genotype estimated through the Bradford method was entered against the sample name in the MS Excel file with three replications.

Correlation and statistical analysis

The experimental plot was raised during the summer of 2023 at the Department of Pulses, CPBG, TNAU, Coimbatore. The field was located at an elevation of 426.72 m above mean sea level (MSL), with geographical coordinates of 11.02 °N latitude and 76.92 °E longitude. All the soybean genotypes (88 Nos) were raised in the field. The agronomic practices were carried out as per the recommended package of practices. The type of soil is red, and six irrigations were provided. The fertilizer was applied basally in the ratio of 20:80:40 NPK, and the top dressing was done with 2:1 DAP:potash. Five randomly selected plants were harvested singly and the average yield of 5 plants was worked out for single plant yield. The protein

content worked out using a near-infrared analyzer was correlated with single plant yield. The correlation between protein content and single plant yield was analyzed using R studio version 3.6.0.

Results

The standardization of protein content with diverged genotypes was taken up with the Bradford method. Then the estimation of protein content after standardization was done for 88 genotypes using NIR.

Determination of protein content using the Bradford method

Determination of protein content for 39 genotypes using the Bradford method revealed the presence of sufficiently diverged genotypes. The classification of these genotypes based on protein content was carried out. The genotypes were classified based on variation in protein content, and the results were presented in Fig. 1. The genotypes were classified into 6 groups with 10% variation among them. Accordingly, the 39 genotypes were classified into 6 groups with genotypes containing more than 42% protein content as class 6, < 30–40% as class 5, 20–30% as class 4, and 10–20% as class 3. The results revealed that three genotypes were classified in class 6, which contained protein content of more than 42%, and three genotypes possessed protein with less than 25%.

Calibration of near infrared analyser

Protein content variation was sufficiently present in the genotypes selected for calibration, which falls within a range of 21.62 to 45.94 with an average of 32.79%. The standard graph of the percentage of protein content of the 39 genotypes for calibration is given in Fig. 2. A near-infrared analyzer was calibrated using these 39 genotypes, and a prediction model was developed.

Prediction model

The suitability of the prediction model is based on the determination coefficient and root mean square error values. In this study, the determination coefficient (R-value) was 0.9029 for protein calibration in a near-infrared analyzer, while the root mean square error was (RMSEC) 2.585 and (RMSEP) 2.832 for calibration and prediction. A value of 0.8152 was obtained for the slope. The prediction model selected for protein content calibration was well-suited. The graph with an actual reference value and prediction value is provided in Fig. 3.

Classification of genotypes based on protein content using NIR

The protein content was determined for 88 soybean germplasm accessions and genotypes and the protein values were classified and furnished in Fig. 4. Among the genotypes taken for the study, 17 genotypes have >40% protein content. The majority of the genotypes (38 numbers) have protein content within the range of 30 to 40%, and 31 genotypes were in the range of 20 to 30%. Only two genotypes possessed less than 20% protein content.

