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SDS-PAGE Analyses and Electrophoretic Band Characterization of Three Cultivars of Grass Pea based on Seed Storage Proteins

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ABSTRACT

Background: Grass pea (*Lathyrus sativus* L.) is valued for its high dietary protein content, high degree of adaptability under extreme conditions, disease resistance and low input requirement for its cultivation. This species is represented by several sub-species and important varieties which are not well distinguishable by their morphological characters. The objective of present study was to determine the cultivar differences based on seed storage protein by using SDS-PAGE and to obtain information useful for a breeding program.

Methods: Gel electrophoresis was carried out in the discontinuous buffer system in a vertical electrophoresis apparatus and electrophoretic data were documented by using a gel documentation system. Seed protein sub-fractions (albumins, globulins, glutelins and prolamins) from seeds were extracted and quantified. ODAP (*β-N-oxalyl-L-a,β-diaminopropionoc acid*) an undesirable and toxic non-protein amino acid (responsible for 'neuro-lathyrism' disease) content was estimated.

Result: The differences among cultivars were observed and clearly identifiable from their protein banding patterns in view of in number of bands, position of the bands and molecular weight of the bands *etc*. Quantity of total protein and sub-fractions of protein (albumins, globulin, glutelins and prolamins) of seed storage proteins also showed the variation among the three cultivars. ODAP content estimated in three grass pea cultivars was found very low ranging which is highly desirable for cultivation.

Key words: Grass pea, SDS-PAGE, Seed storage protein.

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is a member of the *Vicieae* tribe and family *Fabaceae*, consists of about 160 annual and perennial grain legume crop plants and many of them are economically important. This species is represented by several sub-species and important cultivars. Grass pea (*khesari/Teora/Lakh/Lakhadi* in India) is prized for its high dietary protein content (26-32%), adaptability to harsh environments, disease tolerance and low input requirements for cultivation (Urga *et al*. 2005). Despite its drought resistance, grass pea is unaffected by excessive rainfall and can be grown in flood-prone areas, including those with poor soils and thick clay (Sinha, 1980). Grass-pea can easily be grown on marginal land under normal as well as adverse environmental conditions. Because of its ease of cultivation, grass pea is considered a traditional common crop by more than 100 million people in drought-prone areas of Asia and Africa and cheapest food legume for low-income families and rich source of protein and good qualities of essential amino acids. Grass pea also contains an undesirable and toxic non-protein amino acid called ß-*N-oxalyl-L-α, ß-di-aminopropionic acid* (ODAP) which can lead to'neuro-lathyrism' a disease causing paralysis of lower limbs if consumed in high quantity (300-400 g/day) as a staple food for a long period of time (for continuous 2-4 months) (Singh and Rao, 2013; Mondal and Puteh, 2014; Basaran *et al*. 2016; Ozbek Yazici *et al*. 2020). The ODAP content of different grass pea genotypes ranged from 1.55 to 20.8 mg/g seeds (Arslan *et al*. 2017). Its seeds also contain a high amount of free L-homoarginine (1.8±0.06 g/100 g of seed flour), which may act as a precursor of lysine (1.76±0.11 g/100g of seed flour, $C_6H_{14}N_2O_2$) (Mariame Cisse *et al*. 2013). Lysine is an essential amino acid and the building blocks of protein.Lysine helps to produce collagen in the body and it is essential for the development of carnitine, a nutrient that aids in the conversion of fatty acids into energy and lowers cholesterol. It also appears to have antiviral properties. As a fodder crop it is both palatable and extremely nutritious and for sheep its high lysine content promotes improved wool quality (Tubby, 2009).

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Grass pea seed proteins are synthesized at a particular stage during seed development to be utilized during germination. These proteins are therefore called storage proteins. Seed storage proteins are highly physiologically stable, being unaffected by environmental conditions and easily to handle. The major storage seed proteins are albumins (water soluble), globulins (salt soluble), glutelins (alcohol soluble) and prolamins (acid or alkali soluble) *etc*. of seeds and these proteins display a number of characteristic features (Osborne, 1924; Katsube *et al*. 1999). Thus, it was reported that biochemical and molecular analysis, particularly of electrophoretic analysis of seed proteins as revealed by electrophoretic band profiles of seed proteins of SDS-PAGE (Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis) have provided valid evidence for detecting and assessing relationships even at the level of cultivars (Ladizinsky and Hymowitz, 1979; Kamel *et al*. 2003; Javaid *et al*. 2004; Çelebi *et al*. 2009; Hameed *et al*. 2009; Jukanti *et al*. 2017). So, the objective of present study was to determine the differences among three important cultivars of *Lathyrus sativus* based on seed storage protein by using SDS-PAGE and to obtain information useful for a breeding program.

