Indian J. Genet., 68(3): 283-287 (2008)

Seed protein profiles and cultivar identification in garden pea (*Pisum sativum* L.)

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(Received: April 2006; Revised: July 2008; Accepted: August 2008)

Abstract

Electrophoretic patterns of seed proteins of 23 genotypes of garden pea (*Pisum sativum* L.) when analyzed by SDS-PAGE were placed in 22 groups on the basis of banding pattern. All the genotypes showed different banding patterns except PMR-43 and Azad Pea-3 which fell in the same group and showed similar banding pattern. The similarity between two genotypes might be possible due to their derivation from the cross of same parental combination. On the basis of distance matrix and UPGMA analysis, dissimilarity between genotypes was established and most dissimilar genotypes *viz*., Bonneville vs PMR-43, Bonneville vs Azad Pea-3, Bonneville vs VL-7 and NDVP-9 vs IP-3 (Pant Uphar) were identified. Seed protein electrophoresis is thus useful in identifying pea cultivars and in breeding programme.

Key words: Seed protein profiles, electrophoresis, dissimilarity index, *Pisum sativum*

Introduction

The increasing numbers of varieties with similar morphological characteristics have created the need for better and newer systems of identification and characterization of crop varieties. Electrophoretic pattern have been lately used towards identification of cultivars, genomes, genera and taxa, genetic control of polymorphism and for tracing the phylogeny of various plants groups [1, 2]. These approaches corroborate the conventional methods used for such studies. However, the advantage of electrophoresis over other methods is that it is repeatable and provides unique pattern for protein bands for different cultivars. Therefore, the present investigation was conducted to assess variation in 23 genotypes of garden pea employing electrophoresis.

Materials and methods

Twenty-three genotypes of garden pea (*Pisum sativum* L.) from the germplasm being maintained at the Vegetable Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar from the *Rabi season* of 2001-02 were analysed by SDS-polyacrylamide gel electrophoresis [3]. The extraction procedure as given by Matta and Gatehouse [4] and Mishra *et al.* [5] with slight modification was followed. The procedure developed by Laemmli [6] was followed for preparing and running the gel. For assessment of molecular weight of different protein bands, PMW-B Marker (3500-20500 Da) was used. The presence and absence of bands were used for dissimilarity index and unweighted pair group method using arithmetic averages (UPGMA) analysis.

Results and discussion

The seed proteins of 23 pea lines on SDS gels (Figs. 1 & 2) exhibited the existence of 29 protein bands located in six zones (A, B, C, D, E and F). Zone A representing the heaviest molecular weight protein was sub divisible into eight sharp and distinct bands i.e. A_1, A_2, A_3, A_4, A_5, A_6, A_7 and A_8. Among these A_5 and A_8 were comparatively darker bands. Similarly, Zone B representing mostly sharp bands, was sub divisible into four bands i.e. B_1, B_2, B_3 and B_4. B zone had thinner and lighter band. The next zone C representing dark to lighter bands with few faint bands was sub divisible into six bands *viz*., C_1, C_2, C_3, C_4, C_5, and C_6. The next zone D characterized by lighter to faint bands, was sub divisible into three bands i.e. D_1, D_2 and D_3. Similarly, zone E representing mostly sharps bands, was sub divisible into five bands i.e. E_1,

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E_2, E_3, E_4 and E_5. Among these, E_1 and E_5 were comparatively thicker and darker bands. The last zone F was characterized by comparatively lighter bands, was sub divisible into three bands i.e. F_1 F_2, and F_3. Thus, a total of 29 bands could be resolved in seed protein (Fig. 2).

Total 11 bands i.e. A_4, A_5, A_7, A_8, B_1, B_3, C_1, C_2, C_6, E_1 and E_2 were common in all the genotypes under study. These bands can serve as a source of reference for inter-gel or inter-laboratory comparison. Thicker band E_5 was absent in PSM-3, IP-3, Bonneville and Arkel, whereas E_4 band was present in IP-3, HUVP-3, KS-225, EFF. The lines IP-3, NDVP-12, Bonneville and PMR-34 showed absence of band E_3, C_5, B_4 and F_2, respectively. Therefore, the dissimilar groups were mainly due to the absence or presence of bands.

