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Seed protein characterization and isozyme diversity for cultivar identification in grasspea (*Lathyrus sativus* L.)

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Abstract

The characterization of seed protein of various geographically divergent grasspea genotypes was done by protein fractionation, polymorphism and isozymic analysis. No positive correlation was found between seed size and protein content. Though there was genotype-specificity for different levels of protein fractions, globulin level proved to be the highest and prolamine the least. SDS-PAGE of different grasspea genotypes, even of same geographic origin revealed variation in number, width and intensity of bands. The multigene inheritance of seed protein expression could be identified from the zymograms. Similarity percentage between genotypes was also calculated. In general, geographical origin does not influence genotype specific seed protein content and its polymorphism. Observations suggested the polymorphic nature of esterase and peroxidase isozyme, which provided worthy information, regarding genetic variability among the cultivars under experiment.

Key words : Grasspea, seed storage protein, protein fraction, protein polymorphism, isozymes.

Introduction

Classical identification of cultivars and more so the germplasm diversity based on standard morphological markers has proved to be inadequate because of the wide spectrum of phenotypic variation and their interaction with environment [1]. In such instances electrophoretic patterns of seed protein can be used effectively to decipher the similarities and differences between cultivars and genotypes [2, 3]. Protein markers have been successfully used for varietal identification for several crops[4-7]. Isozymes also provide useful evidences in the study of variation within cultivars in terms of intensity of common bands and presence or absence of other bands [8].

In this study, seed proteins of grasspea genotypes were characterized by analyzing seed protein fractions and studying protein polymorphism by SDS-PAGE. This was supported by the analysis of two isozymes esterase and peroxidase from leaf tissues by Polyacrylamide Gel Electrophoresis (PAGE).

Materials and methods

Four divergent genotypes comprised the experimental materials. Of them Nirmal was developed at Berhampur, West Bengal and Hoogly Local, an indigenous type is cultivated in the district of Hoogly, W.B. The other two P24 and P28 of IARI, New Delhi are grown in northern parts of India.

Seed protein content of genotypes was estimated following Lowry *et al.* [9]. Protein fractionations were done by the method of Osborne [10] modified by Dannielson [11] and Shewry *et al.* [12]. To study seed protein polymorphism, one dimensional SDS-PAGE (15% separating gel and 4% stacking gel) was carried out following Laemmli [13] in a mini vertical gel system (BIOTECH). For this purpose total protein was extracted after suspending seed flour for 45 minutes in an extraction buffer (Tris-HC1, pH 6.8), followed by centrifugation at 10,000 rpm at 4°C for 20 minutes. Then the protein sample was heated for 2-3 minutes in boiling water and gradually cooled. 100 µg of protein was loaded with micropipette along with 10 µl sample buffer containing bromophenol blue in each sample well. A molecular weighed marker (BSA, 66 K Da) was also incorporated into the gel to determine the molecular weight of the bands. The gel was run at 20 mA for 2 hours followed by staining in Coomassie Brilliant Blue R250 over night. Relative mobility (Rm) of the protein band was determined. For the study of isoenzymes esterase and peroxidase, native PAGE was done with 7.5% gel. Electrophoresis of esterase was done according to the method of Kahler and Allard [14]. For this purpose 1 g of fresh leaf sample from 10 days old seedlings was macerated in Tris-citric acid buffer

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(pH 7.8) at 4°C. The homogenates were centrifuged at 10,000 rpm for 30 minutes at 0°C. 50 μg protein was loaded in each lane and the gel was run at 20.4 mA for 2 hours. The gel was stained using Fast Blue RR salt and α-naphthyl acetate. For the study of peroxidase also 1 g leaf sample from 10 days old seedlings was macerated in phosphate buffer (pH 7.0) at 4°C for 30 minutes. Staining was done according to the method proposed by Welter and Dyck [15] using ortho dianisidine. Relative mobility (Rm) values were calculated both for esterase and peroxidase bands.

Results and discussion

Seed weight protein content and protein fractions - Different genotypes recorded variable 100-seed weight and seed protein content (Table 1). Hooghly Local with minimum 100-seed weight displayed highest seed protein content. Nirmal on the other hand, recorded minimum seed protein content though it had comparatively higher seed weight. Negative correlation (r = –0.10) was observed between seed size and seed protein content. Neither there was a positive correspondence between widely geographically divergent genotypes and seed protein content or seed size. Albumin and glubulin were two major protein fractions of which globulin was the highest in the genotypes, prolamine fraction was the least (Table 1). This corroborates the earlier observations of Bera and Bera [16] in black gram and Dana [17] in green gram. However, P/28 a genotype evolved at IARI with higher seed weight and fairly high seed protein content maintained very high level of different protein fractions, particularly albumin and globulin. Such genotype specificity for different protein fractions would be an important parameter for varietal characterization.

