International e-Conference on New Horizons And Multidisciplinary Applications In Science And Technology
In Association withInternational Journal of Scientific Research in Science and Technology
Volume 9 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)

Protein Profile Pattern in Gamma Irradiated Wild Pea

Dr. M. J. Keche[1]
[1]Department of Botany, SGBAU, Rajarshee Shahu Science College Chandur Rly, Dist Amravati, Maharashtra, India

ABSTRACT

Genetic Variation in germplasma has important role in identification of varieties. Electrophoreticpattern of protein fraction are directly related to the genetic background of the protein and be used to certify the genetic make-up SDS-PAGE (SDS-Polyacrilamide gel electrophoresis) is valid technique increasingly being utilized as an approach for species identification, Each variety or an approach for species identification .Each variety or a group of varieties exhibit characteristic protein banding Pattern thus on the basis of the pattern they can be identified accordingly.

Keywords: SDS- PAGE, Protein, Banding pattern

I. 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% lupin. They can therefore , be considered a good substitution to animal protein of these legume containing amino acid and plant breeding have to consider this problem in any improvement programme (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 (Amirsh and tavakloi 1970).
Field pea is significant pulse crop in both India and Australia. There are number of similarities that include nationally co-ordinate breaking programmer, moisture stress in rained crops low yield. Significant of powdery mildew and an intrest in developing lodging resistant varities. (Barum et.al 2000). Peas are of great nutritional importance due to their high content of protein, complex carbohydrate, dietary fiber minerals, vitamins and antioxidant compound. Although pea widely used in animals nutrition (Hedly,2001). Human consumption of pea is lower than that of other traditionally more accepted pulses (Hedly, 2001, Schneider 2002). Nevertheless in 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 2001).

Copyright: © the author(s), publisher and licensee Technoscience Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited

II. METHODS AND MATERIAL

Preparation of Seed Sample:

For extraction of proteins individual seeds, irradiated with particular gamma dose were ground to fine powder with mortal and pestle. To extract protein in 0.01 gm of seed flour, 400 ml of the protein extraction buffer (0.05M, Tris-HCL, 0.2%) SDS, 5M urea and 1% b-mercaptoethanol, was added to the tube and mixed well by vortex. Then centrifugal at 15,000 rpm for 5 min at room temperature. The extracted crude protein was recovered as clear supernatant and was stored at 20 C for further use.

SDS-page (Sodium dodecyl Sulphate-Polyacryalamide gel electrophoresis.)

The components used in the formation of this gel are known to be neurotoxic thus the care has to be taken while preparing gel. The most commonly used components to synthesis the matrix are acrylamide monomer and N-N methylene these free radical can activate acrylamide monomers inducing them to react with other acrylamide molecules forming long chain is the process. Theses chain become cross linked the presence of bis-acrylamide. TEMED acting as a cataylst for gel formation.

Material:

a) Sample buffer(Lamnilli buffer):

1.5 M Trisc HCL - 417 ul

Glycerol - 800 ul

10% SDS - 200 mg

β – mercaptoethanol - 362 ul

0.002% bromophenol blue -0.2 mg

(make the volume 10 ml pH-7.0± 0.2

b) Gel buffer (tris glycine buffer)

Tris base - 1.514

Glycine -7.207

SDS - 500 mg

(make up the Volume 500 ml and adjust pH 8.6±0.2)

c) Acrylamide Gel

Acrylamide - 297

N-N methylene bis acrylamide – 1%

TEMED -

APS (Ammonium per Sulphate) – 10%

A) Resolving buffer 1.5 M tris- HCL

B) Stacking gel 1 M tris HCL

d) Staining Dye

Methanol - 40 ml

G-Acetic acid - 10 ml

Distilled water - 50 ml

Coomassive brilliant blue R-250 dye- 0.2 gm

e) Destainer-

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

Preparation of Gel:
Seed protein were analyzed through slab type SDS-PAGE followed by Lammli (1970) using 11.2% polyacrylamide gel. Electrophoresis was carried out at 100v for two and half hours. In order to check reproducibility of the method two separate gels were run under similar conditions. After electrophoresis gels were stained with 0.2% (w/v) Coomasive brilliant blue for 5 hrs and then distained for 2 days on gyratory shaker.
Properly distained gels were read on gel read on gel documentation system and molecular weight of individual bands calculate with respect to marker band using ALPHA imager softwear. Consistency of result was ensured by analyzing a minimum of 7 seed sample irradiated with different doses from one accession. Further electrophoresis was repeated at least twice on each protein extract only constant bands were taken into account.

Data Analysis:
The gels were analyzed on gel documentation system for its position. On (releative mobility) values, molecular weight determination. The gels were scored as presence (1) or absences (0) of band of comparable size. Pair wise similarities betweenaccessions were calculating using Jaccards coefficient. The cluster analysis was performed on similarity matrix by UPGMA method (Sneat and Sokal 1973).