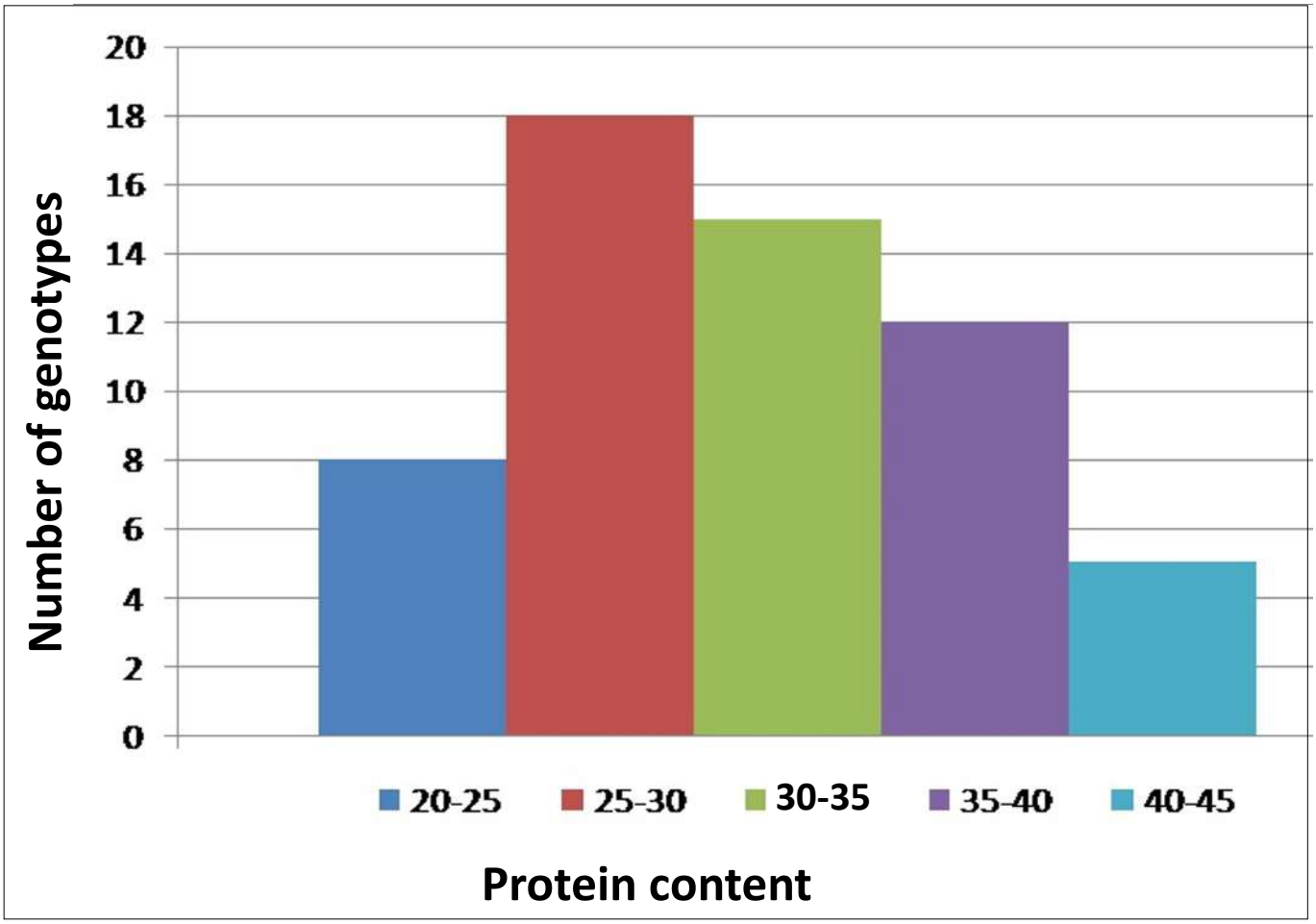

Fig. 1. Histogram showing the protein content of selected soybean genotypes.