Table 1. Seed weight, soluble protein content and different protein fractions in grasspea genotypes

Genotype	100 seed wt(g)	Soluble protein (%)	Fractions (% of total protein)			
			Albumin	Globulin	Glutelin	Prolamin
Nirmal	7.12	21.80	26.64	33.46	20.90	0.10
P24	6.45	22.93	26.68	30.52	19.88	0.16
P28	8.33	23.72	27.10	34.30	20.50	0.18
Hooghly Local	6.21	24.61	26.40	32.70	20.70	0.21

Protein polymorphism - SDS-PAGE showed differences in number of bands, band width and intensity for different genotypes of grasspea [Fig. 1]. The genotypes exhibited maximum of 19 bands. Sampat [18] also reported 18-25 protein bands in different *Vigna* species. Two genotypes of IARI, New Delhi, namely P24 and P28 differed in their band number in which band 19 was missing in P24 and P28 but additional two bands '11' and '13' were absent in P28. On the other hand, two genotypes of West Bengal displayed a different kind of situation. Seed protein of Nirmal expressed all the 19 bands, but in Hoogly Local band '11' was missing. Thus genotypes of same geographic origin displayed different band numbers for their seed protein. With reference to molecular weight (compared with the standard BSA of 66 K Da) and respective Rm values (Table 2), it is possible to draw some kind of approximation regarding their molecular weights and several protein groups were envisaged. Grasspea genotypes represented broadly five different protein groups and within each group a good deal of differences existed as revealed by their band width and staining intensity. However, band '1', '2' and '3' might be considered representing one protein group, where band no. 1 would likely to have molecular weight little above 66 K Da while band '2' and '3' would have within the range of 60-55 K Da. Band '4' represented 2nd group of protein, which was distinctly different because of its broad width and highly intense staining (Fig. 1). It would have the molecular weight around 45 K Da. Third group represented maximum number of bands (5 to 13) with varying width and intensity pattern. They represented molecular weight in the range of 35-21 K Da. The fourth group comprising of four different bands represented also similar width but different intensity, their molecular weight would be in the vicinity of 15 K Da. The fifth group with two bands would likely to represent the molecular weight in the range of 10-12 K Da. The above discussion indicated the presence of

Table 2. SDS-PAGE banding pattern of seed storage proteins in four grasspea genotypes

Band No.	Rm values	Genotypes				Band intensity	Band width
		P/24	P/28	Hooghly Local	Nirmal		
1	0.200	+	+	+	+	F	N
2	0.234	+	+	+	+	F	N
3	0.268	+	+	+	+	F	N
4	0.230	+	+	+	+	I	B
5	0.398	+	+	+	+	I	IM
6	0.441	+	+	+	+	I	IM
7	0.468	+	+	+	+	I	IM
8	0.489	+	+	+	+	F	N
9	0.524	+	+	+	+	F	N
10	0.551	+	+	+	+	F	N
11	0.572	+	-	-	+	I	N
12	0.613	+	+	+	+	I	N
13	0.648	+	-	+	+	F	N
14	0.717	+	+	+	+	I	IM
15	0.751	+	+	+	+	I	IM
16	0.786	+	+	+	+	I	IM
17	0.827	+	+	+	+	F	IM
18	0.895	+	+	+	+	F	N
19	0.944	-	-	+	+	F	N

Where F = Faint, I = Intense, N = Narrow, B = Broad, IM = Intermediate

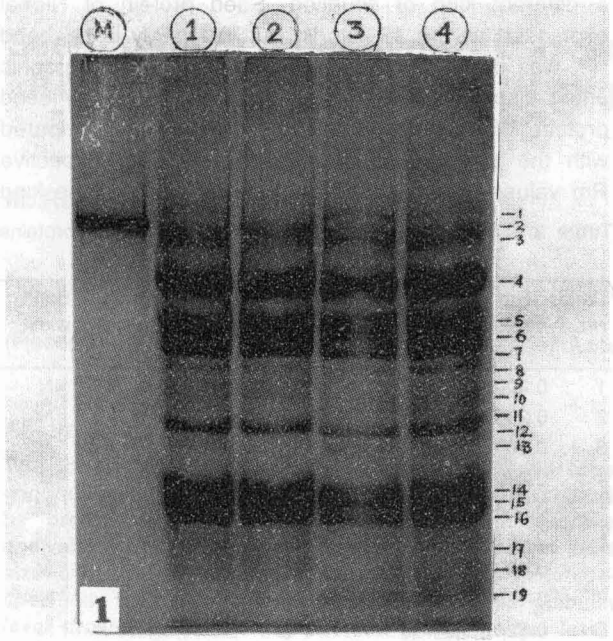

Fig. 1. SDS-PAGE electrophoretic pattern of seed storage proteins from (M) BSA, (1) P24, (2) P28, (3) Hooghly Local, (4) Nirmal

