



## Research Article

### Seed Protein Diversity Assessment and Genetic Diversity among Different Soybean [Glycine max (L.) Merrill] Accession

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#### Abstract

A field research was conducted at the field of National Cattle Research Program, Rampur, Chitwan from July 2015 to November 2015. The objectives of the study were to find out high protein content, high yielding and desirable traits of soybean accessions. Fifteen soybean accessions including six released varieties obtained from National Grain legume Research Program, Nepalgunj were carried out in Randomized Complete Block Design (RCBD) with three replications. No. of nodules, days to flowering, days to maturity, plant height, numbers of fruiting nodes/plant, number of branches/plant, numbers of pods/plant, numbers of seeds/plant, seed diameter, test weight and grain yield were recorded and a seed sample of 250 gm from each plot was analyzed for protein content at Department of Food Technology and Quality Control Centre, Babarmahal, Kathmandu. There was highly significant difference among the accessions in days to flowering, days to maturity, plant height, no. of fruiting nodes/plant, no. of pods/plant, no. of seeds/pod, seed diameter, test wt, grain yield and seed protein (%). Higher seed protein concentration were found in the accession PK-7394 (43.17%), LS-77-16-16 (41.33%) and PK-327 (42.11%) among fifteen soybean accessions. Accession lang-beakong had significantly highest grain yield (3.36 ton/ha) followed by PK-7394

(3.17 ton/ha) which was at par. Accession PK-7394 was considered as the best accession in terms of both grain yield and protein content prospects.

**Keywords:** Correlation; Nodule; Morphology; Protein; Soybean

#### Introduction

Soybean [Glycine max (2n=2x=40)] is an important member of family leguminosae and sub-family papilionaceae. It is an annual herb mainly grown for seed from which oil and protein are extracted. Domestication of the soybean is believed to have originated in the northern and central regions of China as long as 5000 years ago, with the first documented use of the plant by a Chinese emperor. Soybean cultivation spread throughout Japan, Korea, and Southeast Asia, although the USA and Brazil account today for most of the soybean production of the world. Soybean primarily, an industrial crop, cultivated for oil and protein [1]. As the world population expands, there will be a greater pressure for the consumption of plant products [2]. Today soybean is considered one of the most economical and valuable agricultural commodity because of its unique chemical composition and multiple uses as food, feed and industrial materials. Soybeans have the highest protein content among cereal and other legume species, and the second highest oil content among all food legumes. Soy protein contains the essential amino acids, which closely match the requirements for humans or animals. Furthermore, soybean also contains many biological active components like isoflavones, lecithin, saponins, oligosaccharides and phytosterols. Many of these components act as anti-cancer agents and antioxidants. Considering the facts there is increasing interest towards the health benefits of soy-containing foods, particularly the role of soy protein helps to lowering the incidences of certain cancers. It has been suggested that the high intakes of soy may lower incidence of certain cancers in Asian countries, where soy consumption is high, as compared to Europe or United States of America [3]. Due to its nutritional value along with its affordable low cost, soy protein is the largest commercially available vegetable protein in the world, and it is an important alternative to existing animal derived proteins. Soy proteins are also of particular interest because they impart high functionality in food systems and being used to obtain better quality products. Because of these advantages (economic, nutritive, dietetics, etc.) it is important to develop new soy protein foods or a range of new food formulations with new textures [4].

Soybean is considered a miracle crop due to its multi-advantageous qualities i.e., food, feed, oil, fodder, soil sustainability and medicinal values. It contains about 37-42% of good quality protein, 6% ash, 29% carbohydrate and 17-24% oil comprising 85% unsaturated fatty acid with two essential fatty acids (linoleic and linolenic acid) which are not synthesized by the human body so it is highly desirable in human diet [5].

Nitrogen is the most limiting element for crop growth and usually supplied by application of fertilizer, which brings on substantial costs to farmers and with potentially adverse effects on the environment.

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Being legume it also fixes atmospheric nitrogen into soil making it available to plants. As the best source of protein it truly claims the title “the meat that grows on plant”[6]. Reported that soybean can capture an amount of 300 kg of nitrogen per hectare from the atmosphere. The leguminous plants establish a symbiotic relationship with rhizobia (symbiotic nitrogen fixation) to directly capture N<sub>2</sub> to support plant growth. Nitrogen conversion takes place in a unique organ (root nodule). The development of root nodules commences with a molecular dialogue between the host plant and a compatible strain of rhizobium involving a succession of complex process that lead to profound changes in both symbiosis [7].

