Research Paper

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

# Electrophoretic Partten in Gamma Radiated Wild Pea

Life Science

**KEYWORDS :** SDS PAGE, Electrophoresis Pattern, Gamma radiation, Protein profiling.

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## ABSTRACT

*Genetic variation in germplasm has important role in identification of varieties. Electrophoretic partten of the protein fraction are directly related to the genetic background of the protein and be used to certify the genetic make-up SDS PAGE (polyacridamide Gel Electrophoresis) is valid technique increasingly being utilized as an approach for species identification . Each variety or a group of varieties exhibited species specific protein banding pattern. Thus on the basis of this pattern they can be identified accordingly.*

**Introduction**

In recent year grain legume play important and primary role in the search for vegetable sources of protein owing to the high protein content of seed ranging from 20% in pea to 40% in lupine. They can therefore be considered a good substitution to animal protein of those legume containing amino acids and plant breeding have to consider this problem in any improvement programmes (summerfield and Roberts 1985). Mutagenesis started utilizing experimental mutagen in altering seed protein in many cereals both quantitatively and qualitatively with a view to bridge protein gap cause of malnutrition (Amirshahi and Tavakoli, 1970).

Field pea is signified pulse crop in both Indian and Australia. There are number of Similarities that include nationally Co-ordinate breeding programmes moisture stress in rained crop, low yield , significant of powdery mildew and a interested in developing lodging resistant varieties (Baum et.al 2000). Peas are of great nutritional important due to their high content of protein, complex carbohydrate dietary fibre minerals, vitamins and antioxidant compound. Although pea widely used in animal nutrition (Hedly, 2001). Human consumption of pea is lower than that of other traditionally more accepted pulses (Hedley 2001, Schneider 2002). Neverthless in recent year the wealth of nutrient available from the pea and its beneficial functional properties have prompted increasing interest and demand for this legume for the food preparation oriented to generative and infant nutrition (Davidson et.al 2002).

**Materials and Methods**

**Prepration of Seed Sample:**

For extraction of protein individual seeds irradiated with particular gamma dose were ground to fine powder with mortar and pestle. To extracted protein in 0.01 gm of seed flour , 400ul of the protein extraction buffer(0.05M Tris-HCL, 0.2% SDS 5M urea and 1% B-mercaptoethanaol) was added to tube and mixed well by vortex. Then centrifuged at 15,000 rpm for 5 min at room temperature. The extracted crude protein were recoverd as clear supernatant and was stored at 20 c for furher uses.

**SDS- PAGE ( Sodium Dodecyl Sulphate- Polyacryalamide Gel Electrophoresis).**

The components used in the formation of this gel are known neurotoxic thus the car has to be taken while preparing gel. The most commonly used component to synthesis the matrix are acrylamide monomer and N-N methlyene these free radical acan activate other acrylamide molecules forming long chain is the process .These Chain become cross linked the presence of tir acrlyamide .TEMED acting as a catalayst for gel formation.

**Material:-**

**Sample buffer (lammili buffer)**

1.5M Tris HCL- 417 ul
Glycerol - 800ul
10% SDS - 200mg
B- mercaptoethanol- 362ul
0.002% bromophenol blue-0.2mg
(make the volume 11ml PH- 7.0+-0.2

**Gel buffer (tris glycine buffer)**

Tris base- 1.514
Glycine-7.207
SDS-500mg
(make up the volume 500ml and adjust PH 8.6+- 0.2)

**Acrylamide gel**

Acrylamide -29%
N-N methylene bis acrylamide-1%
TEMED-10%
APS (Ammonium persulphate)-10%
Resolving buffer 1.5M tris-HCL
Stacking gel 1m Tris HCL

**Staining Dye**

Methanol- 40ml
G.Acetic acid
Distilled water-50ml
Commassive brilliant blue R- 250 dye-0.2gm

**Destainer**

Methanol-40ml
G Acetic acid-10 ml
Distilled water-50ml
**SDS (10%)-** Dissolve 1gm SDS in 10 ml D.W

**Preparation of gel:-**

Seed protein were analysed through slab type SDS- page followed by lammlie (1970) using 11.25%. Polyacrylamide gel. Electrophoresis was carried out at 100ul for two and half hours. In order to check reproducibility of method two separate gel were run under similar condition. After electrophoresis gels were stained with 2.2% (w/v) coomassie brilliant blue on a gyratory shaker.