MATERIALS AND METHODS

Plant material

The seeds of 3 different cultivars of grass pea (Fig 1 A-C) namely B-1(Nirmal), flower initiation time 58 days), Prateek (flower initiation time 67 days) and BIO L-212 (Ratan), flower initiation time 52 days) were officially procured from Pulses and Oilseeds Research Station, Berhampore, West Bengal, India. These cultivars were chosen and selected as experimental materials only on the basis of their flowering time (an agronomic character). 100 seed weights of three cultivars were 32.4 gm (Prateek), 35.5gm (Nirmal) and 42.5 gm (Ratan) respectively.

Extraction of seed proteins

From each cultivar's 0.2 g dry seed was homogenised in chilled 2 ml of 0.2 M phosphate buffer in a pre-chilled pestle and mortar (pH-8.2). The extracts were centrifuged at 4°C for 15 minutes at 10,000 rpm. The crude proteins were extracted and the supernatant was used for protein profiling. By using a dye-binding assay, the protein concentration of extracts was determined immediately and directly from the supernatant using the method of Bradford (1976). A standard curve of 595 nm absorbance versus 10-80 µ g of BSA was also drawn and the amount of protein in the sample was computed using this curve. Repetition of same experiment was done 3 times in order to check the reproducibility of the method.

Seed protein sub-fractions (albumins, globulins, glutelins and prolamins) from *Lathyrus* seeds were extracted and quantified as described by Vaz *et al*. (2004).

SDS-PAGE

To denature the protein, the supernatant was mixed (1:1) with 2× sample buffer (Laemmli, 1970) and heated in a 1.5 ml eppendorf tube in a water bath at 85°C for 3 minutes just before starting the electrophoresis process. Then the protein samples were then run through a one-dimensional SDS-PAGE in a 1 mm thick gel slab (4% stacking gel= 2.5 cm height and 10% resolving gel= 5.5 cm height). The gel was 8 × 7.3 cm^2 in total.

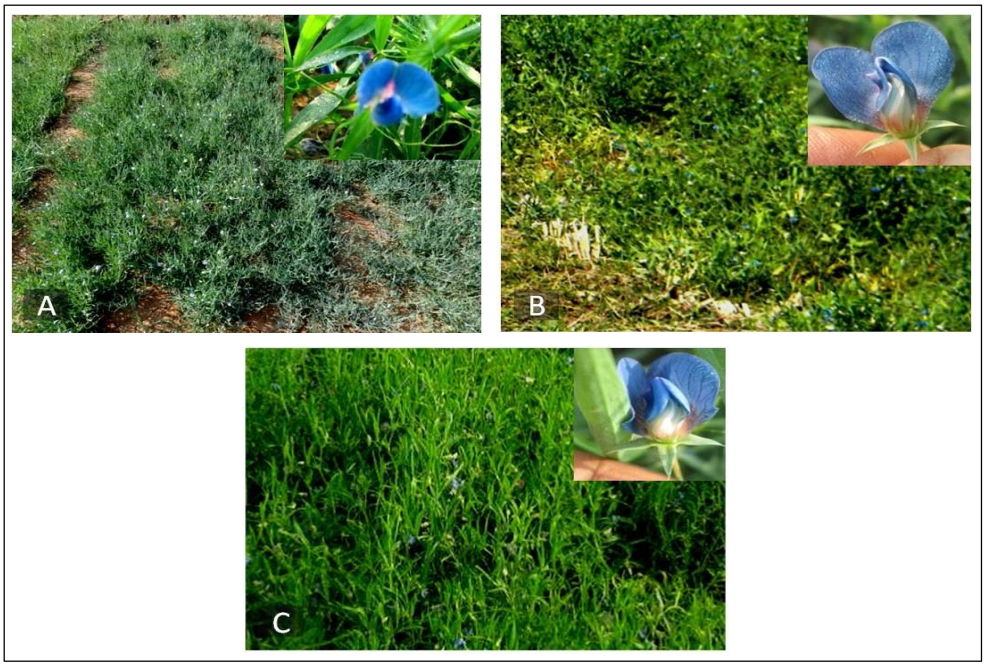

Fig 1: Showing field grown plant of (A) Variety BIO L 212 Ratan, (B) Variety Prateek and (C) Variety B-1 Nirmal, with flowers (inset).