Latest statistics indicated that the area of soybean in Nepal was 23757 ha with an average productivity of 1.18 ton/ha (MOAD, 2015). In Nepal, soybean is being used from household consumption to commercial uses. Now soybean is considered as important crop because it is used as an important ingredient in feed industries and the soybean demands is growing rapidly. Everyday Nepal imports raw or processed soybean in the estimated worth value 1-2 corer NRs from abroad (Brazil, Argentina, Ethiopia, India etc.). With the quest of its importance, improved soybean varieties along with modern production packages are required to boost soybean production in Nepal.

The objectives of the research was

- To find out the high protein content soybean accession
- To find out the high yielding soybean accession

## Materials and Methods

The study was conducted at the research field of National Cattle Research Program, Rampur, Chitwan, from July, 2015 to November, 2015. The experiment was carried out in Randomized Completely Block Design with fifteen treatment and three replications. The plot was 8m<sup>2</sup> (4mx2m). The experimental materials were included as six released varieties and nine pipeline accession of the soybean which were obtained from the National Grain Legume Research Program, Khajura, Nepalgunj, and Bank Table 1.

S.N.	Accession name	Accession type	Origin
1	Cobb	Released variety	USA
2	Hardee	Released variety	USA
3	AGS-376	Pipeline variety	
4	Puja	Released variety	India
5	LS-77-16-16	Pipeline variety	
6	Seti	Release variety	Taiwan, china
7	Ransom	Released variety	USA
8	Tarkari-Bhatmas-1	Released variety	China
9	PK-7394	Pipeline variety	
10	PK-327	Pipeline variety	
11	PI-94159	Pipeline variety	
12	F-778817	Pipeline variety	
13	IARS-87-1	Pipeline variety	
14	Iang-beakong	Pipeline variety	
15	TGX1485-1D	Pipeline variety	

**Table 1:** List of soybean accessions used for this experiment.

## Method of Protein Determination

Sample of 250 gm from each plot was sent to the lab of Department of Food Technology and Quality Control Centre (DFTQC), Babarmahal, Kathmandu for the seed protein content calculation for which kjeldahl method is used. It is assumed, in general protein contains 16% nitrogen which means that each gram of nitrogen determined reflects a protein content of  $100 \div 16 = 6.25$ g. The factor 6.25 has been worked out based on a number of studies on amino acid profile. During reporting the result, it is therefore customary to mention the factor (usually 6.25) used in the calculation. The principle for determination of nitrogen and crude protein is as follows:

A known weight of the sample was transferred to 250 ml Kjeldahl flask for determination of nitrogen by Micro-kjeldahl method. Into the flask, catalyst mixture (potassium sulphate + mercuric oxide) and concentrated H<sub>2</sub>SO<sub>4</sub> were added. The mixture was boiled and digestion was continued until the color of the digest was colorless. The volume of the digest was made up to a known volume. Similarly a blank without the sample was run. The reduced nitrogen extracted by steam distillation from a definite volume of the digest was collected in boric acid solution. The nitrogen present in the boric acid solution was estimated by titrating with 0.02 N HCl using mixed indicator (methyl red and methylene blue). The blank distillation and titration were carried out and calculation was done. In this way nitrogen (% dry basis) is determined and finally protein content on seed is calculated by the formula

$$\text{Protein (\% dry basis)} = [\text{Nitrogen (\% dry basis)} \times 6.25]$$

## Statistical analysis

Data entry, processing and computation of mean standard deviation for all the quantitative traits were performed using Microsoft Office Excel. Data was analyzed by using R program 3.3 versions along with MS-excel 2010. Analysis of variance was performed for all traits in order to test the significance of variation among genotypes. The data was analyzed for mean, coefficient of variation (CV %), LSD value and correlation coefficient. UPGMA clustering was done using Minitab 14.

## Cluster analysis

Cluster analysis is a type of multivariate technique whose primary purpose is to group individuals or objects based on characteristics they possess, so that individuals with similar description are mathematically gathered into the same cluster. The resulting cluster of individuals should exhibit higher within clusters homogeneity and between clusters heterogeneity. Thus, if the classification is successful, individuals within cluster should be closer when plotted geometrically and different clusters shall be apart. Among various agglomerative hierarchical methods, Unweighted Paired Group Method using Arithmetic Averages (UPGMA) is most commonly adopted clustering algorithm, followed by the Ward's minimum variance method (Ward, 1963). The accessions were clustered using days to flowering, days to maturity, plant height, no of fruiting nodes/plant, pods/plant, seeds/pod, seed diameter, grain yield and protein concentration as variables.

