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Quantitative estimation of protein and minerals in the selected cultivars of *Vicia faba*

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ABSTRACT

Vicia faba provides an excellent source of vegetable protein, aminoacids and minerals, as an essential supplement of cereal-based human diet and is being also used as feed and fodder, showing an appreciable nutritional value of this crop. Twelve Vicia faba cutivars have been analysed to evaluate the protein and minerals content. The protein percentage of different cultivars included in this study have 30 to 32 per cent or more than this. Estimation of alkali earth metals like Mg, Ca, Sr, Ba and Cd, alkali metals like Na, K, Pb and transitional elements like Mn, Fe, Cobalt, Nickel and Zinc were also taken up. Results obtained on the ground of quantitative estimation of these classified minerals have been shown that certain minerals are very essential and significant whereas fortunately certain minerals have been found to have poor amount in all the taxa and someone causes toxicity after a critical limit. Results from the presence of protein and minerals either in high amount or in less amount in the selected taxa of Vicia faba can serve very important and extremely needful for the selection of cultivars regarding the taxonomic and chemical divergence for further utilization.

Keywords : Chemical divergence, nutritional value, *Vicia faba.*

Faba bean (*Vicia faba* L.) an annual diploid legume (2n= 2x= 12) belonging to Family Fabacea is the seventh most produced legume world wide with a yield of 3,503, 300 tons (Zohary *et al*., 2012). It is believed to be one of the oldest domesticated legumes, since oldest seeds have been found in Syria (Tanno and Wilcox, 2006). *Vicia faba* is a facultative cross pollinating plant with outcrossing rates varying between 1-55 per cent depending on the environment (Alghamdi *et al*., 2012) The protein content of *Vicia* is high 2-25 per cent and is used for human and animal consumption especially in Asian and African countries (Ali *et al*., 2014). *Vicia faba* is known for its role in maintaining soil fertility and nitrogen fixation and is able to grow in diverse climate and varying soil conditions (Kaur *et al*., 2014; Sans *et al.*, 2007). Moreover, it can be utilized throughout the year, as it can be consumed in both raw and processed forms. In the human diet it is mostly the seed grain that is consumed, while the pods are used as feed. However, as the pods also provide macro-, micro and non-nutrient phytochemicals, they have potential to be used as a source of functional compounds (Mateos-Aparicio *et al*., 2010). The nutritional importance of fava bean is prominent (approx. 250 g protein per kg seed; Macarulla *et al*., 2001) and it offers a valuable amount of energy: 320 kcal per 100 g dry weight (Ofuya and Akhidue, 2006). The legume has also therapeutic potential as it provides L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor to the neurotransmitter catecholamine and a drug used to treat Parkinson's disease (Ramya and Thaakur, 2007). Fava bean is a rich source of fiber and non-nutrient secondary metabolites shown to be beneficial to human health (Aune *et al*., 2011). Vicilin and legumin are the major protein portions in fava bean, and overall in other beans, followed by albumins, prolamins, and glutelins (Nikokyris and Kandylis, 1997).

Vicia faba has become important pulse crop and as a great source of vegetable protein, as such selected for study in terms of nutritional value. The present investigation is on the line of basic research where some selected cultivars of *Vicia faba* have been selected for thorough screening in terms of nutritional value. The intention was to furnish basic information which would provide an impetus to break through in the yield of this pulse through proper choice of variety, use of good quality seeds, recommended package of agronomic practice and also for future genetic endeavors since it is a rich source of proteins, fiber, and other non-nutrient compounds considered beneficial for health. Apart from this since it is a popular source of proteins in many Asian countries including ours we need to work on its potential as a predominant source of proteins in the diet (Multari *et al*., 2015). Fava bean cultivation therefore can make a valuable contribution towards protein self-sufficiency and can provide a more environmentally friendly substitution for industrial N-fertilizers with associated improvements in resource efficiency and production costs. From both a food security and environmental

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sustainability perspective, encouraging both production and consumption of fava bean is a timely and important target (Multari *et al*., 2015). The present study conducted is extremely significant in the current scenario, since pulses and legumes have been an integral part of Indian cropping patterns and thus are critical for both farmers and consumers. In addition the United Nation also has extended support for such works, therefore augmenting the role of a legume such as *Vicia faba* as a versatile crop for food security and nutrition (ICRISAT, 2016). Presence of protein and minerals also brings forth the glimpses of chemical divergence between related taxa and therefore, the quantitative estimation have been carried out. Quantification and comparison of minerals and proteins in different cultivars would help us in choosing the most feasible and high yielding variety. Since *Vicia faba* is a versatile crop with positive influence on soil and in terms of sustainable food production, using different cultivars and analysing their nutrient profile can help us in creating region-specific informative databases for *Vicia faba* (Oliveira *et al*., 2016).

