

Identification of Lentil Varieties by Seed Protein Electrophoresis

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ABSTRACT Twenty five varieties of lentil obtained from IIPR, Kanpur were analyzed for varietal identification through electrophoresis of seed protein. In all 15 bands were recorded with maximum relative mobility of 0.90. Presence or absence of any particular band helps in demarcation and identification of a variety. Similarity index was calculated to have an idea about evolutionary relationship among varieties in this experiment which varied from 53.3% to 93.3%.

Keywords : Lentil, protein banding, similarity index

Identification and characterization of crop varieties / genotype are important for seed trade. The traditional method usually record expression of morphological characters at different growth stages of the crop. The expression of these morphological characters is not stable over the year and location due to high interaction with environment. The phenological characters are influenced significantly by environmental fluctuation, whereas qualitative traits are limited and require specific time to judge the expression for distinctness. Moreover, it also becomes difficult to distinguish closely related varieties by morphological characters alone. The use of protein electrophoresis is a well-established and reliable method for varietal identification [1]. Therefore, the present investigation was conducted to access the electrophoretic variation in 25 varieties of lentil, under active seed multiplication chain in India.

MATERIALS AND METHODS

Pure seed of 25 lentil varieties (Table 1) obtained from IIPR, Kanpur were produced at Jabalpur. The seed was ground in a mortar and pestle after removing the seed coat and defatted by a mixture of Chloroform, Methanol and Acetone (2: 1:1). One ml Tris- Glycine extraction buffer (pH 8.3) was added to 0.5 g of defatted powder and left over night. After extraction the mixture was cleared by centrifugation at 10,000 rpm for 10 minute at 4°C. In the clean

suspension, 10% solution of SDS (10µl), mercaptoethanol (10µl) with bromo-phenol blue (10µl) was added. The mixture was kept for 10 minute in water bath at 80°C. The solution (10µl) was loaded into discontinuous SDS polyacrylamide (SDS-PAGE) gel with 8% stacking and 13% running gel [2]. Gel stained with Coomassive brilliant blue (0.1%) was scored for presence or absence of bands in each variety. Relative mobility (RM) and similarity index (SI) between two bands were measured.

RESULTS AND DISCUSSIONS

In all 15 bands were recorded in SDS-PAGE electrophorogrames (Table 2). Presence or absence of particular band in any variety helps to demarcate it with others. In all 15 bands are observed with maximum number of bands *i.e.*, 15 in LL 699, while minimum *i.e.*, 10 are present in variety JL3, PL5, PL234 and PL639. It is recorded that band number 1(0.28), 5 (0.55), 6 (0.58), 9 (0.69) and 15 (0.90) are common in all the varieties. This may be used as a source of reference for comparison of inter gel or inter laboratory results. Band number 2 (0.34) is present in only 8 varieties (Asha, IPL81, L4076, LL147, LL699, NDL1, PL4 and VL1). In the same manner absence of band is also used as distinguishing trait as it differentiate varieties from others *e.g.*, band number 3 (0.39) is absent in variety Asha, K75 and PL639; band number 13 (0.84) is absent in NDL1; band number 10 (0.72) and 14 (0.88)

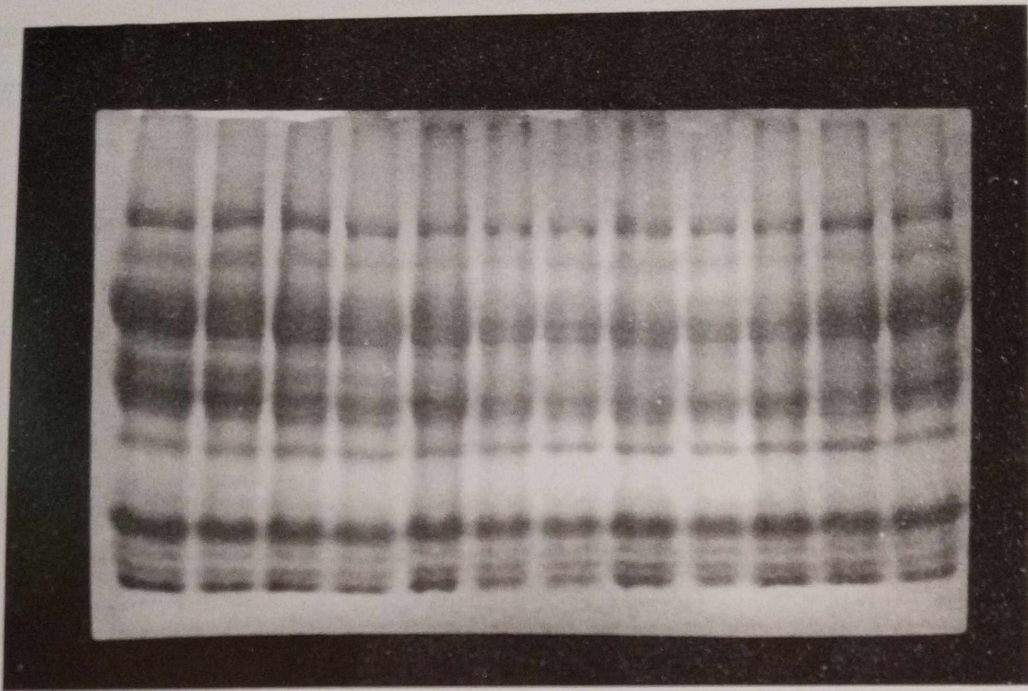
Table 1. Parentage and source of lentil varieties used in the study