The importance of this experiment for the characterization of germplasm could be realized from the fact that all the 3 leafless genotypes *viz*., HUVP-3, NDVP-250, and PSM-3 fell into different groups. This difference was due to presence of bands E_4 and D_1 in HUVP-3, A_2 in NDVP-250 whereas absence of bands D_1 and C_4 in PSM-4 and D_1 in NDVP-250 and B_2 in HUVP-3. Thus, the differences between banding patterns of those three otherwise indistinguishable genotypes were quite distinct. Similarly PMR-31, RP-3, Stomp, Punjab Ageta-6 and PSM-4 were round seeded having dissimilar protein profiles. Similar results have been reported by Weeden [7] and Shridhar and Ram [8] in French bean.

The cultivars which were indistinguishable on the basis of simple identifiable morphological traits like growth habit, flower colour etc. could be distinguished through electrophoretic patterns. For example, all the cultivars except PMR-43 and Azad Pea-3 under present investigation, fell in different zones and showed different banding patterns. The two genotypes PMR-43 and Azad P-3 fell in the same group and showed similar banding

Table 1. Dissimilarity indices (%) among different genotypes of garden pea

Genotype		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.	PSM-3	0																						
2.	PMR-31	37.1	0																					
3.	PMR-34	52.5	52.5	0																				
4.	PMR-43	52.5	45.5	45.5	0																			
5.	FC-1	55.7	49.1	41.5	32.2	0																		
6.	Azad P-1	41.5	41.5	49.1	32.2	37.1	0																	
7.	Azad P-3	52.5	45.5	45.5	0.0	32.2	32.2	0																
8.	IP-3	49.1	55.7	49.1	49.1	45.5	52.5	49.1	0															
9.	Bonneville	41.5	41.5	55.7	61.6	58.7	58.7	61.6	58.7	0														
10.	PMR-19	45.5	37.1	37.1	26.3	41.5	41.5	26.3	49.1	55.7	0													
11.	VL-7	45.5	45.5	37.1	26.3	32.2	32.2	26.3	41.5	61.6	26.3	0												
12.	NDVP-12	49.1	49.1	32.2	32.2	26.3	37.1	32.2	37.1	58.7	32.2	18.6	0											
13.	NDVP-9	52.5	52.5	52.5	58.7	55.7	55.7	58.7	61.6	55.7	52.5	52.5	55.7	0										
14.	KS-168	52.5	52.5	45.5	52.5	49.1	55.7	52.5	55.7	55.7	45.5	45.5	49.1	26.3	0									
15.	HUVP-3	37.1	37.1	45.5	52.5	49.1	49.1	52.5	55.7	41.5	45.5	45.5	49.1	37.1	37.1	0								
16.	RP-3	45.5	45.5	45.5	52.5	41.5	55.7	52.5	41.5	49.1	45.5	45.5	41.5	45.5	37.1	37.1	0							
17.	KS-245	55.7	49.1	41.5	49.1	45.5	52.5	49.1	58.7	58.7	41.5	41.5	45.5	32.2	32.2	41.5	49.1	0						
18.	NDVP-250	49.1	49.1	55.7	55.7	52.5	58.7	55.7	52.5	58.7	49.1	49.1	52.5	41.5	41.5	41.5	32.2	45.5	0					
19.	Stop	55.7	55.7	49.1	49.1	45.5	52.5	49.1	52.5	58.7	49.1	41.5	45.5	32.2	32.2	41.5	41.5	37.1	37.1	0				
20.	EFF	41.5	41.5	41.5	49.1	45.5	52.5	49.1	52.5	45.5	41.5	41.5	45.5	41.5	32.2	18.6	32.2	37.1	37.1	37.1	0			
21.	PA-6	49.1	49.1	49.1	49.1	37.1	52.5	49.1	37.1	52.5	49.1	41.5	37.1	49.1	41.5	41.5	18.6	52.5	37.1	37.1	37.1	0		
22.	Arkel	45.5	45.5	45.5	45.5	41.5	49.1	45.5	49.1	55.7	37.1	37.1	41.5	37.1	26.3	37.1	26.3	41.5	32.2	32.2	32.2	32.2	0	
23.	PSM-4	49.1	49.1	55.7	55.7	52.5	52.5	55.7	58.7	58.7	49.1	49.1	52.5	32.2	41.5	41.5	41.5	45.5	26.3	37.1	45.5	45.5	32.2	0