MATERIALS AND METHODS

Twelve cultivars of *Vicia faba* from different sources as detailed out in table 1 were selected for the study. Estimation of crude protein, mineral content etc. are discussed below :

Table 1: Germ plasms of *Vicia faba* under investigation

Serial No	Cultivars	Source	Remarks
1	DHB – 94	Agriculture College Dholi, Pusa Samastipur	Seasonal *rabi* crop.
2	DHB – 95	”	”
3	DHB – 96	”	”
4	DHB – 97	”	”
5	DHB – 98	”	”
6	DHB – 99	”	”
7	DHB – 100	”	”
8	HV – 1	Hisar Agriculture University	”
9	HV – 2	”	”
10	JV – 2	Agriculture College Dholi, Pusa Samastipur	In water stressed condition
11	Ranchi local	Birsa Agriculture University, Ranchi	”
12	Bhagalpur local	Agriculture College, Sabour	”

Crude protein estimation

The technique of Oser (1965) was employed for quantification of crude protein. The protein percentage could be known after multiplying nitrogen percentage (16.25) since protein content stands as 16 per cent of the total nitrogen content. The extraction of crude protein in each case was done by grinding 1 gm of seeds in 5 ml of distilled water for each of the selected taxon. 2 ml of the mixture of the solution is taken in a tube and 3 ml of 10 per cent TCA was added to it and the mixture was centrifuged and the supernatant contained protein nitrogen. Both the fractions were separately digested in 2 ml of conc. H_2SO_4. A little of catalyst containing of $CuSO_4$, K_2SO_4 and FeO_2 in ration of 1:8:1 was added. A few drops of H_2O_2 were added to the soluble fraction whose colour changed to apple green. The coloured solution denoted the completion of digestion process. For each digest, the volume was raised to 15 ml by adding distilled water. In separate tubes, 1 ml of the final digests were taken. In each tube 14 ml of distilled water was added and 2 ml of NaOH was also added for 30 minutes. These tubes were placed on ice bath and 3 ml of freshly made Neecler's reagent was added in each of them. The optional density was known at 490 nm using spectronic-30-spectrophotmeter.

For quantifying crude protein, a standard curve for nitrogen was obtained dissolving 66 mg of ammonium sulphate in 100 ml of distilled water. From this stock, 0.2, 0.4, 0.6, 0.8 and 1.0 was measured separately which will constrain 28, 56, 84, 112 and 140 g of nitrogen respectively. The volume of each was made to 15 ml after adding distilled water. The optical density was recorded at 490 nm on spectrophotometer against a blank consisting of distilled water which help this crude nitrogen content in each taxon, can be calibrated.

Mineral content estimation

The spectrophotometric determination of some alkaline earth metals and transition elements in selected twelve cultivars of *Vicia faba* was done by following the methods of Kohn, 1969; Chauhan *et al.* ,1980 and Malik *et al.*,2000. For each taxon, 2 g of seeds was dried at 80ºC for 48 hrs. The seeds were then crushed into fine powder. Each sample was divided in two halves, first half was used for the mineral nutrition and second half was preserved for the lab record. 1 gm of powder was kept in an oven at 105ºC for 3 hrs and then allowed to come in room temperature. The ash was put in a beaker containing 20 ml of conc. HNO_3 and left overnight for predigesting. A mixture of 4 ml of 1 per cent conc. H_2SO_4 and 7 parts of perchloric acid was added to the beaker containing predigested material. The beaker was put on

a sand bath for further digestion. After the subside of copious fumes of H_2SO_4, 10 ml of double distilled water was added to the beaker, a clear solution then appeared and volume was raised to 25 ml by adding double distilled water. The absorbance of portion of each solution was measured at its respective absorption maximum, against a reagent blank prepared under the similar conditions.

