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Heterosis and protein profiling through SDS-PAGE in vegetable pea

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ABSTRACT

59 genotypes (14 lines, 3 testers and 42 F_1s) of vegetable pea were studied for heterosis and 17 parents were characterized through SDS-PAGE. PMR-53 × PSM-3 showed highest economic heterosis for most of the traits studied including the yield and yield attributing characters. Seed proteins profiling showed ten groups on different banding patterns in three zones (A, B, C and D). The UPGMA analysis showed that VRP-32, VRP-16 and PMR-53 and PMR-19, Arka Ajit, PSM-4, PMR-62, PMR-31, PMR-60, Nepal Pea, VP-266, PMR-32, E-6, AP-3, Arkel, PSM-3 and VL-7 formed two different clusters. However, PSM-4, PMR-62, PMR-31 and PMR-60; Nepal Pea, VP-266, PMR-32 and AP-3, Arkel, PSM-3 and VL-7 were three different neighbouring groups.

Key words: Heterosis, SDS-PAGE, Vegetable pea (*Pisum sativum* L.).

INTRODUCTION

Pea is an important vegetable crop due to its high nutritive value, particularly proteins, 7.2g/100g (Singh, 10) and other health building substances like carbohydrates, calcium and phosphorous. The major objective of vegetable pea breeding is to develop high yielding pure line varieties according to market demand having attractive pod shape, size and colour. Higher productivity is one of the most important objectives of any breeding programme. The amount of success in such programme depends upon the selection of desirable genotypes and availability of genetic information about yield and its associated attributes. The success of selecting desirable genotypes depends upon the nature of genetic variability which is created by employing suitable breeding methods. For generating the desirable genetic variability, the choice and use of appropriate parental material and breeding methodology are important.

For recombination breeding approach, the information on combining ability of the parents offers useful clues regarding the choice of productive parents and hybrids for further use. Therefore, the determination of combining ability of parental lines is the pre-requisite of most breeding programmes. The nature and magnitude of two kinds of combining abilities, i.e. general combining ability (gca) and specific combining ability (sca) helps the breeder in adopting appropriate breeding methodology.

Identification of cultivars using classical methods based on morphological and physiological characters has become increasingly difficult because of the large number of lines being released and convergence of these lines on a few of the most desirable characters. Seed protein electophoresis is being utilized as an additional approach for species identification and as a useful tool for tracing back the evolution of various group of plants. Numerous electrophortic methods are available to identify cultivars by protein banding patterns. Sodium dodecyl sulphate polyacryamide gel electrophoresis (SDS-PAGE) provides the best resolution (Smith and Simpson, 12)

The present investigation entitled “Heterosis and protein profiling through SDS-PAGE in Vegetable pea (*Pisum sativum* L)” was therefore, undertaken involving seventeen lines (female parents) with three testers (male parents) in a line x tester design to provide information on combining ability, type of gene action involved in expression of various quantitative characters and cultivar identification through seed protein profiles.

MATERIALS AND METHODS

Seventeen diverse genotypes including 14 lines viz. VRP-32, VRP-16, PMR-53, PMR-19, Arka Ajit, PSM-4, Nepal Pea, VP-266, E-6, PMR-62, PMR-31, PMR-60, PMR-32, AP-3 and 3 testers viz., Arkel, PSM-3 and VL-7 were taken to raise the F_1s. Crossing was made in winter (rabi) season of 2005-06. All 42 F_1s and 17 parents

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were evaluated in 2006-07 at Vegetable Research Centre, GBPUA&T, Pantnagar in RBD in three replications for fifteen quantitative characters viz., days to first flowering, number of first flowering node, days to first green pod picking, pod length (cm), 100 green pod weight (g), number of seeds per pod, 100 green seed wt. (g), shelling (%), T.S.S. (%), number of green pods per plant, number of primary branches per plant, number of nodes per main stem, plant height (cm), green pod yield per plant (g), dry seed yield per plant (g). The data were subjected to appropriate statistical analysis. Further, seed protein of 17 parents was utilized for sodium dodecayl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

RESULTS AND DISCUSSION

The yield and related traits were most heterotic characters, whereas, number of green pods per plant, number of primary branches per plant, number of nodes per main stem, green pod yield per plant (g), dry seed yield per plant (g) showed high heterosis values (more than 100 %) in desirable direction over mid parent, better parent or standard parent (Table 1). Pod length (cm) and number of nodes per main stem showed significant negative heterosis in most of the crosses, this indicates the reduction in performance of the F_1 over the standard parent.