III. RESULT AND DISCUSSION

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.
The results are shown in **Photoplate 3**
Table a) Rm for standard protein markers

Sr.No	Bands	Positions	Molecular Wt	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 b: Band similarity in *Pisum* mutants

Sr No.	Position	Similar bands in Protein samples	Rm
1	251	5 KR, 10KR	0.523
2	252	20 KR, 25 KR	0.525
3	268	15 KR, 20 KR	0.558
4	270	5 KR, 25 KR	0.562
5	274	15 KR, 25 KR	0.571
6	280	10 KR, 25 KR	0.583
7	281	15KR, 20KR	0.585
8	287	15KR, 20KR	0.598
9	290	5 KR, 25 KR	0.604
10	293	10 KR, 30 KR	0.610
11	294	Control, 20 KR	0.613
12	299	5 KR, 15 KR	0.623
13	300	10KR, 20KR, 30KR	0.625
14	301	5KR, 25KR	0.627
15	304	10 KR,15 KR	0.633
16	310	5 KR, 30 KR	0.646
17	311	10 KR, 15 KR	0.648
18	324	20 KR,25 KR	0.675
19	330	20 KR, 25 KR	0.688
20	337	5KR,10KR,15KR,25KR	0.702
21	345	5 KR,10 KR, 25 KR	0.719
22	350	10KR,20KR	0.729
23	355	10 KR, 25 KR	0.740
24	363	15KR, 20 KR	0.756
25	366	5KR, 25KR	0.762
26	373	10 KR, 25 KR, 30 KR	0.777
27	374	5 KR, 20 KR	0.779
28	380	10 KR, 15 KR, 20 KR	0.792
29	384	5KR, 30KR	0.800
30	387	10 KR, 20 KR	0.806
31	390	5 KR, 10 KR, 25 KR	0.812
32	398	5 KR, 15 KR	0.829
33	404	25 KR, 30 KR	0.842
34	406	5 KR, 15 KR	0.846

35	411	15 KR, 25 KR	0.856
36	412	20KR, 30KR	0.858
37	415	5 KR, 10 KR	0.865
38	418	15 KR, 25 KR	0.871
39	424	25 KR, 30 KR	0.883
40	425	15 KR, 20 KR	0.885
41	430	10 KR, 15 KR, 20 KR	0.896
42	440	5 KR, 10 KR, 15 KR	0.917

Table c: Band variability in *Pisum* mutants

Sr No.	Position	Variable bands in Protein samples	Rm
1	271	10 KR	0.565
2	272	Control	0.567
3	273	20KR	0.559
4	276	Control	0.575
5	279	5KR	0.581
6	284	30KR	0.592
7	285	25KR	0.594
8	286	Control	0.595
9	288	10KR	0.600
10	292	15KR	0.608
11	305	30KR	0.635
12	306	20KR	0.637
13	307	25KR	0.640
14	312	20KR	0.650
15	314	25KR	0.624
16	316	30KR	0.658
17	317	5KR	0.660
18	318	15KR	0.663
19	319	20KR	0.665
20	320	30KR	0.667
21	323	10KR	0.673
22	325	15KR	0.677
23	327	5KR	0.681
24	328	30KR	0.683
25	329	10KR	0.685
26	333	15KR	0.694
27	335	30KR	0.698
28	340	20KR	0.708

29	342	30KR	0.712
30	343	15KR	0.715
31	344	20KR	0.717
32	348	15KR	0.725
33	349	25KR	0.727
34	351	30KR	0.731
35	352	5KR	0.733
36	353	15KR	0.735
36	356	20KR	0.742
37	357	30KR	0.744
38	358	15KR	0.746
39	359	5KR	0.748
40	360	10KR	0.750
41	361	25KR	0.752
42	365	30KR	0.760
43	367	10KR	0.765
44	368	20KR	0.767
45	372	15KR	0.775
46	381	25KR	0.794
47	386	15KR	0.804
48	391	15KR	0.815
49	393	30KR	0.819
50	401	20KR	0.835
51	402	10KR	0.837
52	403	15KR	0.840
53	405	20KR	0.844
54	409	10KR	0.852
55	416	20KR	0.867
56	417	30KR	0.869
57	419	20KR	0.873
58	420	10KR	0.875
59	423	5KR	0.881
60	426	10KR	0.887
61	429	5KR	0.894
62	433	25KR	0.902
63	437	20KR	0.910

The **Table b** andc represent the band similarities and variability obtained amongst the variable gamma doses.

UPGMA (Unweighted Pair Group Matrix of Arithmetic Mean

i. Open the programme 'neighbor'. Type the name of the file distance

ii. Type N and enter .This will change the option Neighborhood join to UPMA
iii. Type Y and enter. This will run the programme and give the Output.
iv. Again two output file will be created as outfile and outree rename these file as a upgmaout and upgmatree
v. Open these in notepad or textpad . Observe these file. You find the distance matrix, input file data, branch length and tree.
vi. Open this file in Treeviev software and observe the tree in different form.