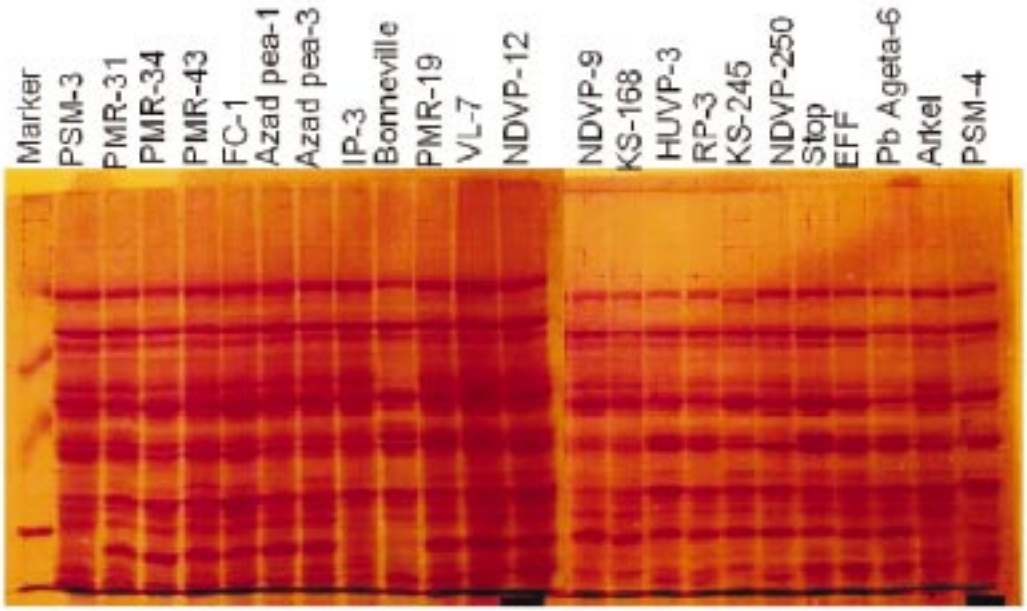

Fig. 1. SDS-PAGE electrophoregrams of seed proteins in garden pea genotypes (0. Marker, 1. PSM-3, 2. PMR-31, 3. PMR-34, 4. PMR-43, 5. FC-1, 6. Azad Pea-1, 7. Azad Pea-3, 8. IP-3, 9. Bonneville, 10. PMR-19,11. VL-7, 12. NDVP-12, 13. NDVP-9, 14. KS-168, 15. HUVP-3, 16. RP-3, 17. KS-245, 18. NDVP-250, 19. Stop, 20. Early Felthum First, 21. Punjab Ageta-6, 22. Arkel and 23. PSM-4)