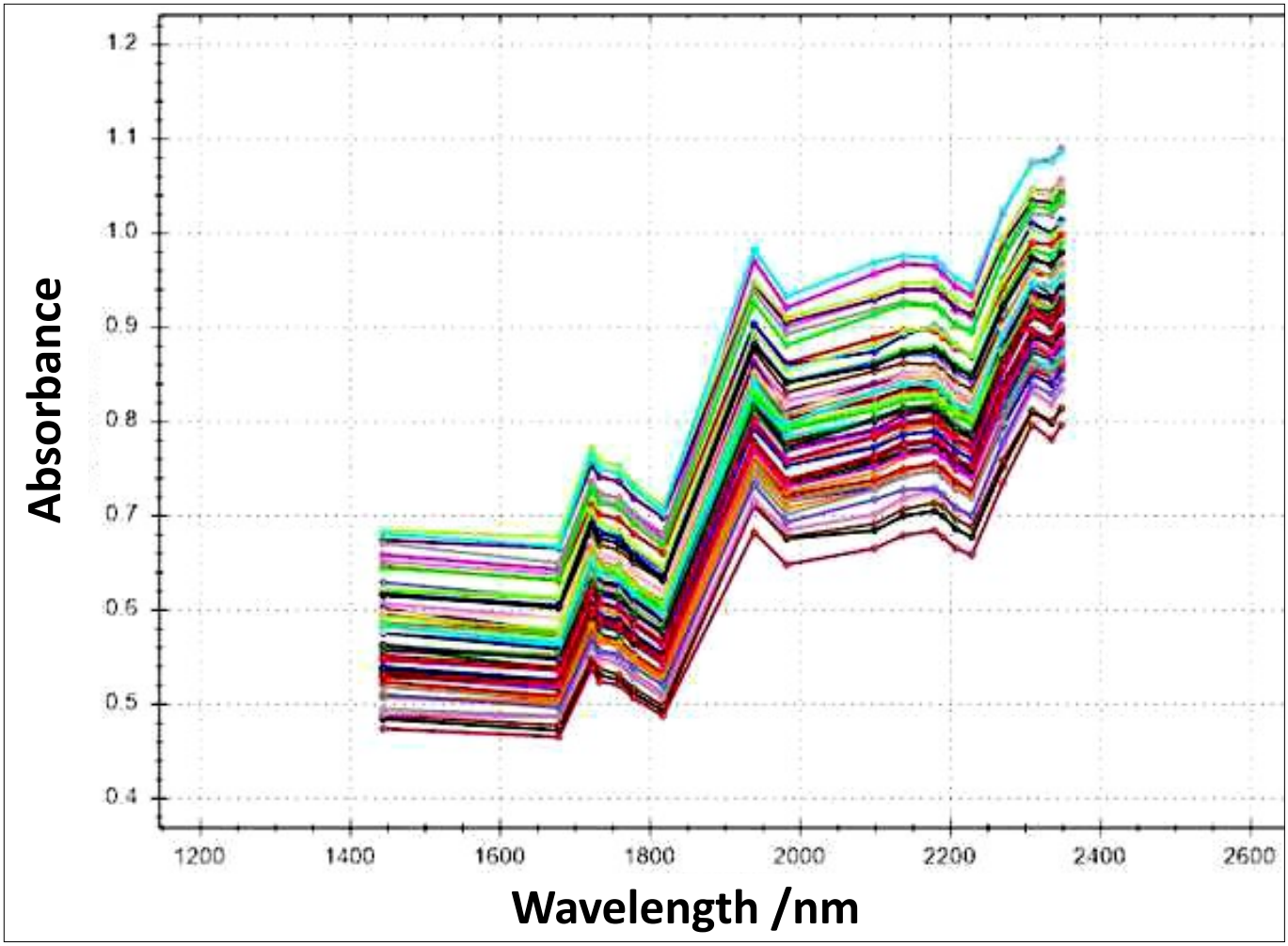

Fig. 2. Standard graph for protein content of 39 genotypes for calibration.