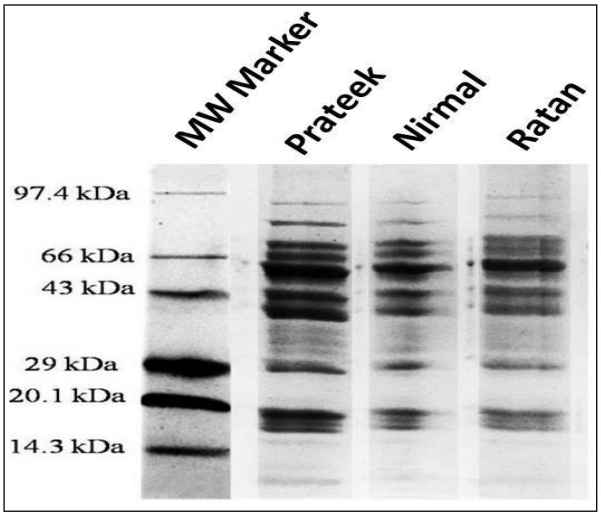

Fig 2: Showing seed storage protein profiles of three varieties of grass pea on 10% SDS polyacrylamide gel {four lanes are marked by MW (molecular weight) marker, Prateek, Nirmal and Ratan at the top}. Lane MW marker (extreme left) contains marker protein showing seven bands with molecular weights 97, 66, 51, 30, 25, 20, 14 kDa (A).

Electrophoresis was performed in a vertical electrophoresis apparatus (Bio-Tech India Pvt. Ltd) using a discontinuous buffer system as per the method of Laemmli (1970). 20 µl protein samples were loaded into each well of the gel using a micro-pipette. Protein molecular weight marker (molecular weight range= 14-97 kDa) from Chrommas Biotech, India, was used in one well of the same gel. To see the movement of protein in the gel, 0.02 per cent bromophenol blue (BPB) was added to the protein sample as a tracking dye. The gel was run in continuous current mode at 10 mA. The gel was then stained with 0.025 % Coomassie brilliant blue (CBB) R-250 for an overnight staining.

Analysis of gel documentation

Finally, the Gel-DocTM XR+ system from Bio-Rad in the United States was used to photograph and scan the gels. Image LabTM software (version 5.0) was used to perform a detailed analysis of protein band patterns in terms of band number, mobility of protein bands, staining intensity, band percentage, lane percentage and molecular weight determination for each band.

