compared to the other higher evolved species. The preferential selection of homorepeats of certain amino acids in proteins of different species is a reflection of the mechanisms to achieve functional and structural versatility by proteins in the course of evolution. As the database analysed is large, we believe that the percentage frequency results will not be biased by multiple entries of single proteins. A similar trend of homorepeat frequency can be expected of more exhaustive forthcoming data as the number of sampling points analysed is quite large.

1. Cantor and Schummel, in *Biophysical Chemistry*, W. H. Freeman and Co., 1980, Part I, pp. 41–154.
2. Doolittle, R. F., *Sci. Am.*, 1985, **253**, 88–99.
3. Doolittle, R. F., *Methods Enzymol.*, 1990, **183**, 99–110.
4. Borivoj Keil, *Methods Enzymol.*, 1990, **183**, 50–60.
5. Zamyatnin, A. A., *Protein Seq. Data Anal.*, 1991, **4**, 57–60.
6. Kolaskar, A. S. and Samuel, S. L., *Protein Seq. Data Anal.*, 1991, **4**, 105–110.
7. Argos, P., *Nucleic Acids Res.*, 1988, **16**, 9909–9916.
8. Kolaskar, A. S. and Kulkarni-Kale, U., *J. Mol. Biol.*, 1992, **223**, 1053–1061.
9. Jones, T. A. and Thirup, S., *EMBO J.*, 1986, **5**, 819–822.
10. Gibrat, G. J., *J. Mol. Biol.*, 1987, **198**, 425–443.
11. Blundell, T. M., *Trends Biochem Sci.*, 1990, **15**, 425–430.
12. McLachlan, A. D., *Symp Quant. Biol.*, 1987, **II**, 411.
13. Shin, M. S., Bargiello, T. A., Clark, B. T., Jackson, F. R. and Young, M. W., *Nature*, 1985, **317**, 445–448.
14. Bairoche, A. and Boeckmann, B., *Nucleic Acids Res.*, **20**, 2019–2022.
15. Pain, P., *Trends Biochem. Sci.*, 1987, **12**, 309–312.
16. Higgins, T. J. V., *Annu. Rev. Plant Physiol.*, 1984, **35**, 191–221.

Received 9 March 1993; revised accepted 28 May 1993

γ-Rays- and EMS-induced leaf mutants in mung bean (*Vigna radiata* (L) Wilczek)

V. P. Singh and Rashmi Sharma

Department of Plant Sciences, Rohilkhand University, Bareilly 243 005, India

A few pentafoliate and tetrafoliate mutants were isolated from the γ-rays and EMS-treated M_2 population. These mutants showed a significant increase in dry matter production, total chlorophyll contents and yield compared to their parents in M_2 and M_3 generations.

THE role of mutation breeding in the induction of leaf mutants of agronomic interest is well established[1]. Being easily discernible and stable phenotypes, the leaf mutants offer interesting experimental material. In a short-duration crop such as mung bean, it is important that the leaf area should expand and reach its optimal level as rapidly as possible for maximum interception of the incident light. Earlier studies indicate that the number of pods per plant, reduction in leaf number per plant and leaf area, and insufficient dry-matter production are the principal factors limiting the yield[1,2]. The present report describes an attempt to study the morphological and physiological components of yield in the tetrafoliate and pentafoliate mutants induced in mung bean cv. PDM-116 and PDM-11.

Dry seeds of mung bean (*Vigna radiata* (L) Wilczek) cv. PDM-116 and PDM-11 obtained from Pulse Directorate, Kalayanpur, Kanpur, were irradiated with different doses of γ-rays (15, 30 and 45 kR) at the Indian Agricultural Research Institute, New Delhi, delivered from a source of ^{60}Co and sown in the field. In another experiment presoaked seeds (12 h in distilled water) were treated with an aqueous solution of chemical mutagen (0.1%, 0.2%, 0.3% ethylmethanesulphonate) for 6 h with intermittent shaking of the mutagenic solution. After the termination of chemical treatments, the seeds were washed in running water and directly sown in the field. Seeds from each M_1 plant were collected and sown in the field in randomized-block single-row design to raise M_2 generation. The mutants isolated from M_2 generation were carried over to M_3 generation to study their breeding behaviour and productivity. The protein content was estimated following the modified Kjeldhal's method[3] and the chlorophyll contents were estimated following Arnon's method[4].