## Results and Discussions

There was variation found in agro-morphological characters among the fifteen soybean accession shown in Table 2. Variation was found mainly in hypocotyl colors, leaflet shape, pubescence density,

pubescence color, flower color, seed coat color, hilum color, surface luster and plant height. Two types of hypocotyls color (green and purple) were recorded. Among the fifteen soybean accessions, eight soybeans had green hypocotyl color and remaining seven had purple hypocotyl. Three different types of leaflets shape were observed. Among them; eleven soybean accessions had intermediate, two accessions had broad and remaining two accessions had narrow type of leaflets shape. Variation was also observed in pubescence color and density too. Among the tested accessions, eight accessions had normal, three had semi-sparse, three had dense and one accession had sparse type of pubescence density. Tawny and grey color of pubescence was observed in eleven and four accessions respectively. Among the fifteen accessions; nine and six accessions had white and purple flower respectively. Three types of seed coat color were observed among the tested accession. Ten accessions had yellow, three accessions had yellowish white and remaining one accession i.e. Tarkari-Bhatmas-1 had green type of seed coat color. Brown, black and grey hilum color was found in six, five and four accessions respectively. Surface lusture was also varied, thirteen accessions had intermediate type of surface lusture and remaining (Hardee and Iang-beakong) accessions had shiny type of surface lusture. There was significant variation found. In plant height also. Significantly higher plant height (57.80 cm) was recorded in Hardee accession and lowest plant height (32.97cm) was recorded in Tarkari-bhatmas-1.

### Agronomic Performances of Soybean Accession

Analysis of Variance (ANOVA) revealed that there was significant difference ( $P \leq 0.01$ ) in days to flowering, Days to maturity, Plant height, nodes/plant, pods/plant, seeds/pod, seed diameter, grain yield and seed protein content [8]. also reported range of variation for days to flowering, days to maturity, plant height, no of pods/plant, 100 seed weight and grain yield. There was no significant difference in nodule number among the accession. Significantly longer days to flowering are observed in Iang-beakong accession (50 days) and significantly shorter days to flowering are observed in PI-94159, PK-327, LS771616, Ransom and Tarkari-bhatmas-1. Similarly AGS-376 accession is recorded as a late mature (127 days) and Tarkari-Bhatmas accession is earlier maturity (94 days). Result showed that Hardee

and IangBeakong accession had significantly higher plant height and LS-771616 had lowest plant height Significantly higher number of pods/plant was observed in Iang Beakong accession (72 pods/plant) and significantly lower number of pods/plant was observed in cobb accession (30 pods/plant). Soybean plant with higher plant height and higher no of nodes might bear higher no of pods/plant. Therefore genotypes with remarkably higher number of pods/plant can be utilized in hybridization of soybean with early flowering and maturing traits for better yield [9,10]. Significantly greater seed diameter was recorded in IARS-87-1 accession (5.38mm) and significantly lower seed diameter was observed in LS-771616 accession (4.39mm). IangBeakong accession showed highest yield i.e. 3.38 ton/ha among the accession and Cobb showed significantly lower yield i.e., 1.72 ton/ha. Result showed that PK-7394 accession had significantly higher protein content (43.17%) among the fifteen accession which was statistically at par with protein content of PK-327 (42.11%) and LS-771616 (41.93%). Substantial genotypic variation for seed protein concentration has been documented by [11-16]. But high seed protein concentration is frequently associated with less yield [17-19] Table 3.

### Correlation of different traits of soybean with protein content

Protein content was significantly and negatively associated with seed diameter and test weight (Table 4). Similar result was showed by Mario et al., (2000). According to him, the negative correlation between seed diameter and protein content % was relatively low, although significant (-0.42) and Burton (1991) reported an estimate of correlation coefficient as high as -0.47 and Johanson et al., (1955) reported an estimate of -0.64 correlation coefficient between protein content and grain yield. Tiniuset al.,(1993) reported correlation values for seed size and protein content in two soybean sub-populations as -0.97 and -0.86. There was non-significant negative correlation between protein content and grain yield found in our study which might be due to 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 decrease in nitrogen supply.