However, shelling (%) showed non-significant response for standard heterosis. The negative heterosis is considered desirable for days to first flowering, number of first flowering node, days to first green pod picking and plant height. The reason for significant negative heterosis may be due to the presence of dominant loci in different direction leading to cancellation of effects. The crosses showing no heterosis indicated that the parents involved in the cross do not differ in gene frequency with respect to character under study (Pandey *et al.,* 8). E-6 × Arkel and E-6 x VL-7 were the best combinations over the standard check 1 and 2 for days to first flowering (-39.56 and -16.76 and -38.80 and -15.72 per cent); Arka Ajit x PSM-3 for pod length (0.32 and 7.59 per cent); PSM-4 x Arkel and PMR-62 x Arkel for 100-green pod weight and number of seeds per pod (71.25 and 104.07 and 14.94 and 23.06 per cent); AP-3 x VL-7 and PMR-31 x VL-7 for 100-green seed weight and number of green pods per plant (20.00 and 25.36 and 168.74 and 39.71 per cent) respectively. The combination PMR-53 × PSM-3 showed the highest values of heterosis over mid parent; better parent and standard parent (check 1 and 2). The crosses E-6 × PSM-3 also expressed significant positive heterosis of the three types. In general, the hybrids with highest yield also expressed heterosis for this trait. The work of Singh and Santhoshi (11) also give credence to the present findings that heterosis in yield was due to more number of primary branches per plant. Green pod yield per plant being a complex trait, is a multiplication product of several basic component traits of yield. The increased pod yield will definitely be because of increase in one or more component traits. Similar findings were reported by Panda *et al.* (6), Tyagi and Srivastava (7), Kumar *et al.* (3) and Shah and Muhammad (9). In the present study, the best performing F_1 hybrid for dry seed yield PMR-53 × PSM-3 showed highest heterosis for number of primary branches per plant over standard parents (check 1). The second best cross for dry seed yield was E-6 × PSM-3; it also showed highest heterosis for number of nodes per main stem. In top ranking heterotic crosses for number of primary branches per plant and number of nodes per main stem PSM-3 was found to more frequently involve.

The above findings indicated that some inbreds had strong heterotic capacity compared to other ones during hybridization process. As the performance of hybrids depended upon the heterotic capability of the parents involved from economic point of view it will be useful to select and utilize the parental inbreds with strong heterotic capability for important traits associated with yield in order to achieve higher gains in F_1 hybrids through exploitation of heterosis.

Studies on the degree of heterosis carried out in 15 important quantitative characters of vegetable pea showed that the mean of hybrids was higher than those of parents for all the characters except number of first flowering node, pod length, shelling (%), number of nodes per main stem and green pod yield per plant. Based on the experiment it was observed that the crosses PMR-53 × PSM-3, E-6 × PSM-3 and PSM-4 × PSM-3 were superior and can be selected as high yielding hybrids over commercial parents.

The seventeen genotypes were distinguished into ten groups on different banding patterns in four zones (A, B, C and D). PSM-4, PMR-62, PMR-31 and PMR-60; Nepal Pea, VP-266 and PMR-32; AP-3, Arkel, PSM-3 and VL-7 fell in three different groups and showed similar banding pattern within the group. The degree of darkness and thickness of various bands in different cultivars are the most commonly reported types of variation, suggesting that formulation of many of the bands in the seed protein profile are under the control of quantitative gene systems. This kind of variation may be due to the lack of separation on the gels of several proteins having similar migration rates.

The index of similarity is the second way of expressing variation in the banding patterns between two gels. Using this index, the similarity in the banding patterns of seventeen vegetable pea genotypes was

Table 1. Range and heterosis values for different quantitative traits in vegetable pea.