S.No.	Variety	Pedigree	Source
1	Asha	Selection from Jorhat local	Behrampur
2	DPI 15	PL 406 X L 4076	IIPR Kanpur
3	DPL62	JLS 1 X LG 171	IIPR Kanpur
4	IPL81	K 75 X PL 639	IIPR Kanpur
5	JL 1	Selection from local germplasm	JNKVV Jabalpur
6	JL 3	Selection from local germplasm	JNKVV Jabalpur
7	K75	Selection from Bundelkhand	CSAUT Kanpur
8	L4076	PL 234 X PL639	IARI New Delhi
9	L4147	(13875 X P4) PKVL 1	IARI New Delhi
10	LH84-8	L9-12 X JLS-2	CCSHAU Hisar
11	LL56	L9-12 X L32-1	PAU Ludhina
12	LL147	PL 284-64 X NP 21	PAU Ludhina
13	LL699	Not known	PAU Ludhina
14	NDL1	Precoz X L9-12	NDUA & T Faizabad
15	PL 4	UPL 175 X (PL 184 X P285)	GBPAU & T Pantnagar
16	PL5	Not known	GBPAU & T Pantnagar
17	PL 77-12	Mutant of BR 25	RAU Dholi
18	PL234	Selection from P 230	GBPAU & T Pantnagar
19	PL406	Selection from P 495	GBPAU & T Pantnagar
20	PL639	L9-12 X T8	GBPAU & T Pantnagar
21	Ranjan	Mutant of B 77	Behrampur
22	Subrita	JLS X T 36	Behrampur
23	VL 1	Selection from local germplasm	VPKAS Almora
24	VL 4	Local selection from Pithoragarh	VPKAS Almora
25	VL103	Local selection from Pithoragarh	VPKAS Almora

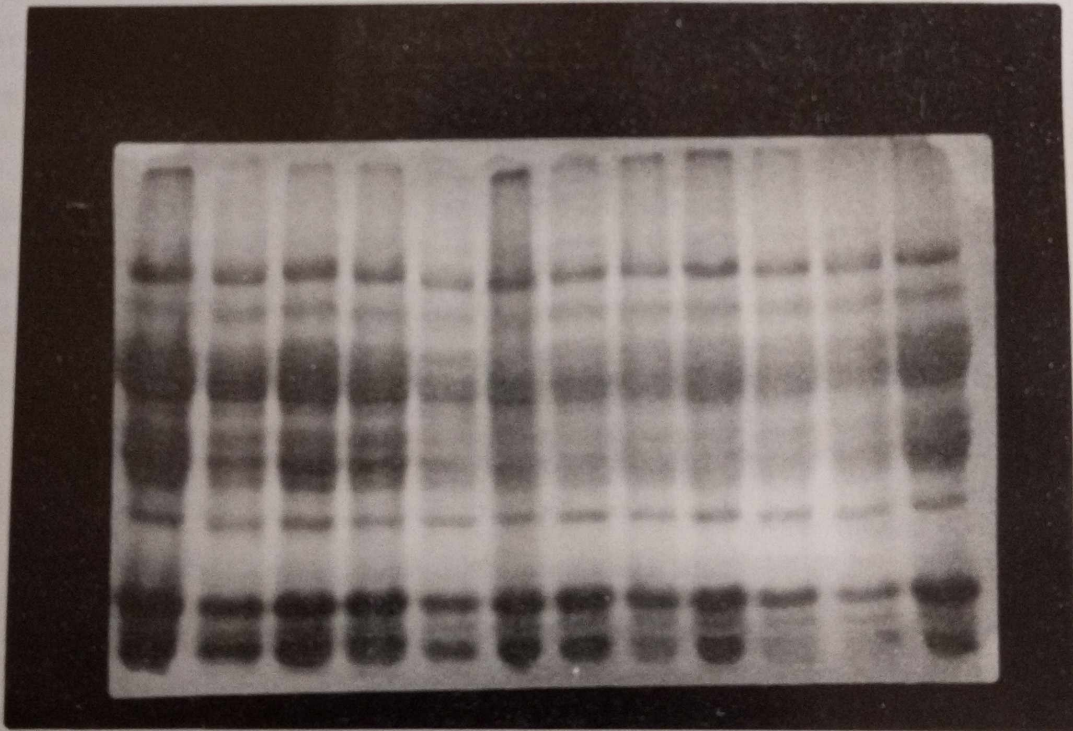
are absent in Subrita; Band number 4 (0.53), 8 (0.68), 11 (0.77) and 12 (0.81) are also absent in many varieties (Fig 1). Hussain *et al.*, [3] recorded similar observations in lentil.

