Relation Between Seed Characterstics And Seed Quality In Bhat (Black Seeded Soybean) Collected From Northern Hills

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Abstract—The 50 genotypes of black soybean and brown seeded soybean called as *Bhat* was analyzed for the protein content and oil content. The studies were conducted with respect to growth habit and seed color and size. In general, black seeds were more in protein and less in oil content in comparison to yellow colored seeds. In present studies it was investigated that whether the growth habit of the black soybean is been related to nutritional superiority in terms of oil and protein percentage. In this study the range of protein percentage was found to be 23 to 43. Accessions MOB-29 having 43% proteins in seed. The correlation studies between protein content, oil content and 100 seed weight was done and it was found to negatively non- significant. The different groups were made according to protein content and oil and studied the relation with growth habit and coat color. In this study we observed evident role of farmer in selection of collected germplasm in genetic enhancement.

Key Words:- Growth habit, Bhat, protein percentage, nutritional quality, hill, seed coat color.

I. Introduction

Soybean (Glycine max (L.) Merrill) is a member of Papilionaceae family and native to Northeastern China and distributed in Asia. It is a major source of protein. In Uttrakhand region of India, soybean varieties are known as Bhat. Based on color, black seeded soybean is known as Kaley Bhat. Similarly, yellowish white and brown seeded soybeans are known as Safed Bhat and Rata Bhat, respectively. Bhat is also considered as treasure house of medicinal properties. Black soybeans and its soy products are the richest sources of isoflavones in the human diet. The consumption of soy, and therefore these isoflavones, generally has been considered beneficial, with a potentially protective effect against a number of chronic diseases [6]. Black soybeans also reduce cholesterol level, inhibit growth of cancerous cells and interesting one that people consuming black soybean in Japan was less effected by pollution and adverse effects of radiation [1]**.** In hilly areas of Bhat primarily consumed because of its delicious taste rather than of nutritional superiority. It is also seen that the Bhat contain some medicinal properties. It is seen that oil of Bhat is cholesterol-free and contain linolenic acid, which has been found to prevent heart disease. Bhat also contains isoflavones such as genistein and daidzein that have been found to have antioxidant, antitumor, and estrogenic activity [6]. The Bhat variants used in this study was collected from Kumaon and Garwal hills of Uttrakhand (approximately 8 different districts) such as Dharchula, Ranikhet, Karanparyag, Chaukutiya, Didihat etc.

II. Material Methods

A set of 48 accessions with 2 checks was evaluated during 2011 at G.B. Pant University of Ag. and Tech. Pantnagar (Uttarakhand) located at 243.84 meters above the mean sea level. The university falls under the subtropical zone and situated in the Tarai region at the foothills of Shivalik range of the Himalayas. Seeds for the present investigation were obtained from the trial planted in Randomized Complete Block Design (RCBD) with three replications. The seed samples were collected from fully matured ten random plants for each genotype separately from all replication. The protein was then analyzed for seed protein during 2011-2012 in the Soybean Laboratory of G.B. Pant University of Ag. and Tech. Pantnagar.

Extraction of protein was done by grinding the seeds of each genotype seperately which were dried to 4-5% moisture level in oven set at 108°C for 6 to 8 h. dried powdered seeds were taken for analysis. The grounded product was centrifuged with extraction buffer at 10000 g at 4°C for 20 minutes and the supernatant was collected.

Quantification of protein was done following the method of Bradfort method, [3]. We used Bovine Serum Albumin (BSA) as standard. The method relies on principle of the binding of the dye Coomassie Blue G250 to the protein molecule. The cationic form of the dye, which predominates in the acidic assay reagent solution, has a λmax of 470 nm. The dye binds to protein as the anionic form, which has a λmax of 595 nm. Thus the amount of dye bound to the protein can be quantified by measuring the absorbance of the solution at 595 nm. The dye appears to bind most readily to arginine residues (but not to the free amino acid) of the protein. Hence the absorbance of light by the dye-protein complex at 595 nm is proportional to the amount of protein bound (over a limited range); i.e., there is a linear relationship between absorbance and the total protein concentration of the sample over a narrow range. The spectrophotometer used for analysis was double beam UV-VIS spectrophotometer: 2202.

Similarly, the oil was extracted by grounded seed sample through Soxhlet extraction apparatus and quantified using formula [7]

$$\text{Percent crude fat} = \frac{\text{Weight of fat soluble material}}{\text{Weight of sample}} \times 100$$

III. Result And Discussion

Seed protein and oil percentage obtained from 50 genotypes were analyzed statistically in RCBD. Analysis of variance for seed protein and on il of revealed statistically significant variance ratio for protein and oil percentage among 50 accessions. The results, therefore, indicated that the difference in seed protein content among Bhat germplasm studied so far might be due to genetic cause and not due to environmental or error factors. The results show that the

protein has negatively non significantly correlated with the Oil content (-0.070) and 100- Seed weight (-0.026).

Based on the seed protein content, 50 accessions were classified in three groups. Group I contained 21 genotypes with seed protein content more than mean ± Sem (33.756 ± 0.858). Group II included 10 genotypes, which were at par with mean (mean ± Sem), in seed protein content whereas 19 genotypes with seed protein per cent less than the mean (mean ± Sem) formed group III (Table 1). Seven genotypes were noted to be of determinate type and 14 were of indeterminate type plant growth habit among the genotypes containing seed protein more than the mean seed protein content with 15 black, 5 yellow and 1 chocolate coats respectively. Genotypes of group II, which were at par with mean value in seed protein, consisted of 2 determinate types and 8 indeterminate type of plant growth habit. The III group composed of 4 determinate types and 15 indeterminate types of plants. Thus, out of 50 accessions, 13 were of determinate types whereas 37 were indeterminate type of growth habit and 37 were of black seed coat, 10 with yellow and 3 with chocolate coat. (Table-I).