S.No.	Character	Range of heterosis				Best two heterotic combinations for different characters (Heterosis in parenthesis)			
		MP	BP	SP		Relative heterosis	Heterobeltiosis	Standard heterosis Check 1	Check 2
				Check 1	Check 2				
1	Days to first flowering	-10.52 to 32.62	-16.77 to 61.81	-39.56 to13.96	-16.76 to 56.94	N. Pea × Arkel (-10.52) E-6 × Arkel (-9.33)	E-6 × Arkel (-16.77) PMR-31 × Arkel (-13.71)	E-6 × Arkel (-39.56) E-6 × VL-7 (-38.80)	E-6 × Arkel (16.76) E-6 × VL-7 (-15.72)
2	No. of first flowering node	-13.21 to 23.38	-23.55 to 62.79	-26.36 to 27.27	-15.01 to 46.91	E-6 × PSM-3 (-13.21) N. Pea × Arkel (-9.91)	PMR-53 × VL-7 (-23.55) PMR-53 × Arkel (-22.73)	E-6 × PSM-3(-26.36) E-6 × VL-7 (-24.55)	E-6 × PSM-3 (-15.01) E-6 × VL-7 (-12.91)
3	Days to first green pod picking	-7.75 to 5.31	-7.75 to 6.61	-7.75 to -3.88	-4.03 to 4.04	PSM-4 × PSM-3 (-7.75) PMR-19 × PSM-3 (-5.81)	PMR-53 × PSM-3 (-7.75) PSM-4 × PSM-3 (-7.75) PMR-53 × VL-7 (-5.81)	PMR-53 × PSM-3 (-7.75) PSM-4 × PSM-3 (-7.75) PMR-19 × PSM-3 (-5.81)	All non significant
4	Pod length (cm)	-11.42 to 11.30	-18.48 to 17.81	-20.80 to 0.32	-15.06 to 7.59	VRP-32 × VL-7 (11.30) VRP-32 × Arkel (4.55)	VRP-32 × PSM-3 (17.81) VRP-32 × VL-7 (16.90)	A. Ajit × PSM-3 (0.32) A. Ajit × Arkel (-4.26)	A. Ajit × PSM-3 (7.59) A.Ajit × Arkel (2.68)
5	100-green pod weight (g)	-64.02 to 85.05	-67.42 to 104.07	-67.77 to 71.25	-61.59 to 104.07	PMR-53 × VL-7 (85.05) PMR-31 × Arkel (78.69)	PSM-4 × Arkel(104.07) PMR-53 × VL-7(96.90)	PSM-4 × Arkel (71.25) PMR-53 × VL-7 (51.67)	PSM-4 × Arkel (104.07) PMR-53 × VL-7 (80.74)
6	No. of seeds per pod	-26.00 to 25.34	-31.70 to 27.84	-25.04 to 14.94	-19.74 to 23.06	PMR-62 × Arkel(25.34) PMR-60 × Arkel(23.97)	PMR-60 × Arkel (27.84) PMR-62 × Arkel (27.78)	All non-significant	PMR-62 × Arkel(23.06)
7	100-green seed weight (g)	-23.53 to 28.24	-32.84 to 33.33	-35.72 to 20.00	-32.84 to 25.36	VRP-32 × Arkel (28.24) VP-266 × PSM-3 (21.00)	VRP-32 × Arkel (33.33) VP-266 × PSM-3 (26.56)	All non-significant	VRP-32 × Arkel (25.36)