Properly destained gel ever read on gel documentation system and molecular weight of individual bands calculated with respect to marker band using ALPHA imager software consistency of result was ensured by analyzing a minimum of electrophoresis was repeated at least twice on each protein extract. only constant band were taken into account.

**SDS- Gel Electrophoresis**

The protein samples were subjected to SDS- PAGE to the band pattern in mutant seeds of wild *Pisum* variety. The separated pattern of protein bands were observed for each set. The Rm is relative mobility of each protein was determined using following formula.

$$Rm = \frac{\text{Distance between origin and protein bands}}{\text{Distance between origin and tracking dye}}$$

The molecular weight and Rm value of each protein sample was determined on the gel documentation system taking protein markers as standard. The molecular weight and Rm value of each protein sample was determined on the using standard protein marker.

**Observation table:**

**Table 1 : Rm for standard protein markers**

| Sr.No | Bands | Positions | Molecular Weight | Rm |
|---|---|---|---|---|
| 1 | 1 | 320 | 97,400 | 0.667 |
| 2 | 2 | 341 | 66,000 | 0.710 |
| 3 | 3 | 357 | 43,000 | 0.744 |
| 4 | 4 | 397 | 29,000 | 0.827 |
| 5 | 5 | 412 | 18,400 | 0.858 |

**Table 1a: Protein band pattern for 5 KR gamma dose**

| Sr.No. | Bands | Positions | Molecular Weight | Rm |
|---|---|---|---|---|
| 1 | 1 | 251 | 296955.41 | 0.523 |
| 2 | 2 | 270 | 215455.61 | 0.562 |
| 3 | 3 | 279 | 185079.27 | 0.581 |
| 4 | 4 | 290 | 153706.08 | 0.604 |
| 5 | 5 | 299 | 132035.59 | 0.623 |
| 6 | 6 | 301 | 127651.03 | 0.627 |
| 7 | 7 | 310 | 109653.95 | 0.646 |
| 8 | 8 | 317 | 97426.59 | 0.660 |
| 9 | 9 | 327 | 82291.98 | 0.681 |
| 10 | 10 | 337 | 69506.30 | 0.702 |
| 11 | 11 | 345 | 60723.59 | 0.719 |
| 12 | 12 | 352 | 53954.05 | 0.733 |
| 13 | 13 | 359 | 47939.19 | 0.748 |
| 14 | 14 | 366 | 42594.87 | 0.762 |
| 15 | 15 | 374 | 37212.65 | 0.779 |
| 16 | 16 | 384 | 31430.93 | 0.800 |
| 17 | 17 | 390 | 28402.54 | 0.812 |
| 18 | 18 | 398 | 24813.64 | 0.829 |
| 19 | 19 | 406 | 21678.23 | 0.846 |
| 20 | 20 | 415 | 18621.89 | 0.65 |
| 21 | 21 | 423 | 16268.86 | 0.881 |
| 22 | 22 | 429 | 14701.34 | 0.894 |
| 23 | 23 | 440 | 12209.29 | 0.917 |

**Table 1b: Protein band pattern for 10 KR gamma dose**

| Sr.No. | Bands | Positions | Molecular Weight | Rm |
|---|---|---|---|---|
| 1 | 24 | 250 | 302012.28 | 0.521 |
| 2 | 25 | 271 | 211848.06 | 0.565 |
| 3 | 26 | 280 | 181980.33 | 0.583 |
| 4 | 27 | 288 | 158985.58 | 0.600 |
| 5 | 28 | 293 | 146113.73 | 0.610 |
| 6 | 29 | 300 | 129824.80 | 0.625 |
| 7 | 30 | 304 | 121345.68 | 0.633 |
| 8 | 31 | 311 | 107817.91 | 0.648 |
| 9 | 32 | 323 | 88042.20 | 0.673 |
| 10 | 33 | 329 | 79559.28 | 0.685 |
| 11 | 34 | 337 | 69506.30 | 0.702 |
| 12 | 35 | 345 | 60723.59 | 0.719 |
| 13 | 36 | 350 | 55807.26 | 0.729 |
| 14 | 37 | 355 | 5128898 | 0.740 |
| 15 | 38 | 360 | 47136.50 | 0.750 |
| 16 | 39 | 367 | 41881.67 | 0.765 |
| 17 | 40 | 373 | 37846.34 | 0.777 |
| 18 | 41 | 380 | 33627.19 | 0.792 |
| 19 | 42 | 387 | 29878.39 | 0.806 |
| 20 | 43 | 390 | 28402.54 | 0.812 |
| 21 | 44 | 397 | 25236.19 | 0.827 |
| 22 | 45 | 402 | 23193.01 | 0.837 |
| 23 | 45 | 409 | 20607.43 | 0.852 |
| 24 | 47 | 415 | 18621.89 | 0.865 |
| 25 | 48 | 420 | 17114.22 | 0.875 |
| 26 | 49 | 426 | 15465.25 | 0.887 |
| 27 | 50 | 430 | 14455.18 | 0.896 |
| 28 | 51 | 440 | 12209.29 | 0.917 |