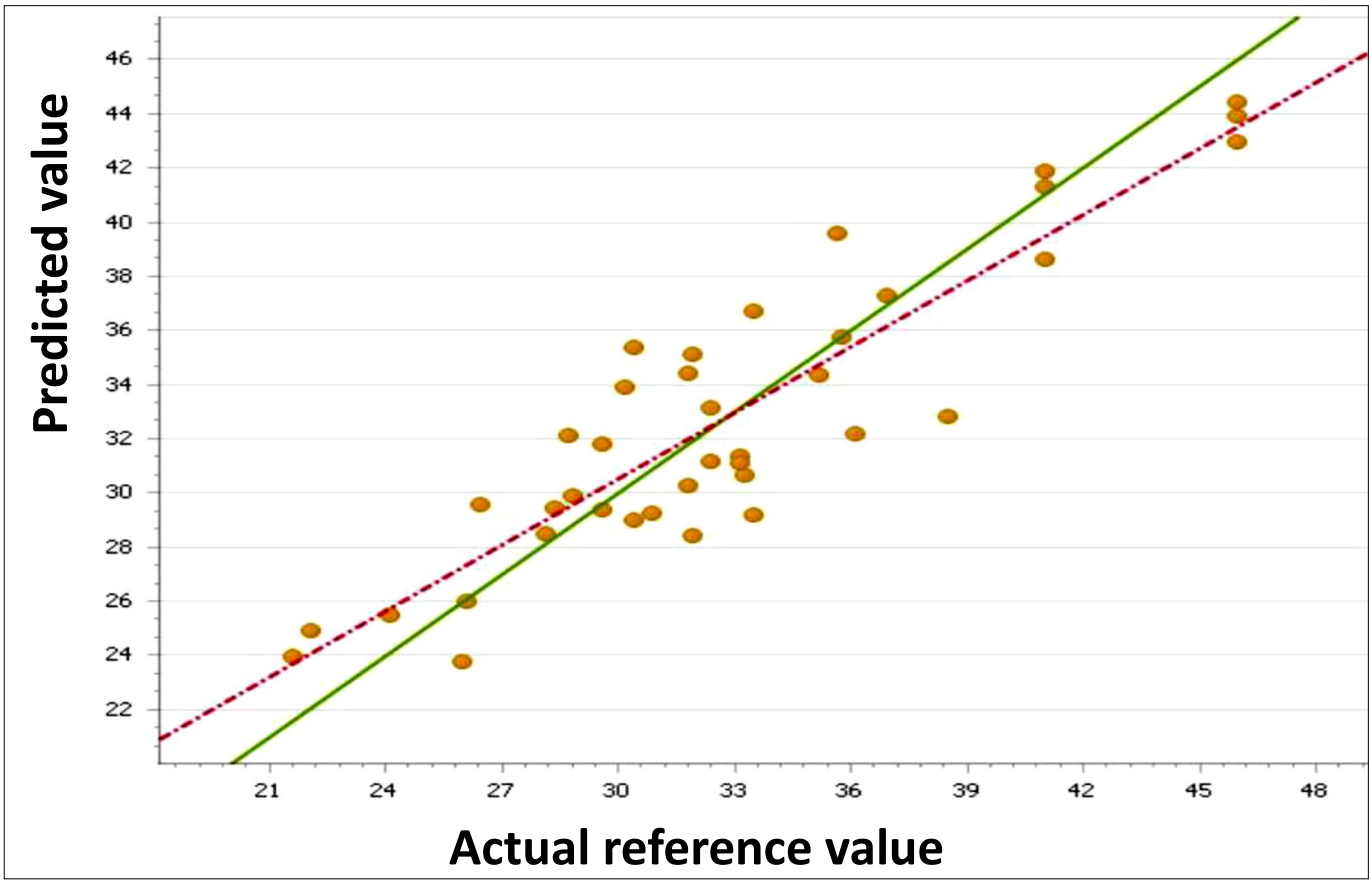

Fig. 3. Measured (wet lab) actual values versus predicted Near infrared analyser protein content (%) values in grounded soybean seed samples.