8	Shelling %	-15.24 to 19.31	-12.86 to 28.66	-16.73 to 18.72	-15.01 to 21.06	All non significant	VRP-16 × VL-7 (28.66)	All non significant	All non significant
9	T.S.S. (%)	-63.56 to 41.67	-63.20 to 61.91	-44.24 to 106.06	-63.16 to 36.14	VP-266 × PSM-3 (33.33) PMR-32 × VL-7 (41.67) PMR-62 × Arkel (84.85)	VP-266 × PSM-3 (61.91) N. Pea × VL-7 (51.11)	N. Pea × VL-7 (106.06) VP-266 × PSM-3 (106.06)	VP-266 × PSM-3 (36.14) N. Pea × VL-7 (36.14)
10	No. of green pods/plant	-2.97 to 260.76	37.50 to 273.83	33.90 to 168.74	-37.75 to 39.71	PMR-31 × VL-7 (260.76) PMR-60 × VL-7 (225.66)	E-6 × Arkel (273.83) PMR-31 × VL-7 (265.39)	PMR-31 × VL-7 (168.74) N. Pea × Arkel (163.79)	PMR-31 × VL-7 (39.71)
11	No. of primary branches per plant	-15.09 to 130.77	-11.77 to 173.81	-50.00 to 50.00	0.00 to 200.00	PSM-4 × VL-7 (130.77) VP-266 × Arkel (84.09)	PMR-53 × VL-7 (173.81) PSM-4 × VL-7 (150.00)	PMR-53×PSM3 (50.00) PMR-53 × VL-7 (47.44)	PMR-53 × PSM-3 (200) PMR-53 × VL-7 (194.87)
12	No. of nodes per main stem	-11.20 to 168.74	-20.93 to 56.73	-32.64 to 16.93	-25.67 to 29.02	E-6 × Arkel (168.74) PMR-19 × PSM-3 (163.79)	E-6 × PSM-3 (56.73) E-6 × Arkel (50.48)	Arka Ajit × VL-7 (16.93)	Arka Ajit × VL-7 (29.02) PSM-4 × Arkel (22.41)
13	Plant height (cm)	-20.35 to 32.16	-22.30 to 52.83	-36.62 to 15.81	-23.83 to 39.16	All non-significant	All non-significant	VRP-32 × PSM-3 (-36.62) PMR-60 × VL-7 (-27.99)	All non-significant
14	Gross yield per plant (g)	-48.06 to 103.98	-52.84 to 158.86	-14.85 to 174.59	-44.84 to 174.59	PSM-4 × PSM-3 (103.98) PMR-53 × PSM-3 (51.05)	PSM-4 × PSM-3 (158.86) PMR-53 × PSM-3 (147.39)	PSM-4 × PSM-3 (174.59) PMR-53 × PSM-3 (153.90)	PSM-4 × PSM-3 (174.59) PMR-53 × PSM-3 (64.47)
15	Dry seed yield per plant (g)	-62.25 to 153.38	-67.19 to 212.79	-54.50 to 79.23	-67.90 to 26.46	PMR-53 × PSM-3 (190.86) E-6 × PSM-3 (153.38)	PMR-62 × PSM-3 (212.79) PMR-53 × PSM-3(208.58)	PMR-53 × Arkel (79.23) PMR-53 × PSM-3 (78.49)	All non-significant