**Table 1c: Protein band pattern for 15 KR gamma dose**

| Sr. No. | Bands | Positions | Molecular Weight | Rmm |
|---|---|---|---|---|
| 1 | 52 | 251 | 296955.41 | 0.523 |
| 2 | 53 | 268 | 222856.08 | 0.558 |
| 3 | 54 | 274 | 201383.77 | 0.571 |
| 4 | 55 | 281 | 178933.27 | 0.585 |
| 5 | 56 | 287 | 161692.94 | 0.598 |
| 6 | 57 | 292 | 148601.91 | 0.608 |
| 7 | 58 | 299 | 132035.9 | 0.623 |
| 8 | 59 | 304 | 121345.68 | 0.633 |
| 9 | 60 | 311 | 107817.91 | 0.648 |
| 10 | 61 | 318 | 95798.25 | 0.663 |
| 11 | 62 | 325 | 85118.55 | 0.677 |
| 12 | 63 | 333 | 74363.10 | 0.694 |
| 13 | 64 | 337 | 69506.30 | 0.702 |
| 14 | 65 | 343 | 62809.32 | 0.715 |
| 15 | 66 | 348 | 57724.13 | 0.725 |
| 16 | 67 | 353 | 53050.65 | 0.735 |
| 17 | 68 | 358 | 48755.55 | 0.746 |
| 18 | 69 | 363 | 38490.83 | 0.756 |
| 19 | 70 | 372 | 33627.19 | 0.775 |
| 20 | 71 | 380 | 30387.19 | 0.792 |
| 21 | 72 | 386 | 27926.97 | 0.804 |
| 22 | 73 | 391 | 24813.64 | 0.815 |
| 23 | 74 | 398 | 22804.67 | 0.829 |
| 24 | 75 | 403 | 21678.23 | 0.840 |
| 25 | 76 | 406 | 19923.11 | 0.846 |
| 26 | 77 | 411 | 17702.05 | 0.856 |
| 27 | 78 | 418 | 15728.61 | 0.871 |
| 28 | 79 | 425 | 15728.61 | 0.885 |
| 29 | 80 | 430 | 14455.18 | 0.896 |
| 30 | 81 | 440 | 12209.29 | 0.917 |

**Table 1d: Protein band pattern for 20 KR gamma dose**

| Sr. No | Band | Position | Molecular wt. | Rm |
|---|---|---|---|---|
| 1 | 82 | 252 | 29983.25 | 0.525 |
| 2 | 83 | 268 | 222856.08 | 0.558 |
| 3 | 84 | 273 | 204813.14 | 0.559 |
| 4 | 85 | 281 | 178933.27 | 0.585 |
| 5 | 86 | 287 | 161692.94 | 0.598 |
| 6 | 87 | 294 | 143667.22 | 0.613 |
| 7 | 88 | 300 | 129824.80 | 0.625 |
| 8 | 89 | 306 | 117316.10 | 0.637 |
| 9 | 90 | 312 | 106012.62 | 0.650 |
| 10 | 91 | 319 | 94194.21 | 0.665 |
| 11 | 92 | 324 | 86568.03 | 0.675 |
| 12 | 93 | 330 | 78227.15 | 0.688 |
| 13 | 94 | 340 | 66073.02 | 0.708 |
| 14 | 95 | 344 | 61757.65 | 0.717 |
| 15 | 96 | 350 | 55807.26 | 0.729 |
| 16 | 97 | 356 | 50430.20 | 0.742 |
| 17 | 98 | 363 | 44808.18 | 0.756 |
| 18 | 99 | 368 | 41180.41 | 0.767 |
| 19 | 100 | 374 | 37212.65 | 0.779 |
| 20 | 101 | 380 | 33627.19 | 0.792 |
| 21 | 102 | 387 | 29878.39 | 0.806 |
| 22 | 103 | 394 | 26547.51 | 0.821 |
| 23 | 104 | 401 | 23587.96 | 0.835 |
| 24 | 105 | 405 | 22047.38 | 0.844 |
| 25 | 106 | 412 | 19589.52 | 0.858 |
| 26 | 107 | 416 | 18310.09 | 0.867 |
| 27 | 108 | 419 | 17405.65 | 0.873 |
| 28 | 109 | 425 | 15728.61 | 0.885 |
| 29 | 110 | 430 | 14455.78 | 0.896 |
| 30 | 111 | 437 | 12843.70 | 0.910 |