In M_2 generation, 1.66% and 2.50% tetrafoliate mutants in variety PDM-116 and PDM-11, respectively, and 0.83% pentafoliate mutants in both these varieties were isolated from the mutagen-treated population. The highest frequency of induced mutants was reported in γ-rays-treated population of both the varieties. It was interesting to note that the tetrafoliate mutants were recorded from γ-rays-treated population and pentafoliate mutants were recorded from EMS-treated population only.

The leaf characteristics of the mutants and their productivity are given in Tables 1 and 2, respectively. In M_2 and M_3 generation, the leaf area increases significantly in both the mutants along with the dry-matter production and total chlorophyll contents per plant. The total yield in the induced mutants was significantly higher, the high-yield contributing factor being the number of pods per plant. The protein contents remain unaltered in tetrafoliate and pentafoliate mutants except in the pentafoliate mutant isolated from variety PDM-116, where a significant increase was observed in the protein content and the yield. A similar result of the induction of desirable leaf mutants by the use of various physical/chemical mutagen in pulses has been reported earlier[2,5–7]. The M_3 segregation population of the mutants showed a 3:1 segregating ratio, confirming that the mutant character is controlled by a single recessive gene (Table 3).

Table 1. *Leaf characteristics of mung bean mutants*

Mutant/control	Treatment	Frequency (%)	No of leaves per plant	Leaf area (cm^2)	Fresh weight (g/plant)	Dry weight (g/plant)	Chlorophyll count (µg/ml)
M_2 generation							
Variety PDM-116							
Tetrafoliate	30 kR γ-rays	1 66	14	70.24	1 412	0.48*	2191 72
Pentafoliate	0 2% EMS	0 83	15	74 46*	1 892	0.68*	2210 68
Trifoliate (control)		—	12	66 39	1.268	0 27	2270 40
Variety PDM-11							
Tetrafoliate	45 kR γ-rays	2 50	15	54 35*	3 205	0.48*	1131.73
Pentafoliate	0.1% EMS	0.83	17	60.99	3 368	0.56*	1121.80
Trifoliate (control)		—	10	61.35	1 382	0 39	1114 60
M_3 generation							
Variety PDM-116							
Tetrafoliate			21	81.53*	2 062	0.62*	2169.82
Pentafoliate			19	86.38*	2.246	0.74*	2197.72
Trifoliate			10	68.39	1 986	0.38	2289.88
Variety PDM-11							
Tetrafoliate			13	69.12*	1 421	0.48	1118 18
Pentafoliate			15	82.36*	1 928	0.58*	1124 24
Trifoliate			8	62 46	1 124	0.42	1077.90

* Significant at 1%.

Table 2. Grain yield, yield components and grain protein per cent in induced mung bean leaf mutants

Mutant/control	Treatment	Frequency (%)	Pods/plants	100 seed weight per plant (g)	Grain yield per plant (g)	Protein per cent
M_2 generation						
Variety PDM-116						
Tetrafoliate	30 kR γ-rays	1 66	54.0 ± 1.64**	4.09 ± 0.280	17.14 ± 1 64**	21 80 ± 0.42
Pentafoliate	0.2% EMS	0.83	63.0 ± 1 89**	4 10 ± 0 312	18 42 ± 2 04**	25.13 ± 0 72*
Trifoliate (control)		—	24 0 ± 1.13	4 18 ± 0 370	9 13 ± 0.77	22 42 ± 0.34
Variety PDM-11						
Tetrafoliate	45 kR γ-rays	2.50	61 0 ± 2.89**	3 01 ± 0.182	17.32 ± 1 82**	21.20 ± 0 62
Pentafoliate	0.1% EMS	0 83	70 0 ± 2.36**	3 12 ± 0.294	20 36 ± 2.19**	21.60 ± 0 89
Trifoliate (control)		—	26 0 ± 1 80	3.32 ± 0 210	10 85 ± 1.12	20.82 ± 0 37
M_3 generation						
Variety PDM-116						
Tetrafoliate	—	—	38 0 ± 0 56**	4.12 ± 0 301	13 28 ± 1.24**	22 06 ± 1 08
Pentafoliate	—	—	54 0 ± 0 67**	3 97 ± 0.320	17 14 ± 1 12**	26 42 ± 0 89*
Trifoliate (control)	—	—	18 0 ± 0 52	4 07 ± 0 315	8 26 ± 0 43	20 80 ± 1 02
Variety PDM-11						
Tetrafoliate	—	—	74 0 ± 1 42**	3 14 ± 0 210	12 43 ± 1 08*	21 92 ± 1 12
Pentafoliate	—	—	76 0 ± 1 15**	3 10 ± 0 250	18 63 ± 1 26**	24 87 ± 0 62
Trifoliate (control)	—	—	24 0 ± 1 13	3 12 ± 0 220	9 68 ± 1.84	21 49 ± 0 84