S.N	Genotype Name	Hypocotyl color	Leaflets Shape	Pubescence Density	Pubescence Color	Flower Color	Seed coat color	Hilum color	Surface lusture	Pht(cm)
1	Cobb	Green	Intermediate	Dense	Brown	White	Yellow	Brown	Intermediate	45.37
2	Hardee	Green	Intermediate	Dense	Brown	White	Yellow	Brown	Shiny	57.8
3	AGS-376	Green	Broad	Dense	Brown	White	Yellow	Black	Intermediate	52.56
4	Puja	Green	Intermediate	Normal	Brown	White	Yellow	Black	Intermediate	43.12
5	LS-77-16-16	Purple	Narrow	Normal	Grey	Purple	Yellowish white	Grey	Intermediate	26.79
6	Seti	Green	Intermediate	Normal	Brown	White	White	Brown	Intermediate	41.85
7	Ransom	Purple	Intermediate	Normal	Brown	Purple	Yellow	Black	Intermediate	40.54
8	Tarkari-Bhatmas-1	Purple	Intermediate	Normal	Grey	Purple	Green	Black	Intermediate	32.97
9	PK-7394	Purple	Narrow	Sparse	Brown	Purple	Yellowish white	Brown	Intermediate	54.84
10	PK-327	Purple	Intermediate	Semi Sparse	Grey	Purple	Yellowish white	Grey	Intermediate	27.89
11	PI-94159	Green	Intermediate	Normal	Brown	White	Yellow	Brown	Intermediate	37.13
12	F-778817	Green	Intermediate	Semi Sparse	Brown	White	Yellow	Grey	Intermediate	35.25
13	IARS-87-1	Green	Broad	Semi Sparse	Brown	White	Yellow	Black	Intermediate	39.99
14	Iang-beakong	Purple	Intermediate	Normal	Brown	Purple	Yellow	Brown	Shiny	57.05
15	TGX1485-1D	Purple	Intermediate	Normal	Grey	White	Yellow	Grey	Intermediate	47.1

Table 2: Agro-morphological characteristics of 15 soybean accessions.

Genotype	DF	DM	Nodnum	Pht (cm)	Nodes	Branch	Pods	Seeds	TW	SD(mm)	GY	Protein (%)
Cobb	42	125	48	45.37	11	4	30	1.93	143	5.12	1.72	39.73
Hardee	45	126	53	57.8	13	5	59	1.9	123	4.87	2.9	41.13
AGS-376	43	127	55	52.56	10	5	54	2.03	133	4.86	2.8	40
Puja	42	117	44	43.12	9	4	40	2	140	4.99	2.2	36.66
LS-77-16-16	40	100	45	26.79	9	4	44	2.26	100	4.39	1.76	41.93
Seti	40	101	38	41.85	11	3	39	1.8	130	5.05	2.03	41.65
Ransom	40	103	55	40.54	10	3	37	2.03	140	4.98	2.07	37.94
Tarkari-Bhatmas-1	40	94	42	32.97	9	2	35	1.96	153	5.17	2.15	35
PK-7394	50	110	50	54.84	12	3	70	1.96	113	4.76	3.17	43.17
PK-327	40	101	47	27.89	9	4	48	2.3	105	4.39	2.02	42.11
PI-94159	40	116	38	37.13	9	4	44	2.03	130	5.13	2.29	38.11
F-778817	43	117	47	35.25	9	4	42	2.16	153	5.1	2.58	35.93
IARS-87-1	43	117	40	39.99	11	4	49	2.23	157	5.38	3.07	39.65
Iang-beakong	50	112	42	57.05	13	4	72	1.96	117	4.89	3.36	34.85
TGX1485-1D	43	117	52	47.1	11	3	42	2.03	127	5.08	2.13	40.07
Mean	42.73	112.24	46.37	42.68	10.46	3.69	46.95	2.04	130.89	4.94	2.42	39.19
P value	0	0	0.89	0	0.0063	0.0995	0.01	0	0	0.001	0	0
F value	7.13*1029	896.71	0.52	8.88	3.006	1.7563	2.74	4.1317	9.93	3.92	7.55	31.35
CV%	1.60E-14	0.53	26.12	13.48	17.96	29.65	33.98	8.13	7.35	4.78	1.53E-14	2.1
LSD0.05	1.14E-14	0.99	20.26	9.62	3.14	1.83	26.69	0.27	16.1	0.39	6.17E-16	1.38

**Table 3:** Agronomical performance of fifteen soybean accessions.

**Notes:** DF=Days to flowering, DM=Days to maturity, Nodnum=Nodule Number, Pht=Plant height, TW=Test Weight, SD=Seed Diameter, GY=Grain Yield