Volume : 5 | Issue : 3 | March 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

**Table 1e: Protein band pattern for 25 KR gamma dose**

| Sr. No | Band | Position | Molecular wt. | Rm |
|---|---|---|---|---|
| 1 | 112 | 252 | 291983.25 | 0.525 |
| 2 | 113 | 270 | 215455.61 | 0.562 |
| 3 | 114 | 274 | 201383.77 | 0.571 |
| 4 | 115 | 280 | 181980.33 | 0.583 |
| 5 | 116 | 285 | 167246.77 | 0.594 |
| 6 | 117 | 290 | 153706.08 | 0.604 |
| 7 | 118 | 297 | 136570.75 | 0.619 |
| 8 | 119 | 301 | 127651.03 | 0.627 |
| 9 | 120 | 307 | 115351.77 | 0.640 |
| 10 | 121 | 314 | 102492.23 | 0.624 |
| 11 | 122 | 324 | 86568.03 | 0.675 |
| 12 | 123 | 330 | 78227.15 | 0.688 |
| 13 | 124 | 337 | 69506.30 | 0.702 |
| 14 | 125 | 345 | 60723.59 | 0.719 |
| 15 | 126 | 349 | 56757.61 | 0.727 |
| 16 | 127 | 355 | 51288.98 | 0.740 |
| 17 | 127 | 361 | 46347.25 | 0.752 |
| 18 | 129 | 366 | 42594.87 | 0.762 |
| 19 | 130 | 373 | 37846.34 | 0.777 |
| 20 | 131 | 381 | 33064.14 | 0.794 |
| 21 | 132 | 390 | 28402.54 | 0.812 |
| 22 | 133 | 396 | 25665.94 | 0.825 |
| 23 | 134 | 404 | 22422.83 | 0.842 |
| 24 | 135 | 411 | 19923.11 | 0.856 |
| 25 | 136 | 418 | 17702.05 | 0.871 |
| 26 | 137 | 424 | 15996.45 | 0.883 |
| 27 | 138 | 433 | 13741.17 | 0.902 |

**Table 1f: Protein band pattern for 30 kr gamma dose.**

| Sr no | Band | Position | Molecular Wt. | Rm |
|---|---|---|---|---|
| 1 | 139 | 284 | 170094.81 | 0.592 |
| 2 | 140 | 289 | 156323.55 | 0.602 |
| 3 | 141 | 293 | 146113.73 | 0.610 |
| 4 | 142 | 300 | 129824.80 | 0.625 |
| 5 | 143 | 305 | 119313.88 | 0.635 |
| 6 | 144 | 310 | 109653.95 | 0.646 |
| 7 | 145 | 316 | 99088.73 | 0.658 |
| 8 | 146 | 320 | 92617.04 | 0.667 |
| 9 | 147 | 328 | 80914.09 | 0.683 |
| 10 | 148 | 335 | 71893.70 | 0.698 |
| 11 | 149 | 342 | 63878.90 | 0.712 |
| 12 | 150 | 351 | 54872.83 | 0.731 |
| 13 | 151 | 357 | 49585.80 | 0.744 |
| 14 | 152 | 365 | 43320.22 | 0.760 |
| 15 | 153 | 373 | 37846.34 | 0.777 |
| 16 | 154 | 384 | 31430.93 | 0.800 |
| 17 | 155 | 393 | 2699.09 | 0.819 |
| 18 | 156 | 399 | 24398.16 | 0.831 |
| 19 | 157 | 404 | 22242.83 | 0.842 |
| 20 | 158 | 406 | 21678.23 | 0.846 |
| 21 | 159 | 412 | 19589.52 | 0.858 |
| 22 | 160 | 417 | 18003.50 | 0.869 |
| 23 | 161 | 424 | 15996.45 | 0.883 |