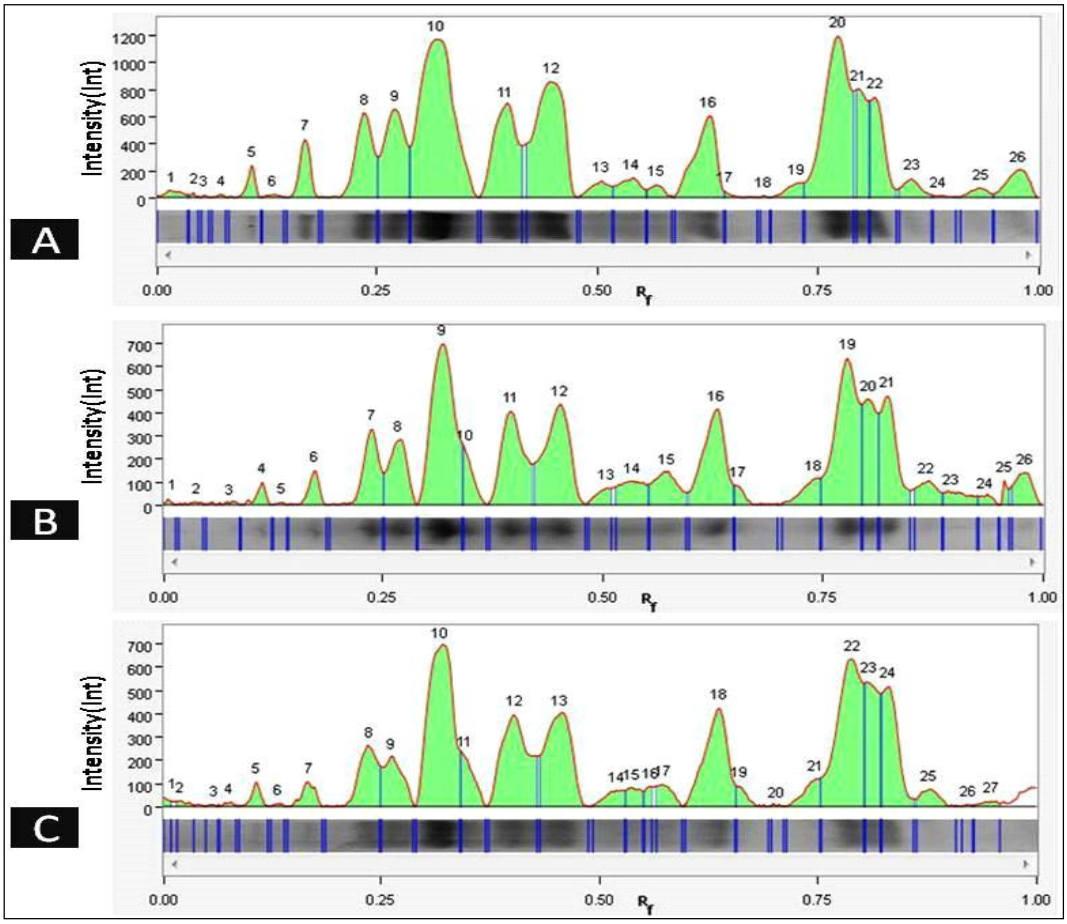

Fig 3: Showing Gel Doc data of individual lane profile showing the number of peaks of different heights obtained on the basis of the intensity of bands against their respective relative mobility (Rf value) of bands of (A) Variety Prateek, (B) Variety Nirmal and (C) Variety Ratan.

Quantitative assay of ODAP

This study was conducted according to the protocol described by Karadag *et al*. (2010).

RESULTS AND DISCUSSION

The total seed protein of three grass pea varieties was analysed using one-dimensional denaturing SDS–PAGE, revealing qualitative and quantitative inter-varietal differences in terms of total number of protein bands, location, thickness, staining intensity (Fig 2), relative mobility of bands and molecular weight. The Gel Doc data/ electrophorogram (Fig 3 A-C) of total protein profiles showed the number of peaks of different heights obtained on the basis of the staining intensity of protein bands against their respective relative mobility (Rf value) of bands of the studied cultivars.

During protein profiling of total proteins of experimental cultivars, B-1(Nirmal) and Prateek showed 26 protein bands while 27 protein bands were found in case of BIO L-212 (Ratan). The result shows the relative mobility (Rf value) and molecular weight of protein bands varied from 0.002 to 0.979 kDa and 14.3 to 97.4 kDa (Table 1-3). Quantitative variation of total seed protein and major storage seed protein among different varieties of grass pea studied in the present experiment was also found and is shown in Table 4.

The quantities of total seed proteins in the present study (Table 4). Prateek cultivar *(*78.9 µg/ml) has highest total protein content whereas B1 (Nirmal) (65.4µg/ml) has lowest total protein content. Present study also reports intermediate quantity of total seed proteins (73.2 µg/ml) in BIO L-212 (Ratan) cultivar. Present results showed that B1 (Nirmal) and BIO L-212 (Ratan) have the highest albumin content (3.6 µg/ml and 4.5 µg/ml, respectively). Nirmal (7.2 µg/ml) and Ratan (8.0 µ g/ml) had higher globulin content than the other studied variety. Furthermore, it was determined that Ratan has the highest glutelin content (3.4 µg/ml) and the highest prolamin amounts (0.41 µg/ml) among the varieties studied. However, prolamin amounts of all studied taxa were quite low.

In this endeavour, ODAP content estimated in three grass pea cultivars was found very low (Table 4) ranging from 0.06 to 0.08%. These low-toxin (<0.1%) varieties are highly desirable for cultivation.

The SDS-PAGE for water-soluble storage seed protein electrophoresis was used to investigate primarily the

Table 1: Profile analysis of eletrophoretically separated seed protein bands of B-1(Nirmal) variety of *Lathyrus sativus*.