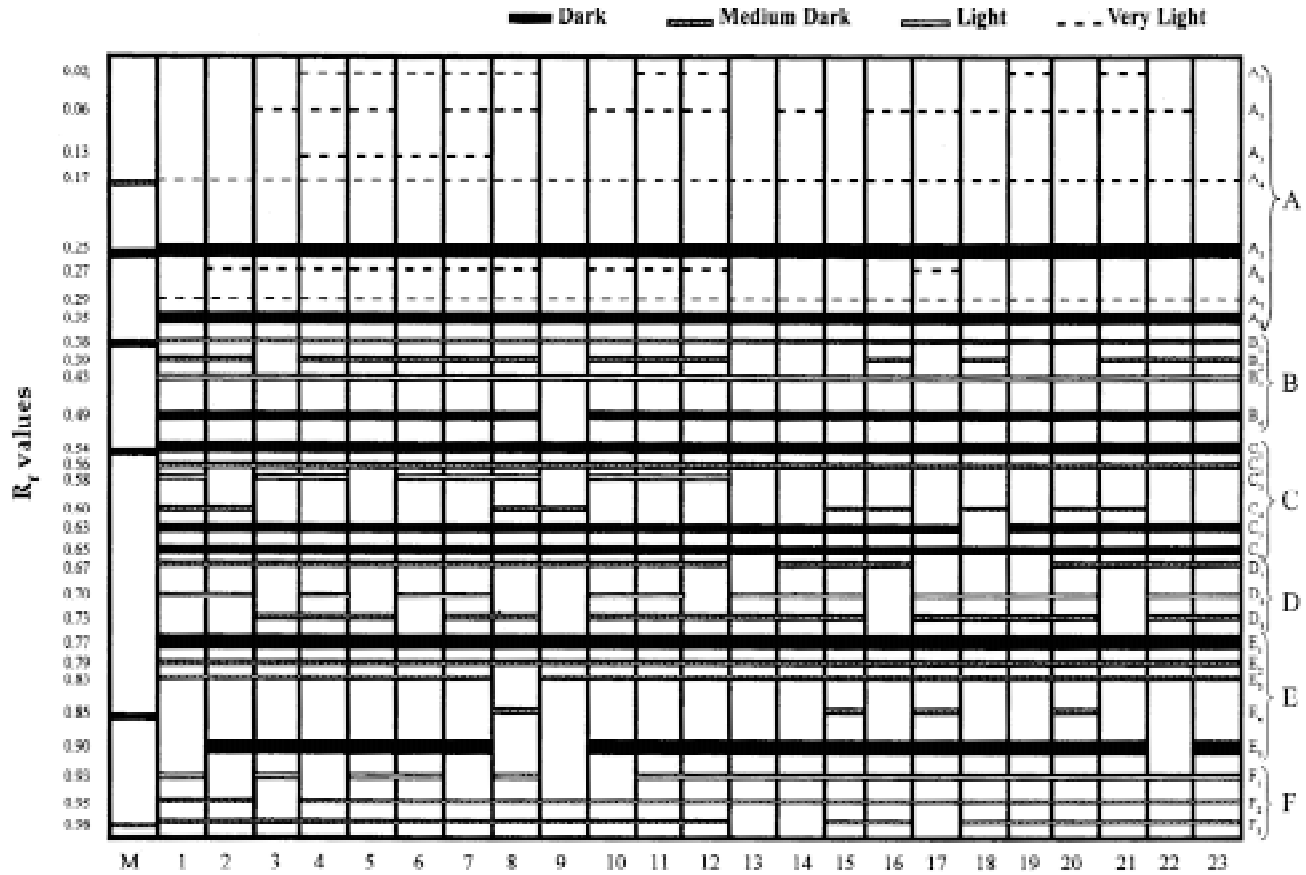

Fig. 2. Diagramatic SDS-PAGE electrophoregram of 23 garden pea genotypes

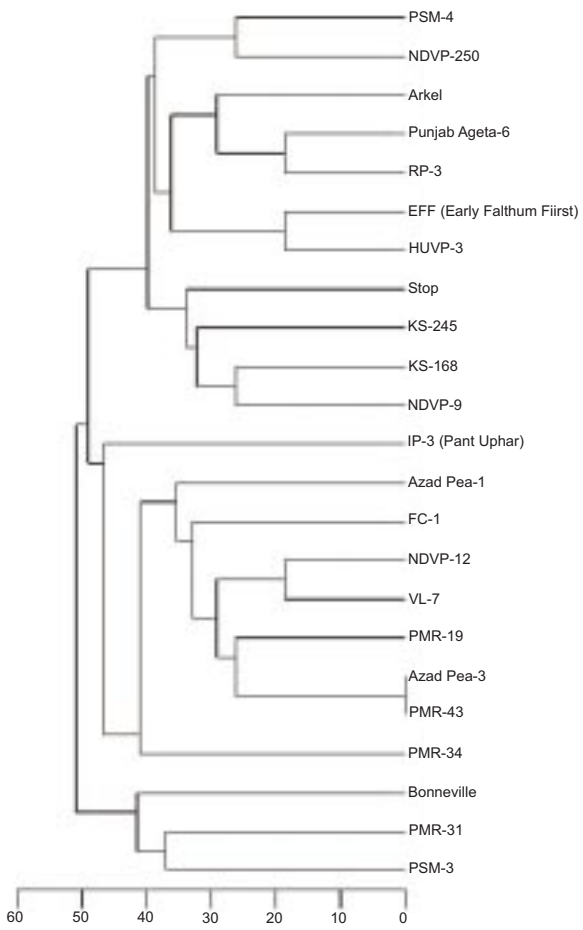

Fig. 3. Dendrogram (Percent Dissimilarity) showing different cluster groups of garden pea genotypes on the basis of seed protein profiles

pattern. The similarity between two genotypes might be due to their derivation from the cross of same parental combination.

PSM-3, a recently released variety from Pantnagar showed dissimilarity from other cultivars except IP-3, Bonneville and Arkel with respect to thicker band under zone E_5. However, PSM-3 could be distinguished from Arkel, IP-3, and Bonneville on the basis of banding patterns of other zones. In zone A, the genotype KS-245 had A_6 band which was thicker than other genotypes which indicates dissimilarity at genotypic level. In cultivar PSM-4, C_4 band was absent and C_5 had thicker band than others.

Therefore, SDS-PAGE can be used as a successful technique to distinguish majority of the varieties from each other. Cooke [9], Hussain *et al.* [10], Suska [11] and Mishra *et al.* [12] also applied electrophoresis as a tool for varietal identification in peas.The dissimilarity index was calculated in order to find out the degree of divergence among different genotypes under study and their evolution relationships. The results presented in Table 1 inferred that dissimilarity value were in the range of 0.0% (Azad Pea-3 vs PMR-43) to 61.6% (Bonneville vs PMR-43, Bonneville vs Azad