Characters	Nodnum	GY(ton/ha)	SD(cm)	Test wt(gm)	Protein(%)
Nodnum	1	0.030ns	-0.155ns	-0.023ns	0.067ns
GY(ton/ha)		1	0.129ns	0.023ns	-0.117ns
SD(cm)			1	0.740**	-0.35*
Test wt (gm)				1	-0.479**
Protein(%)					1

**Table 4:** Correlation of different traits with protein concentration.

**Notes:** \*= Significant & ns= Non-significant

According to previous finding correlation between seed protein content and grain yield was negative [13,18,20]. The negative correlation of protein content of seed with grain yield, seed diameter and test weight indicated that it would be very difficult to identify a soybean accession having higher grain yield simultaneously with higher protein content. Increase in one trait would result in reduction of the other, that is simultaneously increase or decrease of both traits would be difficult. Breeding for high seed protein concentration was based on increase in assimilates supply per seed. This increase however was more related with less seed set than more leaf area. This might be the link between high protein concentration and lower yield.

Nodule number was positively correlated with grain yield and protein content (Table 3). It might be due to nitrogen fixation process by the help of nodule present in roots of soybean plant. Due to the nitrogen fixation process, nitrogen content in the soil may increase and ultimately increase in yield also.

## UPGMA clustering

The accessions were clustered using days to flowering, days to maturity, plant height, no of fruiting nodes/plant, pods/plant, seeds/pod, seed diameter, grain yield and protein concentration as variables. The dendrogram is presented in Figure 1. The critical examination of the dendrogram revealed five clusters with minimum of 29.35% similarity level in UPGMA clustering. Clusters were obtained on the basis of similarity percentage and related characters.

Cluster 1 consisted of one soybean accession i.e. Cobb. This cluster had lowest no of pods/plant and grain yield. Cluster 2 consisted of two soybean accession. It includes Hardee and AGS-376. This cluster contained late matured accession which had higher plant height and higher concentration of protein and relatively higher grain yield. Cluster 3 consisted of five soybean accessions. It include Puja, TGX1485-1D, PI-14159, F-778817 and IARS-87-1. These soybean accessions had intermediate value for days to flowering, days to maturity, plant height, no of pods/plant, grain yield and protein concentration.

Cluster 4 consisted of five soybean accessions. It include LS-77-16-16, PK-327, Seti, Ransom and Tarkari-Bhatmas-1. These were early matured accessions which had lowest plant height, no of fruiting nodes/plant and seed diameter. These accessions had intermediate value for protein concentration and grain yield. Cluster 5 consisted of two soybean accessions including PK-7394 and Iang beakong. These accessions had longer days to flowering, highest plant height, pods/plant, nodes/plant as well as grain yield among the accessions and intermediate value for the protein concentration.

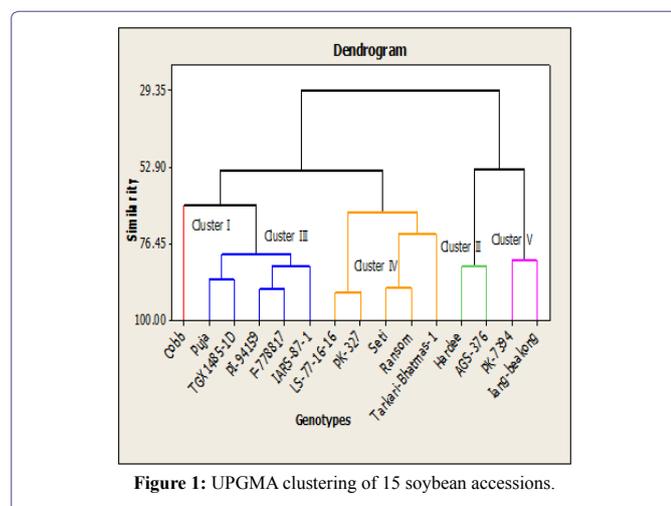


Figure 1: UPGMA clustering of 15 soybean accessions.

## Conclusion

High protein content is an important specialty trait for soybean breeding programs. Soybean is an important ingredient for livestock, poultry and other industry and contributes more than 70% of the protein consumed by humans. In this study PK-7394 (43.17%), LS-77-16-16 (41.33%) and PK-327 (42.11%) were identified as high protein content accessions. Among the studied accessions, Iang-beakong had significantly higher grain yield (3.36 ton/ha) followed by PK-7394 (3.17 ton/ha). In the correlation analysis, grain yield showed positive and highly significant association with days to 50% flowering, days to maturity, plant height, no of nodes/plant. These traits can be considered for selection programs aimed for yield improvement. In terms of both protein content and grain yield, PK-7394 accession was found superior at Rampur locality.

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