Similar comparisons were done for oil content also where seed oil content was classified in three groups. Group I contain 24 genotypes with 8 each of determinate type and indeterminate while genotypes of group II, consisted of 1 variant with indeterminate growth habit. The III group composed of 8 determinate types and 17 indeterminate types of plants. 37 were of black seed coat, 10 with yellow and 3 with chocolate coat. (Table-II). Also it evident that among all genotype the determinate type, around 53% genotypes were with protein content more than the mean seed protein content whereas among the indeterminate types only 37% of the accessions were with seed protein surpassing the mean seed protein value. But if we see top 5 ranking genotype of protein content (Table- III) we can't get clear cut evidence appeared to support that determinate type of genotypes are superior than indeterminate types of genotypes in terms of seed protein content. Thus determinate type of Bhat contained high frequency of high protein genotypes in comparison to indeterminate types of genotypes may or may not be true. However, looking at the protein content with respect to seed coat color it appears to be non-significant relation. In respect with oil content highest oil content were present in (18.47%) i.e. DSM-76 and the one with lowest protein content (10.00 %) i.e. MOB-6.

There was no significant relation of oil content with either of growth habit or coat color. The genotype with highest protein content (43.20%) i.e. MOB-29 and the one with lowest protein content (22.37%) i.e. MOB-45 were of indeterminate type in growth habit. The result clearly indicated that the selection practiced in black soybean over the years for both determinate and indeterminate types by hill farmers had led to the evolution of a genotype with high nutritional quality and low anti nutritional factor despite of their growth habit. The protein deficiency disease kwashiorkor, which is prevalent in India, was rarely seen among children in Kumaon Hills. This may be due to regular consumption of black soybean in their daily meals [4].

50 accessions studied were ranked according to their seed protein content out of these, 5 high ranking genotypes were chosen and are depicted along with some morphological parameters in Table III. These genotypes seem to be promising and potential genetic resource for seed protein content in soybean.

IV. TABLES

Table I. Seed protein based grouping of Bhat (black seeded soybean) germplasm from Northern hills

Group	Range of protein (%)	Number of genotype	Growth Habit		Seed coat color		
			Determinate	Indeterminate	Black	Yellow	Chocolate
I	> (33.756± 0.858) (µ ±SEm)	21	7	14	15	5	1
II	= (33.756± 0.858) (µ±SEm)	10	2	8	9	1	0
III	< (33.756± 0.858) (µ ±SEm)	19	4	15	13	5	1

Table II. Oil content based grouping of bhat (black seeded soybean) germplasm from Northern hill

Group	Range of oil (%)	Number of genotype	Growth Habit		Seed coat color		
			Determinate	Indeterminate	Black	Yellow	Chocolate
I	> (13.8 6± 0.334) (µ ±SEm)	24	8	16	19	5	0
II	= (13.8 6± 0.334) (µ ±SEm)	1	0	1	1	0	0
III	< (13.8 6± 0.334) (µ ±SEm)	25	8	17	18	5	2

Table III- Bhat (black seeded soybean)genotypes as potential genetic resource for seed protein

Genotypes	Protein content %	Growth Habit	Pubescence type	Flower color	Seed coat color	Hilum color
MOB-29	43.20	Indeterminate	Tawny	Purple	Black	Brown
MOB-13	41.73	Indeterminate	Tawny	Purple	Black	Brown
MOB-25	41.43	Indeterminate	Tawny	Purple	Black	Brown
RCP BHATT 332	40.63	Indeterminate	Tawny	Purple	Black	Yellow
SOYBEAN 377	40.33	Indeterminate	Tawny	Purple	Yellow	Black

V. REFERENCES

[1] Akizuki, T., 1972. The Concentric of Death. Kodansha, Tokyo.
[2] Bhartiya, A.; Aditya, J.P.; Singh, G.; Gupta, A.; Agarwal, P. K; Bhat J.C. 2011. Assessment of genetic diversity in indigenous and exotic Collections of black soybean (Glycine max (l.) Merrill). SABRAO Journal of Breeding and Genetics. 43 (1): 81–90.
[3] Bradford MM. 1976. Anal. Biochem. 72:248-254.
[4] Hymowitz, T. 1970. On the domestication of soybean. Economic Bot.24: 408-421.
[5] Miladinovic, J.; Hrustic, M.; Vidic, M.; Tactic, M. and Balesevic, T. S. 2004. Interrelationship between yield, oil content and vegetation period on protein content in new soybean varieties seeds. Zbornik-radova-Naucni-Institute-i-Povrtarstvo. 40 : 227-234.
[6] Munro, I.C.; Harwood, M.; Hlywka, J.J, Stephen, A.M, 2003. Soy isoflavones: a safety review, Nutrition Reviews 61: 1-33
[7] Ranganna, S. 1977. Handbook of analysis and quality control for fruit and vegetable products. New Delhi, Tata McGraw-Hill. 1112 p.
[8] Shah, N.C. 2006. Black soybean: An ignored nutritious and medicinal food crop from the kumaon region of India. Asian Agri-Hist. 10: 33–42.
[9] Surlan-Momirovic, G. 1987. Genotypic and phenotypic correlation and biochemical traits in different varieties of soybean {Glycine max (L.)Merill.}. Polioprivredna Znanstvena Smotra. 76-77, 5-17.