The absorbances of solutions were determined with a compensation spectrophotometer (UVIS PEC – Hilger) with 0.1 mm slit width and 1 cm cells at 23-25^{0}C. The wavelength scale was calibrated with the hydrogen spectral lines at 486 and 656 nm and by use of a didymium glass filter within the wavelength region 400 - 650 nm. The stock solution of 2 x 10^{-4} M tetramethylmurexide (NH_4^+ form) was prepared separately for each series of measurements just before use. All absorbance measurements were carried out with 4 x 10^{-5} M solutions of tetramethylmurexide. The concentrations of alkaline and transition metals were calculated directly from the weights of the analytically pure preparations used.

A Beckman pH meter model Expandometric SS – 2 was used for pH measurements. The pH of the solutions were adjusted using perchloric acid and sodium hydroxide solutions. All other reagents used were of analytical grade.

RESULTS AND DISCUSSION

The protein percentage of different cultivars included in this study has been presented in table 2.

Table 2: Crude protein percentage estimation in 12 cultivars of *Vicia faba*

Serial No.	Cultivars	Protein %
1	DHB – 94	32.06±.28
2	DHB – 95	32.05±.68
3	DHB – 96	32.08±.67
4	DHB – 97	32.08±.19
5	DHB – 98	32.07±.18
6	DHB – 99	32.00±.66
7	DHB – 100	32.04±.19
8	HV – 1	32.03±.22
9	HV – 2	32.06±.26
10	JV – 2	32.03±.26
11	Ranchi local	31.64±.16
12	Bhagalpur local	30.98±.34

Table 3: Quantitative (ppm)estimation of some alkaline earth metals in twelve cultivars of *Vicia faba*

Sl No.	Cultivars	Alkaline earth metals				
		Mg	Ca	Sr	Ba	Cd
1	DHB 94	9.80	18.05	1.40	33.64	5.96
2	DHB 95	9.75	18.02	1.30	33.50	5.94
3	DHB 96	9.65	18.01	1.21	33.45	5.94
4	DHB 97	9.67	17.09	1.14	33.23	4.89
5	DHB 98	9.72	18.07	1.19	33.24	5.87
6	DHB 99	9.60	17.05	1.12	33.28	5.78
7	DHB 100	8.50	16.05	1.10	33.20	5.62
8	HV 1	8.69	17.03	1.19	33.50	5.61
9	HV 2	9.60	17.06	1.12	33.28	5.78
10	JV 2	9.72	18.01	1.40	33.64	5.96
11	Ranchi local	9.62	18.05	1.28	33.59	5.85
12	Bhagalpur local	9.77	18.05	1.36	33.62	5.94

On an average, different cultivars have 32 per cent protein. The highest amount has (32.08%) been recorded in DHB-96, DHB-97 and the lowest in Bhagalpur local (30.98%). The cultivars like HV1 and HV2 and JV2 has been found to have more than 32 per cent of the vegetable protein. Protein percentage estimation clearly suggested that the two local varieties have been relatively poorer in terms of protein content in comparison to rest of the cultivars included in the study. Observation of the table 3 presents profile of some alkali earth metals in twelve cultivars of *Vicia faba*. This included very important alkali earth metals like magnesium (Mg), calcium (Ca), strontium (Sr), barium (Ba) and cadmium (Cd). Maximum amount of Mg was recorded in DHB-94 and Bhagalpur local. DHB-95, DHB-98, JV_2 *etc*. were also found to have an appreciable amount with regard of Mg. As far as calcium is concerned Bhagalpur local, Ranchi local and DHB-94 cultivars have been found to contain the highest amount of Ca and lower amount was recorded in DHB-98.

Barium is another alkaline earth metal which was found to be present in fair amount in all the cultivars. The maximum amount was recorded in cultivars like DHB-94, JV_2 (33.64 ppm) followed by Bhagalpur local (33.62 ppm).Strontium is another alkali earth metal whose profile was found within the critical limit (5 ppm) of toxicity (Mishra, 2000). Fortunately, all the cultivars screened have been found to have very poor amount of it.