Band no.	Mol. Wt. (kDa)	Relative Front (R_f)	*Volume (Int)	**Band %	***Lane %
1	97.4	0.007	11,021	0.1	0.1
2	97.4	0.040	13,161	0.1	0.1
3	97.4	0.076	17,441	0.2	0.2
4	97.4	0.114	76,719	0.8	0.8
5	97.4	0.135	6,955	0.1	0.1
6	97.4	0.173	137,495	1.4	1.4
7	80.6	0.239	454,643	4.7	4.7
8	67.4	0.268	465,771	4.8	4.8
9	56.1	0.318	1,478,205	15.3	15.2
10	51.2	0.344	213,037	2.2	2.2
11	42.8	0.396	826,468	8.6	8.5
12	37.7	0.452	983,437	10.2	10.1
13	33.2	0.505	96,193	1.0	1.0
14	31.1	0.535	243,960	2.5	2.5
15	28.6	0.573	314,045	3.3	3.2
16	26.1	0.630	827,431	8.6	8.5
17	25.1	0.654	77,468	0.8	0.8
18	21.8	0.740	187,678	1.9	1.9
19	20.6	0.779	1,309,787	13.6	13.5
20	19.4	0.803	569,882	5.9	5.9
21	18.1	0.824	695,928	7.2	7.2
22	15.5	0.869	184,361	1.9	1.9
23	14.3	0.896	129,149	1.3	1.3
24	14.3	0.934	49,862	0.5	0.5
25	14.3	0.957	46,973	0.5	0.5
26	14.3	0.979	220,420	2.3	2.3

*volume= the sum of all the intensities within the band boundaries.

**band%= percentage of the band's volume compared to all band volumes in the lane.

***lane%= percentage of the band's volume compared to the entire volume of the lane.

Table 2: Profile analysis of eletrophoretically separated seed protein bands of Prateek variety of *Lathyrus sativus*.

Band no.	Mol. Wt. (kDa)	Relative Front (R_f)	*Volume (Int)	**Band %	***Lane %
1	97.4	0.019	68,373	0.4	0.4
2	97.4	0.043	12,519	0.1	0.1
3	97.4	0.055	4,601	0.0	0.0
4	97.4	0.074	10,914	0.1	0.1
5	97.4	0.109	146,483	0.9	0.9
6	97.4	0.133	21,079	0.1	0.1
7	97.4	0.170	376,961	2.4	2.3
8	81.5	0.237	896,339	5.6	5.6
9	66.0	0.272	1,126,710	7.1	7.0
10	56.1	0.318	3,296,563	20.7	20.5
11	42.8	0.396	1,327,335	8.3	8.3
12	38.0	0.448	2,071,306	13.0	12.9
13	33.2	0.505	181,686	1.1	1.1
14	30.7	0.540	251,022	1.6	1.6
15	28.8	0.567	106,679	0.7	0.7
16	26.2	0.626	1,066,897	6.7	6.6
17	25.5	0.645	23,112	0.1	0.1
18	23.7	0.689	4,387	0.0	0.0
19	22.3	0.727	142,310	0.9	0.9
20	20.7	0.773	2,525,414	15.9	15.7
21	19.9	0.796	737,123	4.6	4.6
22	18.6	0.815	835,777	5.3	5.2
23	16.1	0.856	190,995	1.2	1.2
24	14.6	0.886	15,729	0.1	0.1
25	14.3	0.934	99,403	0.6	0.6
26	14.3	0.978	368,080	2.3	2.3

*volume= the sum of all the intensities within the band boundaries.
**band%= percentage of the band's volume compared to all band volumes in the lane.
***lane%= percentage of the band's volume compared to the entire volume of the lane.

Table 3: Profile analysis of eletrophoretically separated seed protein bands of BIO L 212 (Ratan) variety of *Lathyrus sativus*.