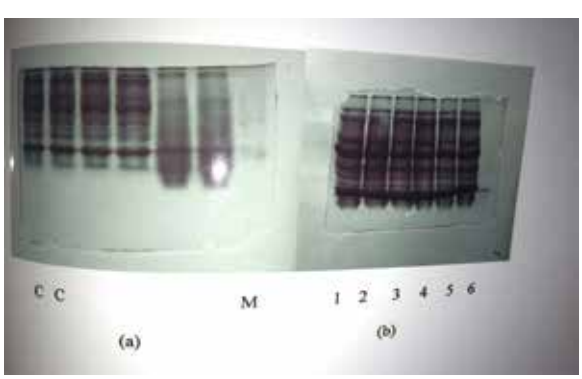


**Fig: SDS gel electrophoresis (Protein banding) Pattern in Wild Pea.**

All Control lane 1-5Kr, lane2-10kr, lane3-15kr, lane4-20kr,lane5-25kr, lane6-30kr.

Band profiles generated by SDS- PAGE were complied onto a data matrix according to presence and absence (0) of selected bands. Cluster analysis was conducted on GS estimates using unweighted pair group method arithmetic average (UPGMA) procedure of the NTSYS-pc programme, version 2.02 (Rohlf, 1998). In the neighour-hood joining and UPGMA method the distance were calculated by using the alogrithemic by Nie and Li (1997). Each band is considered as separate character. The resulting clusters are expressed as dendrogram. The dendrogram present a pictorial representation of the clustering process by indicating the order of individuals groups joined together because of their similarity. The goodness of fit of the dendrogram and genetic similarity (Mental, 1967). The multivariate approach was used to complement the information generated from cluster analysis, because cluster analysis is more sensitive to closely releated individuals, where principle coordinate analysis is more informative regarding distance among major group (Hauser and Crovelo, 1982; Sun *et al.,* 2001)

**Observation and result:**

In present investigation the cluster analysis was performed on similarity matrix by UPGMA method and neighbour-hood joining the distance was calculated by using the algorithm by Nie and Li (1997).

The results can be analyzed as from the above data obtained. The controle set of seeds are having similar protein profile as compared to gamma dose 10 KR as they exhibit the same position in one cluster viz. A as they sets of seeds irradiated with 25KR, 5KR, 15KR, 20KR, 30KR are grouped into different cluster viz B.

While the other cluster which shows the same position of seeds irradiated with 20 KR and 30 KR dose of gamma. As they are in structural positions equivalence, they had identical ties, with each other. In matrix terms, both the row and column vector of an equivalent pair have identical elements. But these protein bands are in far distance as compare to control and 10 KR set of protein patterns. This indicates the higher rate of mutation spectrum from control set. The protein pattern in set of seeds irradiated by gamma doses viz 25 KR has the lower distance as compared to control while 5KR, then 15KR has lower distance to 25 KR. The results indicate that they are having declining from control set of seeds.

Here the genetic divergence can be observed in changed patterns observed with loss of original bands and appearance of novel bands in case of 5KR dose, which shows fruitful result in terms of morphological characters and yield. Over all it is seen that cluster B, revealed more genetic variation than the cluster A. The predicted homology helped in significant homology for the protein bands in each cluster.

**Discussion**

Over past 50 years, the plant varieties coupled with improved management and agronomic inputs have made a significant increase in the yield of major crops (Swaminathan, 1998). A variation is the basic resource to be explored for genetic improvement of any species and hence play an important role in plant improvement programme (Hedegart *et al.*, 1976). Morphological characteristic might themselves are insufficient to distinguished pair of closely related species, geographical races or ecotype because not all genetic differentiation result in morphological differentiation. Thus genetic characterization of normal resources is a better understanding of genetic resources for the implementation of in situ and ex-situ conservation activity (NBPGR).

**Conculsion:**

The wild pea mutant isolated at M or M1 generation revealed that sufficient improvement in total seed protein content and its

quality could be achieved through mutagenesis at lower doses rather than at higher gamma doses. The mutants show typical type of protein variability at each set of dose. All mutant maintained and enhanced their characteristics at M1 generation, which indicates the inheritance of positive traits at lower doses, rather than at higher doses. The significant negative correlation between yield and total protein was exhibited in mutants of higher doses while the mutant at lower doses shows non significant correlation. The later association of high protein content and yield raises the hope of isolation of high protein mutant without endangering the yield.

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