Table 4: Quantitative (ppm)estimation of some alkaline metals in twelve cultivars of *Vicia faba*

Sl No.	Cultivars	Alkaline metals		
		Na	K	Pb
1	DHB 94	1.24	2.94	2.16
2	DHB 95	1.25	3.09	2.00
3	DHB 96	1.33	2.50	1.60
4	DHB 97	1.39	1.80	1.90
5	DHB 98	1.50	3.00	2.10
6	DHB 99	1.40	2.00	2.11
7	DHB 100	1.28	2.95	1.94
8	HV 1	1.33	2.90	1.86
9	HV 2	1.45	2.86	1.96
10	JV 2	1.18	2.22	1.92
11	Rachi local	1.28	2.94	1.86
12	Bhagalpur local	1.10	3.64	1.80

Quantitative analysis of alkaline metals like sodium (Na), potassium (K) and lead (Pb) have also been done and result are presented in table 4. Analysis of table 4 dictates the maximum amount of sodium was sound to be present in cultivar HV_2 and minimum one was measured to be present in Bhagalpur local variety. Potassium is present in plant body either as free ions or in radially exchangeable combinations. This has been found to be the most mobile of the different elements. All the cultivars of *Vicia faba* have found to have very poor amount of potassium. The highest amount has been recorded in Bhagalpur local (3.64 ppm) and the lowest amount is found in DHB-97 (1.80 ppm). Among the other rich cultivars are DHB-95 (3.09 ppms) and DHB-98 (3.00 ppm).

As far as estimation goes, the maximum lead was found in cultivar DHB-94 (2.16 ppm), DHB-99 (2.11 ppm) and DHB-98 (2.10 ppm). Among the lowest values come in cultivar DHB-96 (1.60 ppm) and Bhagalpur local (1.80 ppm). The last parameter of the elements comprises of transitional elements including manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu) and zinc (Zn). Manganese is extremely important element needed for chlorophyll development. It becomes toxic after a critical limit.

Table 5 shows that Mn ranges from 9.45 ppm present in cultivar DHB-99 to 9.80 ppm present in cultivars like DHB-94 and JV_2. As far as Iron is concerned for the preferential basis cultivars like DHB-94, JV_2, DHB-95 and Bhagalpur local are suggested to be preferred. Iron is very important component because of its metabolic utility and structural incorporation. It is needed for chlorophyll synthesis, respiration, photosynthesis and other metabolic pursuits.

Presence of Cobalt is again very critical for the suitability of food grains. As far as the present estimation goes, the element is uniformity distributed bearing two cultivars were the minimum amount was measured. These are DHB-95 having a figure of 9.95 ppm and DHB-96 (9.98 ppm). The highest amount is recorded in cultivar DHB-94 (10.60 ppm).

The highest amount of Ni was found in cultivar DHB-94 (31.50 ppm) and the lowest amount was recorded in DHB-97 (30.11 ppm). Other varieties with appreciable amount include DHB-98, DHB-96, Ranchi local and Bhagalpur local (31.42 ppm).

Copper is another important element which becomes toxic after a critical level. Fortunately in all cultivars screened the amount of copper has been found to be very less. Some class of uniformity has also been recorded in case of zinc. In summation, it can be said that nutritional studies have been very helpful to provide

Table 5: Quantitative (ppm) estimation of some transitional elements in twelve cultivars of *Vicia faba*

Cultivars	Transitional elements					
	Mn	Fe	Co	Ni	Cu	Zn
DHB 94	9.80	3.25	10.60	31.50	3.25	3.21
DHB 95	9.60	3.21	9.95	30.40	3.21	3.18
DHB 96	9.55	3.18	9.98	31.42	3.18	3.16
DHB 97	9.75	3.08	10.55	30.11	3.15	3.14
DHB 98	9.65	3.09	10.41	31.42	3.09	3.07
DHB 99	9.45	3.11	10.42	31.15	3.14	3.18
DHB 100	9.52	3.08	10.45	31.14	3.17	3.16
HV 1	9.55	3.08	10.41	30.85	3.12	3.13
HV 2	9.51	3.07	10.45	30.82	3.09	3.12
JV 2	9.80	3.25	10.51	31.41	3.25	3.21
Ranchi local	9.52	3.08	10.41	31.42	3.23	3.20
Bhagalpur local	9.65	3.18	10.41	31.42	3.17	3.14

some handy information for preferential selection of cultivars.

In view of wide consumption of *Vicia faba*, it was thought to evaluate the nutritional status of the selected cultivars and some of the parameters selected in this regard included total protein and minerals. On this basis some cultivars of DHB-series have been preferred nutritionally.

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