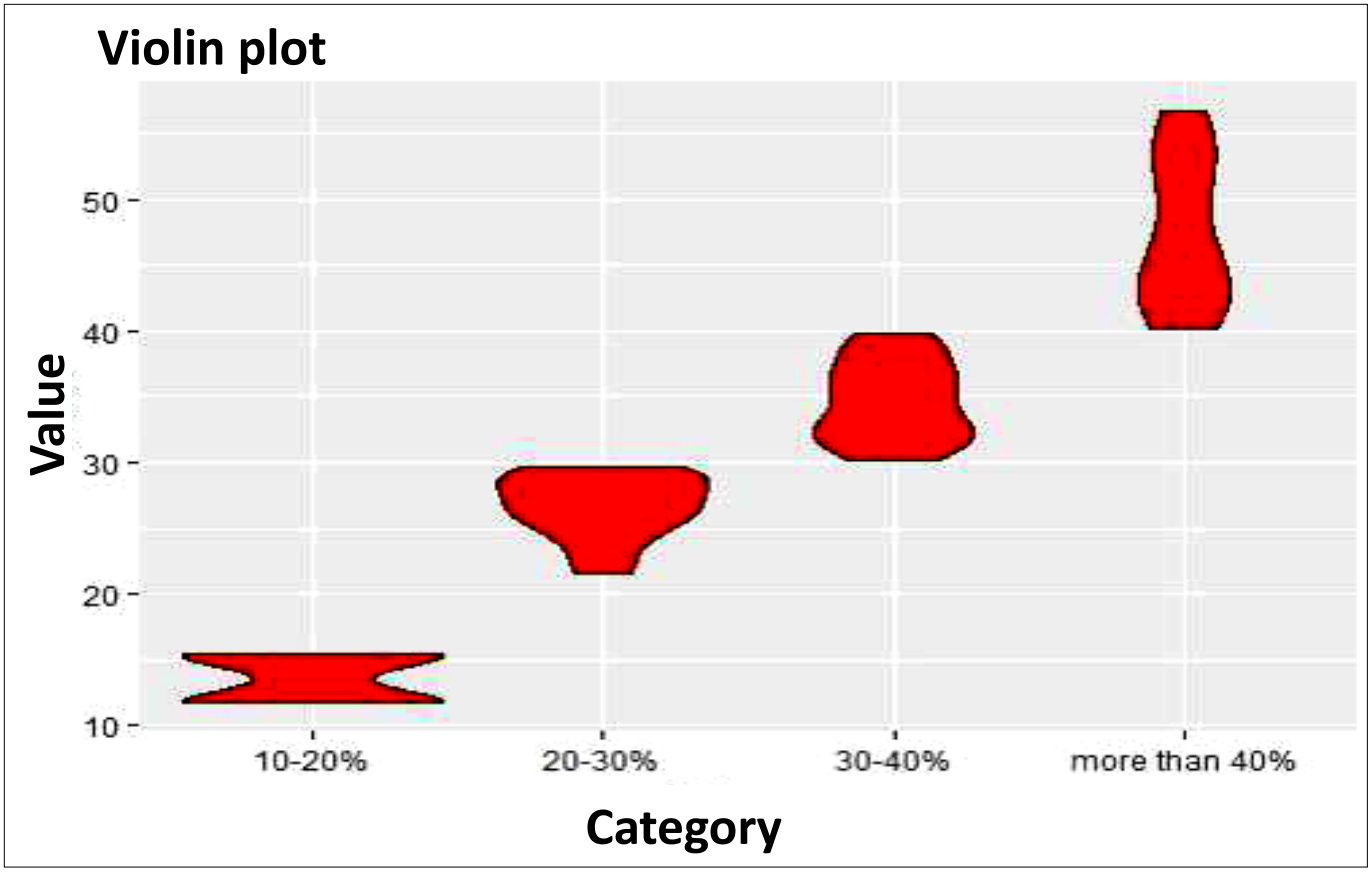

Fig. 4. Categorization of genotypes based on percent protein content.

Correlation of protein and yield

The correlation between protein content and single plant yield was worked out and found to be insignificant. The percent protein values along with single plant yield of various genotypes taken under study are tabulated in Table 1.

Table 1. Genotypes along with percent protein content and single plant yield

S. No.	Genotypes	Protein content g/100 g	Single plant yield (g)
1	Clark	27.67	22.88
2	CO (Soy)3	30.82	28.90
3	CO1	37.03	29.13
4	CSB0806	25.39	12.09
5	JS(SH)8554	30.19	10.19
6	JS(SH)89-2	35.21	16.87
7	JS(SH)91-93	35.78	26.92
8	JS(SH)92-46	33.50	20.03
9	JS(SH)93-44	27.56	25.92
10	JS(SH)99-14	45.94	9.23
11	JS76119	22.08	28.86
12	JS76-1194	26.76	30.48
13	JS89-24	21.74	19.84
14	JS90-29	36.12	30.96
15	JS98-68	38.18	8.12
16	JSSH18608	26.76	28.01
17	LPA52	29.02	26.23
18	LU22	52.96	10.00
19	LU38	44.08	7.83
20	LU46	39.13	9.40
21	LU50	25.45	3.27
22	LU62	42.65	7.87
23	LU65	32.53	15.07
24	LU96	56.66	11.53
25	MAC1281	32.01	20.08
26	MACS 1460	32.48	11.31
27	MACS NRC 1667	34.22	10.58
28	MACS1140	30.87	28.99
29	MACS1188	26.30	28.91
30	MACS1238	33.04	11.89
31	MACS1254	31.78	5.46
32	MACS145	26.42	31.25
33	MACS565	24.14	29.05
34	MAUS1039	28.82	21.11
35	MAUS17	29.51	23.01
36	MAUS311	43.54	10.31
37	MAUS414	24.82	17.33
38	MAUS417	39.65	18.30
39	MAUS55	28.36	15.11
40	MAUS59	33.15	25.31
41	MAUS61	38.52	18.95
42	MAUS71-07	43.31	27.01
43	NRC 132	28.49	18.80
44	NRC142	36.06	10.09
45	NRC2007 I 3	26.30	18.11
46	NRC25	35.66	17.09