Band no.	Mol. Wt. (kDa)	Relative Front (R_f)	*Volume (Int)	**Band %	***Lane %
1	97.4	0.002	21,186	0.2	0.2
2	97.4	0.021	23,005	0.2	0.2
3	97.4	0.059	5,457	0.1	0.1
4	97.4	0.076	14,766	0.2	0.2
5	97.4	0.107	80,464	0.9	0.8
6	97.4	0.133	8,881	0.1	0.1
7	97.4	0.168	126,153	1.4	1.3
8	81.5	0.237	428,963	4.6	4.5
9	70.3	0.261	373,537	4.0	3.9
10	56.1	0.318	1,573,863	17.0	16.6
11	50.9	0.346	251,771	2.7	2.7
12	42.1	0.403	945,238	10.2	10.0
13	37.4	0.455	939,567	10.1	9.9
14	32.1	0.521	118,877	1.3	1.3
15	30.8	0.538	102,720	1.1	1.1
16	29.4	0.559	54,998	0.6	0.6
17	28.6	0.573	132,038	1.4	1.4
18	25.8	0.637	860,280	9.3	9.1
19	24.8	0.661	81,320	0.9	0.9
20	23.2	0.702	5,564	0.1	0.1
21	21.6	0.747	165,315	1.8	1.7
22	20.2	0.791	1,413,577	15.2	14.9
23	19.0	0.808	657,087	7.1	6.9
24	17.6	0.830	732,843	7.9	7.7
25	15.0	0.877	123,264	1.3	1.3
26	14.3	0.922	4,922	0.1	0.1
27	14.3	0.948	32,635	0.4	0.3

*volume= the sum of all the intensities within the band boundaries.
**band%= percentage of the band's volume compared to all band volumes in the lane.
***lane%= percentage of the band's volume compared to the entire volume of the lane.

Table 4: Total protein, major storage proteins amounts and ODAP content of investigated *Lathyrus* varieties.

Variety	Protein amount (µg/ml)	Albumins (µg/ml)	Globulins (µg/ml)	Glutelins (µg/ml)	Prolamins (µg/ml)	ODAP content (%)
B1(Nirmal)	73.2	3.6	7.2	2.0	0.35	0.07
Prateek	65.4	2.2	6.7	1.5	0.16	0.08
BIO L 212 (Ratan)	78.9	4.5	8.0	3.4	0.41	0.06

diversity of seed storage proteins and inter-varietal genetic differences (Emre *et al*. 2015) among three grass pea cultivars. The band patterns show variations in the number of bands, band position and band molecular weight, among other things. Each cultivar's protein profiles exhibit their own electrophoresis pattern, with subunits of varying molecular weight. The differences between varietiesare also observed in both presence and absence of a particular protein band and all three taxa are clearly identifiable from the protein banding patterns. Based on SDS-PAGE results, it can be said that electrophoretic analysis of seed storage proteins demonstrated the similarity between Nirmal and Prateek in terms of same number of protein bands (26) whereas Ratan variety differs by its 27 protein bands. All three cultivars are clearly identifiable from their protein banding patterns in view of both band mobility and relative intensity of the bands.

Furthermore, our present results show that the early flowering characteristics in BIO L-212 (Ratan) cultivar are generally correlated with higher mean levels of total seed protein and glutelin. High seed protein content and early flowering are very important for grass pea breeding strategies, agronomy and improvement (Girma *et al*. 2012). Protein content and seed weight are important traits in grass pea. The approximate ranges in protein content and seed weight of the grass pea collection analysed are 26%-32% and 32.4–42.5 g (100 seeds weight), respectively. Interesting, for values of protein content and 100 seeds weight seems to be the valid evidence of same geographical origin, which could be utilized in breeding programmes after further evaluation and characterization. As a result, genotypes with high protein content and/or 100 seed weight may be useful parents for breeding improved genotypes (Bisignano *et al*. 2003). In other words, strategies for recognize high protein individuals within higher-seeded entries should be suggested.

CONCLUSION

Based on SDS-PAGE results, it can be concluded that electrophoretic analysis of seed storage proteins can be used as powerful tools for grass pea breeding strategies, agronomy and improvement. In this endeavour, ODAP content among three grass pea varieties was found very low, so these varieties are nutritionally safe for food and feed as well as highly desirable for cultivation. Storage protein profiles of each variety showed its own electrophoresis pattern by which they can also be distinguished biochemically particularly on the basis of number of protein bands, band mobility, relative intensity and molecular weight of individual protein band. However, protein content, seed weight, early flowering characteristics and glutelin content could be used in the selection of superior parental genotypes for breeding programmes.

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Author contributions

All authors contributed equally to this paper. Animesh Mondal performed the seed protein extraction, quantitative assay of protein and gel electrophoresis experiments the Gel Doc analysis, interpretations, seed collection and characterizations; Kalyan Kumar De contributed in design, analyses of the results and writing the paper.

Conflict of interest

The authors declare no conflict of interest.

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