Table 2. Seed Protein Profiles as resolved through SDS-PAGE in vegetable pea.

Bands	VRP-32	VRP-16	PMR-53	PMR-19	Arka Ajit	PSM-4	Nepal Pea	VP-266	E-6	PMR-62	PMR-31	PMR-60	PMR-32	AP-3	Arkel	PSM-3	VL-7
A1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B2	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B3	+	+	+	+	+	-	+	+	-	-	-	-	+	-	-	-	-
B4	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B5	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
C1	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C2	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+
C3	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C4	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D2	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-
Total	9	8	9	11	12	12	13	13	10	12	12	12	13	11	11	11	11

+ = Present; - = Abscent

Table 3. Similarity Index (SI %) for seed protein profiles in different genotypes of Vegetable pea.

Genotypes	VRP-32	VRP-16	PMR-53	PMR-19	A. Ajit	PSM-4	N. Pea	VP-266	E-6	PMR-62	PMR-31	PMR-60	PMR-32	AP-3	Arkel	PSM-3	VL-7
VRP-32	100																
VRP-16	92.9	100															
PMR-53	71.4	78.6	100														
PMR-19	71.4	64.3	57.1	100													
A. Ajit	64.3	57.1	50	92.9	100												
PSM-4	64.3	57.1	50	78.6	85.7	100											
N. Pea	71.4	64.3	57.1	85.7	92.9	92.9	100										
VP-266	71.4	64.3	57.1	85.7	92.9	92.9	100	100									
E-6	64.3	71.4	64.3	78.6	71.4	85.7	78.6	78.6	100								
PMR-62	64.3	57.1	50	78.6	85.7	100	92.9	92.9	85.7	100							
PMR-31	64.3	57.1	50	78.6	85.7	100	92.9	92.9	85.7	100	100						
PMR-60	64.3	57.1	50	78.6	85.7	100	92.9	92.9	85.7	100	100	100					
PMR-32	71.4	64.3	57.1	85.7	92.9	92.9	100	100	78.6	92.9	92.9	92.9	100				
AP-3	71.4	64.3	57.1	85.7	78.6	92.9	85.7	85.7	92.9	92.9	92.9	92.9	85.7	100			
Arkel	71.4	64.3	57.1	85.7	78.6	92.9	85.7	85.7	92.9	92.9	92.9	92.9	85.7	100	100		
PSM-3	71.4	64.3	57.1	85.7	78.6	92.9	85.7	85.7	92.9	92.9	92.9	92.9	85.7	100	100	100	
VL-7	71.4	64.3	57.1	85.7	78.6	92.9	85.7	85.7	92.9	92.9	92.9	92.9	85.7	100	100	100	100

analyzed in this investigation (Table 2). A number of genotype pairs have SI values 100 per cent indicating very close relationship between them. Lowest value of similarity index (50%) was shown by PMR-53 with Arka Ajit, PSM-4, PMR-62, PMR-31 and PMR-60 depicting that this was the most diverse groups in evolutionary study.

The UPGMA analysis showed that there are two major groups consisted of (VRP-32, VRP-16 and PMR-53) as I group and rest genotypes (PMR-19, Arka Ajit, PSM-4, PMR-62, PMR-31, PMR-60, Nepal Pea, VP-266, PMR-32, E-6, AP-3, Arkel, PSM-3 and VL-7) as II group (Fig. 1). However, PSM-4, PMR-62, PMR-31 and PMR-60; Nepal Pea, VP-266 and PMR-32 and AP-3, Arkel, PSM-3 and VL-7 were three different neighboring groups.

The importance of this experiment for the characterization of germplasm lines could be realized from the fact that for some genotypes the cultivars which were dissimilar based on morphological features could be easily distinguished through electrophorosis of proteins/ isoenzymes. Similar findings have also been reported by Upadhayay *et al*. (14) in bottle gourd and Yadav *et al.* (15) in muskmelon. Similar results in germplasm lines of vegetable pea have also been reported by many investigators such as Cooke (1), Hussain *et al.* (2), Suska (13), Mishra *et al.* (4) and Mishra *et al.* (5). It can therefore be concluded that the electrophoretic resolution of seed protein in vegetable pea was successful in germplasm identification in most of the cases. Sometimes, the protein profile failed in differentiating between the genotypes which were morphologically dissimilar. The most typical example was the presence of smooth seed surface in cultivars VRP-16, PSM-4, Nepal Pea, VP-266 and PMR-31 in different mega groups along with other wrinkled seed surface types. These two groups contain different cultivars which were distinct from each other with respect to seed shape and other morphological traits. A comparison of genotypes based upon protein profile and morphological traits in groups showing 100 % Similarity Index and morphological features have been depicted in Table 3.

REFERENCES

1. Cooke, R.J. 1983. The characteristics of *Pisum sativum* L. (field pea) cultivars by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. *J. Nat. Inst. Agric. Bot.* **16**: 213-20.
2. Hussain, A.; Ali-Khan, S.T. and Bushuk, W. 1988. Field pea cultivar identification by electrophoretic patterns of cotyledon proteins. *Canadian. Plant Sci*. **68**: 1143-47.
3. Kumar, S.; Singh, K.P. and Panda, P.K. 2000. Heterosis for green pod yield and its components in garden pea. *Haryana J. Hort. Sci.* **29**: 99-101.