47	NRC29	31.90	29.11
48	NRC42	28.70	28.12
49	NRC43	29.61	19.17
50	NRC44	39.32	31.73
51	NRC45	36.24	17.17
52	NRC46	24.14	27.19
53	NRC52	50.57	10.17
54	NRC76	24.71	18.31
55	NRC78	22.99	10.22
56	NRC79	38.39	9.97
57	NRC82	30.41	20.80
58	PK1000	39.09	32.88
59	PK1011	33.27	26.89
60	PK1014	42.06	28.28
61	PK1024	26.08	20.94
62	PK1038	30.98	36.77
63	PK1146	36.58	37.32
64	PK1223	36.92	15.40
65	PK1303	25.96	10.47
66	PK25	30.87	33.44
67	PK257	39.76	35.01
68	PK52	32.60	
69	PK701	21.62	20.03
70	RKS45	45.33	34.09
71	RKS7	37.26	44.73
72	SL443	15.40	28.20
73	SL518	11.75	20.20
74	SL525	54.48	8.53
75	SL794	51.89	26.77
76	SL88W	40.88	25.10
77	TNAU20039	29.46	6.33
78	TNAU20049	40.16	23.93
79	TNAU20056	55.51	14.87
80	UGM74	53.92	10.50
81	UGM77	48.00	3.13
82	VLS53	32.35	12.53
83	VLS69	29.27	31.54
84	VLS70	29.49	31.80
85	VLS75	28.13	23.56
86	WC 67	34.75	32.82
87	WC37	31.78	17.31
88	Williams	27.67	31.98

The genotypes LU96, TNAU20056, and SL525 were observed to have high values of 56.66, 55.51, and 54.48 g protein content/100 g of samples but lesser single plant yield of 11.53, 6.33, and 26.77 g, respectively. Comparing these, the genotype SL525 has a higher yield and protein content. The genotype RKS45 recorded a protein content of 45.33 g/100 g of seeds with a single plant yield of 34.09 g and the genotype Williams had a high single plant yield of 44.67 g but with a protein content of only 27.66 g. Thus, the two genotypes SL525 and RKS45 can be utilized in the hybridization and selection.

Discussion

Traditional methods of protein analysis using wet lab methods are cumbersome and time-consuming. Besides, the accuracy of the results is questionable and needs to be repeated. Hence, alternative, fast, and accurate protein estimation methods have to be incorporated into speed breeding programs. Of late, near infrared analyzer (NIRS) is being used by researchers to determine the protein content in soybean (25–28) and to utilize it in future crop improvement programs. The instrument will be calibrated with the estimated protein of the known genotypes and incorporated into the database. Then, the protein value of the unknown samples will be estimated based on the standard values. Accordingly, in this study, the NIRS is calibrated based on the protein content of the available genotypes. A prediction model has been developed for calibration and validated based on the indices, *viz.,* high determination coefficient value and low root mean square error. The determination coefficient value obtained in this study was similar to that reported (0.88) in a study with 40 soybean genotypes (27). Another report on soybean germplasm predicted a standard error of 0.568 and a determination coefficient of 0.927 (25). A high determination coefficient (0.971) in soybean whole kernel, which can be used for protein estimation, was reported (26). A high value for the determination coefficient (0.98) was reported in grounded soybean flour obtained from germplasm of various countries (28). The present values were in concordance with the previous results showing lower SEC/SEP values and determination coefficient value of more than 0.9, which can be used for accurate protein determination.

The near infrared analyzer works on the principle of monochromator detection of infrared ray absorption, which varies in different compounds. It was found that transmittance levels for high protein samples (>1.6) and low protein samples (<1.4) varied (28). A similar trend was noticed in this study, which was represented in Fig. 3. Compared to the low-protein genotypes, high-protein soybean genotypes exhibited higher absorbance at regions, *viz*. 1325–1475, 1625–1725, 1875–1925, and 2025–2125 nm regions. On the contrary, low-protein genotypes exhibited higher absorbance samples at 1210–1325, 1500–1625, and 1950–2000 nm than the high-protein genotypes. However, in our study, the absorbance showed a variation in different wavelengths. The range of protein content of the 88 genotypes was within the range of 11.76 to 56.66%. The protein content of various genotypes is given as Fig. 5. In a study, a range of 30.58 to 47.00% was reported (28) and in another study, a range of 32.18 to 48.20% was reported (29). Genotypes having protein content up to 56.66% have been obtained in this study, which is similar to the reports with more than 50% of seed protein content (30, 31). Protein content was negatively correlated with oil content and yield (32). A negative correlation between protein content and yield was also reported by other researchers (33–36).