Table- Populations

```
Neighbor-Joining/UPGMA method version 3.65
 Neighbor-joining method
 Negative branch lengths allowed
  +KR-B
  !
  ! +KR-E
  1--3
  ! ! +KR-A
  ! +-5
  !   ! +-KR-C
  !   +-4
  !     ! +-KR-D
  !     +-2
  !       +--KR-F
  !
  +CONTROL
remember: this is an unrooted tree!
Between        And            Length
-------        ---            ------
   1          KR-B           -0.00629
   1            3             0.04788
   3          KR-E            0.00838
   3            5             0.02520
   5          KR-A            0.00513
   5            4             0.02232
   4          KR-C            0.03292
   4            2             0.02633
   2          KR-D            0.03192
   2          KR-F            0.04374
   1          CONTROL         0.00629
(KR-B:-0.00629,(KR-E:0.00838,(KR-A:0.00513,(KR-C:0.03292,(KR-D:0.03192,KR-
F:0.04374):0.02633):0.02232):0.02520):0.04788,CONTROL:0.00629);
```

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

The results can be analyzed as from the above data obtained The control set of seeds are having similar protein profile as compare to gamma dose 10 KR, as they exhibit the same position in one cluster viz. A, as they have coefficients of similarity nearby same (Shown in **Fig- 2**). While the other sets of seeds irradiated with 25 KR; 5 KR; 15 KR, 20 KR and 30 KR are grouped into different cluster viz. B.

While the other cluster which shows the same positions of seeds irradiated with 20 KR and 30 KR dose of gamma. As they are in structural position equivalence, they had they have identical ties, with each other. In matrix terms, both the row and column vectors 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. As results can also be alined with phenotypic variations as it has most divergence from the control set i.e low germination Percentage and lower yield and short height etc.

The protein pattern in set of seeds irradiated by gamma dose viz. 25 KR has the lower distance as compare to control; while 5 KR, then 15 KR has lower distance to 25 KR. The results indicate that they are having declining divergence 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 results in terms of morphological characters and yield. Over all it is seen that the cluster B, revealed more genetic variation than the cluster A. The predicted homology helped in significant homology for the protein bands in each cluster.

IV. CONCLUSION

The plenty of information on genetic fortification through applied mutagenesis of future varieties for higher protein content and quality has been published. Variation in range of total protein, protein sub fractions and band pattern in the parental line of wild pea mutant varieties indicate that the improvement in production with in pea is possible and this is supported by our findings in the mutants deriving from mutational treated by gamma irradiation, where appreciable increase as in their total seed protein has been demonstrated. Nevertheless, protein mutants in exhibit negative correlation between total protein and yield has been reported in cereals (Johnson et al., 1970; Kaul, 1980a) as well as in legumes (Wood et al., 1979; Sjodin et al., 1981a). Such observations reflect difficulty in selecting high protein mutant associated with no change in yield (Gridley and Evans, 1971). The induction of high protein mutant may be attributed to micro mutations with positive effects and low seed yield to micro mutation with negative effect.

V. REFERENCES

[1] . Amrishahi, M.C and Tavakoli, M. (1970). Protein content of different varieties of five species of rules Crops. Improving plant protein by nuclear Techniques (Proc. Symp. Vinna, 1970) I.A.E.A. Vienna, Pp [331-33].

[2] . Barum,M Weeden, N.F.Muehlbauers, F.J., Khal, G Udupa S.M., Eujay, I., Wigand.f., Harrabi, M. and Bouznadz (2000) .Marker technology for plant breeding .In R.Kinght (ed.) Linking Research and Marketing opportunities for pulses in 21 st Century . Kluwer Academic Publishers. The Nether lands. Pp. [421-427].

[3] . Davidsson, L Dimitriou, T Walezyk, T Hurrel, RF (2001). Iron absorption from experimental infant formulas based on pea (Pisum sativum) - protein isolate: the effect of phytic acid and ascorbic acid. Br J Nature: 85: Pp [59-63].

[4] . Davies, D.R, Berry, G.J., Health, M.C and Dawkins, T.C.K (1985). In Pea (Pisum sativum L) (R.J Summerfield and E.H. Roberts eds.) Williams Collins Sons and Co. Ltd. Landon. U.K. Pp [147-198].

[5] . Laemmili, U.K., (1970). Cleavage of structural protein during assembly of the head of bacteriophage T4. Nature 227: Pp. [680-685].

[6] . Summerfield, R.J, and Roberts, E.H. (1985). Gram legume crops. Collins pub. Landon, U.K.

[7] . Wood, D.R Nowick, E.A Fabian, H.J and McClean, P.E (1979). Genetic variability and heritability of available methionine in Colorado dry bean breeding programme. Seed protein improvement in cereals and grain legumes (Proc. Symp. Neuherberg, 1978) I.A.E.A, Vienna, Pp. [69-85]

[8] . Johnson, B.L; Thein, M.M, (1970). Assessment of evolutionary affinities in Gossypium by protein electrophoresis. Am. J. Bet. 57, Pp [1081-1092]