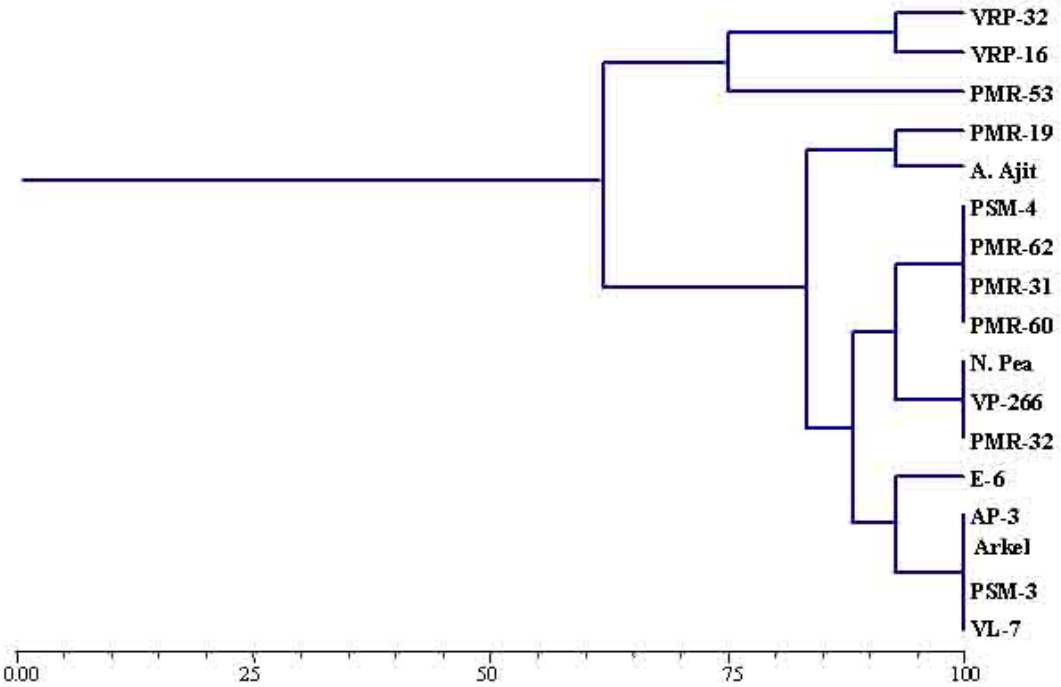

Fig. 1. Dendrogram showing different cluster groups of vegetable pea genotypes on the basis of protein profiles.