Pea-3, Bonneville vs VL-7 and NDVP-9 vs IP-3) which clearly indicated that PMR-43 and Azad Pea-3 had close relationship between them whereas Bonneville was most diverse to PMR-43, Azad Pea-3 and VL-7. It was confirmed morphologically because PMR-43 and Azad Pea-3 both had early maturity, dwarf stature, long pods, wrinkled green colour seeds and high yield, whereas Bonneville with mid maturity and medium tall stature indicated more divergence to PMR-43, Azad Pea-3 and VL-7 all belong to early maturity and dwarf type.

The Unweighted Pair Group Method using arithmetic average (UPGMA) analysis showed that the genotypes falling in the same cluster had less diversity between them (Fig. 2). The low dissimilarity index is likely to be due to similar genes from different parents used in the development of varieties whereas high dissimilarity index showed diversity among genotypes. Similar results were noted using similarity index as a criterion for establishing evolutionary relationships among different genotypes [5.]

The divergence of VL-7 with PMR-43, Azad Pea-3 and PMR-19 (all 18.6%) and KS-168 with Arkel and NDVP-9 (both 18.6) was of very low magnitude and thus a close relationship among them was indicated. It was proved morphemically because all the cultivars had early maturity, dwarf stature and long pods. Thus SDS-PAGE technique for protein profile variations is useful in distinguishing pea genotypes as well as in establishing relationships.

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