polypeptide bands of heterogeneous molecular weight and varying intensity. Significantly the four high molecular weight polypeptide bands (> 66 K Da, 60-55 K Da and around 45 K Da) was universally present in the genotypes and some of the lower molecular weight polypeptide bands were missing atleast in one among P28, Hoogly Local and P24. It appears that several genes might be controlling the expression of the polypeptides. In fact multigene inheritance of seed protein expression is well established in other crops also [19]. Following Whitney *et al.* [20] the similarity percentage of genotypes were worked out. The genotypes P24 and Nirmal recorded similarity percentage close to 95 indicating their high order of closeness in seed protein polymorphism. Significantly they had distinctly different geographical distribution. P24 and Hoogly Local displayed around 80% similarity, suggesting reasonably seed protein divergence between them. Interestingly they also differed in their seed protein content. This study by and large demonstrates genotype-specific protein polymorphism which is not always influenced by geographical origin.

Isozyme diversity - As many as 7 bands were identified (Fig. 2) in case of esterase. None of the genotypes showed variation in band number. Different bands exhibited different mobility on the charged gel indicating different molecular weights of esterase bands. However, bands differed in colour intensity and width. These observations would suggest a polymorphic nature of esterase isozyme. Bands '5' and '6' were similarly intense in Nirmal and Hoogly Local but they were

Table 3. Isozyme banding pattern of four genotypes of grasspea

Band No.	Rm values	Band intensity	Band width
Esterase			
1	0.128	Faint	Broad
2	0.192	Faint	Intermediate
3	0.205	Faint	Intermediate
4	0.230	Intense	Broad
5	0.269	Faint	Intermediate (Hooghly Local and Nirmal) Broad (P/24 and P/28)
6	0.307	Faint	Narrow
7	0.358	Faint	Narrow
Peroxidase			
1	0.080	Faint	Narrow
2	0.200	Faint	Broad
3	0.246	Faint	Broad
4	0.430	Intense	Broad
5	0.471	Intense	Broad

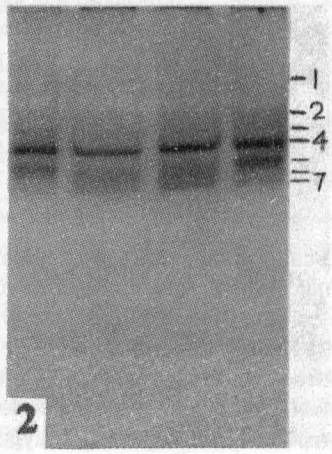

Fig. 2. Electrophoregrams of esterase for (1) P28, (2) Hooghly Local, (3) Nirmal, (4) P24

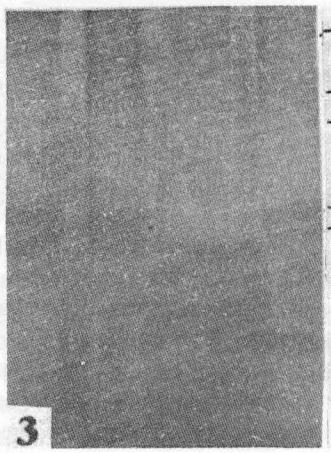

Fig. 3. Electrophoregrams of peroxidase for (1) P28, (2) Hooghly Local, (3) Nirmal, (4) P24

comparatively faint in P24 and P28 indicating a subtle difference between two groups of genotypes. In case of peroxidase five distinct bands were observed (Fig. 3). No observable variations were recorded in either band width or band intensity. However, Rm values indicated different mobility pattern suggesting presence of different molecular weights for the bands. Mobility values ranged from 0.080 to 0.471 indicating a wider range of variability in molecular weights for peroxidase bands. On the other hand, Rm values of esterase bands ranged from 0.128 to 0.358 suggesting a close range molecular weight variation for the esterase bands. It becomes evident that isozymic study under the present set of experiment provides meaningful information which suggests the existence of genetic variability among these cultivars. The polymorphism observed for seed proteins and leaf esterase isozymes among the grasspea cultivars is very informative and useful.

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