Similarity index (SI) is calculated to judge the evolutionary relationship among the genotypes (Table 3). The value of similarity index ranged from

53.3% (Asha-PL232) to 93.3% (DPL15- LL699; IPL81-LL699; LL699-PL4; LL699-VL1). High value of similarity index shows close evolutionary relationship among concerned varieties [4,5]. Varieties those have lesser SI are expected to have more genetic variation, hence variety showing least SI (Asha-PI232) are advocated to be used as parent



1-12



13-24

Fig 1. SDS PAGE Electrophoregrams of seed protein in lentil genotypes Number 1-25 as given in Table 1

Table 2. SDS-PAGE banding pattern of 25 varieties of lentil

S. No.	Variety	Bands														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Asha	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+
2	DPL 15	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
3	DPL62	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+
4	IPL81	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
5	JL1	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+
6	JL3	+	-	+	-	+	+	-	-	+	+	+	-	+	+	+
7	K75	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+
8	L4076	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
9	L4147	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+
10	LH84-8	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
11	LL56	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+
12	LL147	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
13	LL699	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	NDL 1	+	+	+	-	+	+	-	+	+	+	-	+	-	+	+
15	PL 4	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
16	PL5	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+
17	PL 77-12	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+
18	PL234	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+
19	PL406	+	-	+	+	+	+	-	-	+	+	-	-	+	+	+
20	PL639	+	-	-	-	+	+	-	-	+	+	+	+	+	+	+
21	Ranjan	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+
22	Subrita	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+
23	VL 1	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
24	VL4	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
25	VL103	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+

for hybridization programme. Mishra *et al.*, [6] made similar interpretation in pea.

The variation proved to be useful for registration and evaluation of the genetic resources, to evaluate total genetic variation, heterogeneity in the collected material, to identify duplication and to test the genetic purity.

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Table 3. Similarity index (SI) among 25 varieties of lentil based on protein banding pattern

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	73.3	66.6	73.3	60.0	69.2	76.9	92.3	71.4	66.6	66.6	78.5	80.0	71.4	85.7	66.6	78.5	53.3	64.2	84.6	66.6	66.6	73.3	78.5	60.0
2		85.7	86.6	85.7	74.4	78.5	80.0	85.7	92.8	92.8	80.0	93.3	73.3	86.6	92.8	92.8	78.5	78.5	85.7	80.0	92.8	86.6	80.0	85.7
3			73.3	84.6	83.3	76.9	78.5	84.6	92.3	78.5	66.6	80.0	71.4	73.2	92.3	92.3	76.9	91.6	71.4	66.6	78.5	73.3	66.6	71.4
4				73.3	60.0	66.6	80.0	85.7	80.0	92.8	92.8	93.3	85.7	86.6	80.0	80.0	78.5	66.6	73.3	92.8	80.0	86.6	86.6	80.0
5					83.3	76.9	66.6	71.4	92.3	78.5	66.6	80.0	71.4	73.3	92.3	78.5	91.6	91.6	71.4	78.5	78.5	85.7	78.5	84.6
6						75.0	76.9	69.2	76.9	64.2	64.2	66.6	69.2	71.4	76.9	76.9	75.0	90.9	69.2	60.0	64.2	71.4	76.9	69.2
7							71.4	64.2	84.6	71.4	73.3	73.3	76.9	78.5	84.6	71.4	69.2	76.9	91.3	60.0	71.4	66.6	71.4	64.2
8								78.5	73.3	73.3	85.7	86.6	78.5	92.8	73.3	85.7	60.0	71.4	78.5	73.3	73.3	73.3	85.7	66.6
9									78.5	92.3	78.5	80.0	71.4	73.3	78.5	92.3	76.9	76.9	64.2	78.5	78.5	73.3	66.6	84.6
10										86.6	73.3	86.6	78.5	92.3	86.6	85.7	84.6	84.6	78.5	73.3	85.7	80.0	73.3	78.5
11											85.7	86.6	78.5	80.0	92.3	85.7	78.5	71.4	78.5	85.7	85.7	80.0	73.3	84.6
12												86.6	85.7	92.8	73.3	73.3	71.4	60.0	78.5	85.7	73.3	80.0	85.7	84.6
13													80.0	93.3	86.6	80.0	73.3	73.3	80.0	86.6	86.6	93.3	86.6	80.0
14														85.7	78.5	73.3	76.9	64.2	71.4	78.5	66.6	73.3	78.5	71.4
15															80.0	80.0	66.6	66.6	85.7	80.0	80.0	86.6	92.8	80.0
16																84.6	75.0	75.0	90.9	71.4	85.7	71.4	78.5	71.4
17																	76.9	76.9	76.9	81.7	85.7	73.3	92.8	85.7
18																		83.3	72.7	76.9	71.4	76.9	71.4	76.9
19																			66.6	76.9	71.4	83.3	71.4	76.9
20																				64.2	78.5	64.2	71.4	64.2
21																					73.3	85.7	92.8	92.3
22																						80.0	73.3	78.5
23																							80.0	85.7
24																								92.8

Number 1-25 depicting name of varieties as given in Table 1