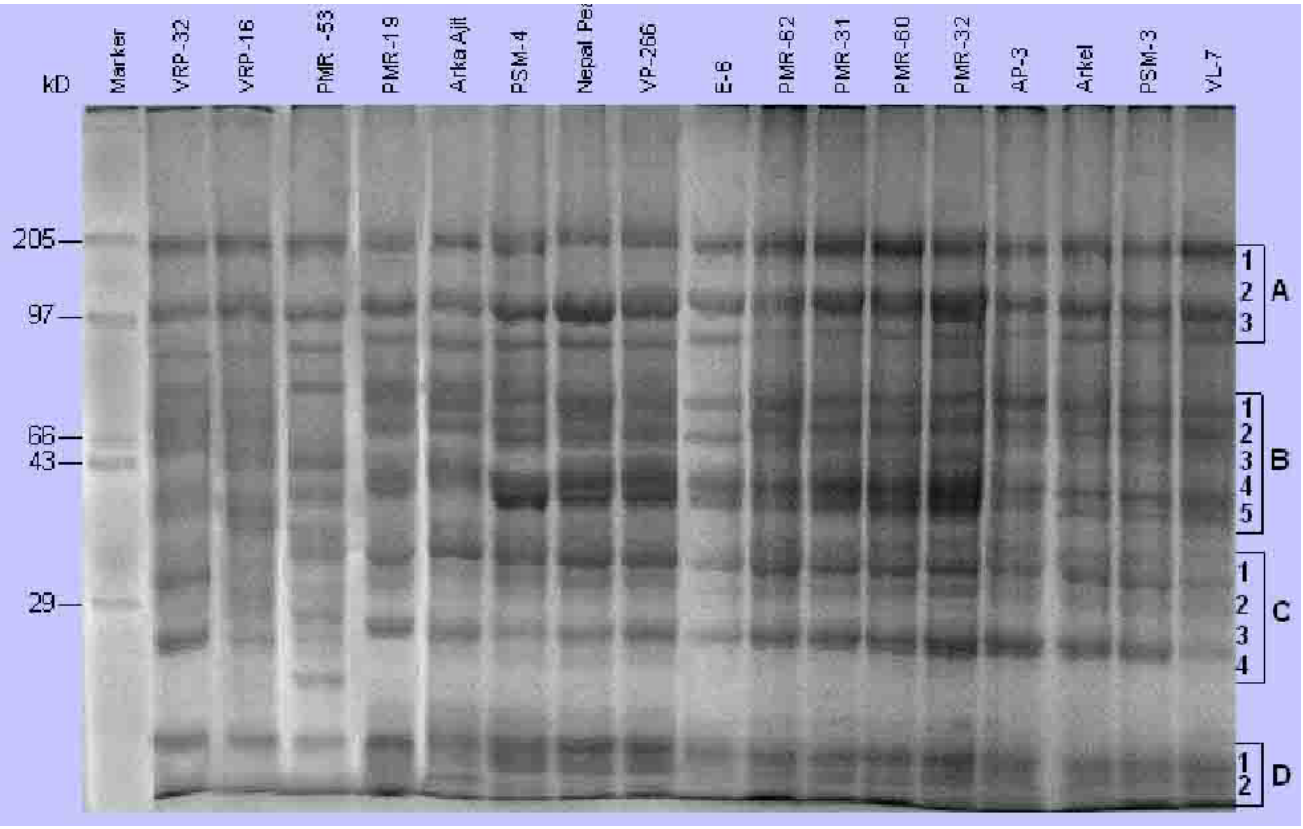

Plate 1. Seed protein profiling of vegetable pea using SDS-PAGE

4. Mishra, S.K., Gupta, S.K.D., Sharma, B. and Kahlon, R. 1998. Diversity in seed protein pattern in garden pea and its wild relatives. *Annals Agri. Res.* **19**: 313-16.

5. Mishra, S.K., Sharma, B., Dasgupta, S.K. and Mathur, N. 2000. Variation in electrophoretic patterns of storage seed proteins in pea (*Pisum sativum* L.). *Annals Agri. Res.* **24**: 88-92.

6. Panda, P.K., Singh, K.P., Kaur, R.M. and Singh, A.K. 1994. Genetic components for yield and its contributing traits in pea. *J. Appl Biol.* **4**: 9-12.

7. Tyagi, M.K. and Srivasvatava, C.P. 1999. Heterosis and inbreeding depression in pea. *Ann. Agric. Bio. Res.* 4: 71-74.

8. Pandey, S., Singh, B., Singh, M. and Rai, M. 2005. Heterosis in cucumber (*Cucumis Sativus* L.). Veg. Sci. **32**: 143-145.

9. Shah, A.H. and Muhammad, Z. 2005. Hybridization of pea varieties. *Sharhad J. Agri.* **21**: 557-62.

10. Singh, B.D. 2007. Plant Breeding Principles and Methods. Heterosis and Inbreeding Depression. Kalyani Publishers, New Delhi. pp. 230-53.

11. Singh, K.N. and Santhoshi, U.S. 1989. Heterosis for yield and protein content in pea. *Legume Res.* **12**: 196-98.

12. Smith D.B. and Simpson P.A. 1983. Relationship of barley proteins soluble in Sodium dodecyl sulphate to malting quality and varietal identification. *J. Cereal Sci*. **1**: 185-97.

13. Suska, H. 1993. Isoenzymes from pea leaves and their use in cultivar identification. *Genetika a Slechteni*. **29**: 27-33.

14. Upadhyay, R., Ram, H.H. and Singh, D.K. 1998. Seed protein electrophoresis in the indigenous cultivars of bottle gourd [*Lagenaria sinceraria* (Mol.) Standl.]. *Veg. Sci.* **25**: 11-13.

15. Yadav, R.K., Ram, H.H. and Kumar, A. 1998. Electrophoretic characterization of indigenous germplasm lines of muskmelon. *Veg. Sci.* **25**: 8-10.

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