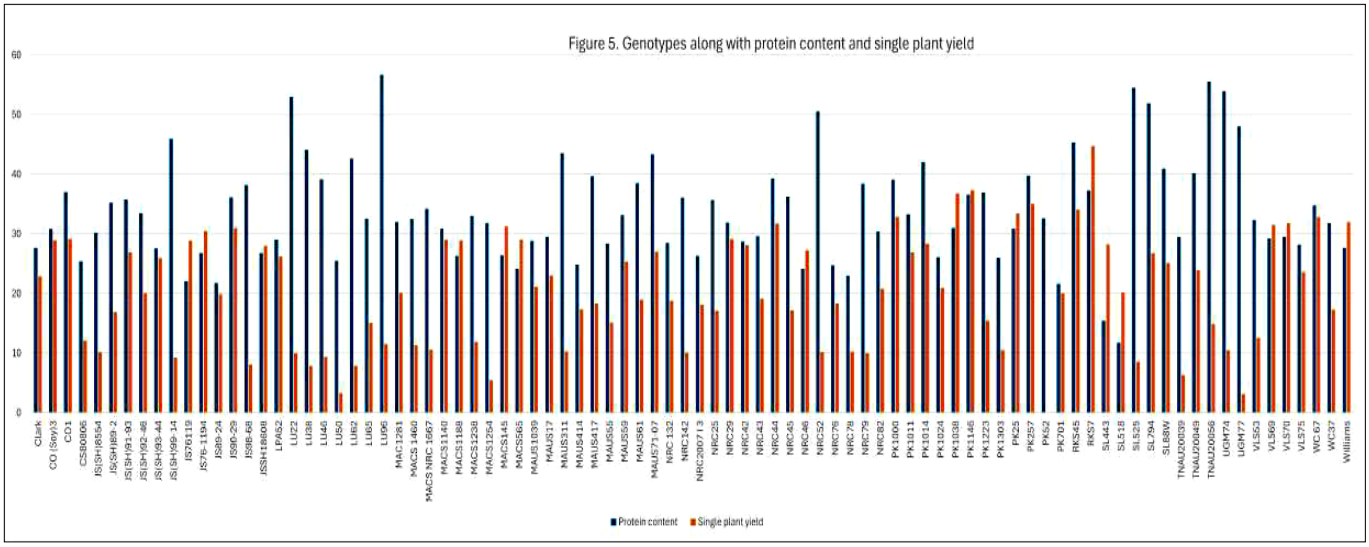

Fig. 5. Genotypes along with protein content and single plant yield.

This correlation is highly deterrent for commercializing the soybean genotypes with high protein content, as the low yield will negatively affect the farmer's income. However, we have observed a non-significant correlation between protein content and single plant yield, which is in concordance with the study in soybeans (37). It was also suggested that high yield along with increased protein and oil content could be achieved, which could be attributed to the genotypes taken for further study. This study provides an effective methodology to calculate the protein content in a short period and reduce the time to select the promising genotypes to be incorporated in the breeding programs. Besides, the trait-specific plant genetic resources can be chosen and utilized in further crop improvement programs.

Conclusion

This paper provides a simple methodology to determine the protein content through NIRS. We can estimate the protein content in a large set of samples in a short period accurately, which helps speed breeding programs. The NIRS is calibrated, and the protein content of 88 genotypes was analyzed through this method. The correlation of protein content with single-plant yield revealed a non-significant relationship, suggesting that an increase in protein content will not directly influence the yield parameters. Accordingly, a genotype, RKS45, with high protein content and single plant yield was chosen for utilization in further breeding programs.

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Authors' contributions

KA planned the work and validated the near infrared spectroscope. PM guided the standardization procedure. RCS and VV determined the protein content in germplasm lines. LK was helpful in standardization.

Compliance with ethical standards

Conflict of interest: : The authors declare that they have no competing interests.

Ethical issues: None

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