* Significant at 5%, ** significant at 1%.

Table 3. Segregating pattern of the induced mung bean leaf mutants in M_3 generation

Mutant	Treatment	Total plants	Normal plants	Mutant plants	χ^2 value
Variety PDM-116					
Tetrafoliate	30 kR γ-rays	19	15	4	0.103
Pentafoliate	0.2% EMS	20	14	6	0.260
Variety PDM-11					
Tetrafoliate	45 kR γ-rays	22	15	7	0.480
Pentafoliate	0.1% EMS	20	16	4	0.260

Table value of χ^2 at 1 degree of freedom and 5% level of significance is 3 841.

1. Gottschalk, W. and Wolff, G., *Plant Breeding*, Springer-Verlag, 1988, publn no. 71, 1988, p. 73.
2. Thakare, R. G., Pawar, S. E., Joshua, D. C., Mitra, R. and Bhatia, C. R., *Induced Mutation – A Tool in Plant Research*, Proceedings of the International Symposium, Vienna 9-13, March 1981, IAEA 213–216.
3. *Official and Tentative Methods of Analysis*, Association of Official and Analytical Chemists, Washington, DE 20044, 1975, 25th edn.
4. Arnon, *Plant Physiol.*, 1949, **24**, 1–14.
5. Vassileva, M., *Experimental Mutagenesis in Plant (Sofia)*, 1978, pp. 440–447.
6. Singh, R. K., Raghuvanshi, S. S and Dhamprakash, *Plant Foods Human Nutrition*, 1988, **38**, 115–120.
7. Khan, M., Iqbal, A. and Rizwi, M.A., *Mutat. Breed. Newsl.*, 1989, **34**, 20.

Received 1 March 1993; revised accepted 15 May 1993

Use of slide agglutination test for the detection of nuclear polyhedrosis virus of silkworm, *Bombyx mori* L.

J. Bhattacharya, N. Krishnan, A. K. Chandra, Om Parkash* and U. Sengupta*

Central Sericultural Research & Training Institute, Berhampore 742 101, India

*Central JALMA Institute for Leprosy, Taj Ganj, Agra 282 001, India

Specific antibody raised against nuclear polyhedrosis virus (NPV) has been used for the detection of the virus disease in silkworm. The agglutination reactions were positive and could be visualized directly up to a titre of antigen 1×10⁸ polyhedra/ml and antiserum 1:2000.

THE agglutination reaction involving clumping of cellular or particulate antigen by specific antiserum is one of the earliest immunodiagnostic methods[1]. Many sophisticated immunodiagnostic methods have been developed subsequently, but these are expensive and require operational skill, thus limiting the commercial use.

Arakawa[2] used latex agglutination test for detection of nuclear polyhedrosis virus (NPV) in silkworm and concluded that, particularly for NPV, the test was not as sensitive as that for other viruses because of low titre of the antivirus IgG. Slide agglutination test, though less precise, is more rapid and can be adopted by all, including the unskilled workers.

In the present study, an attempt has been made to standardize a simple slide agglutination test for detection of NPV of silkworm. Early detection of the NPV-induced disease using this method can be of great help to the sericulture industry for adopting preventive measures.

Polyhedral particles from the haemolymph of NPV-infected silkworm larvae were collected by centrifugation and purified using Percoll towards preparation of inoculum as immunogen for raising antiserum. Purified polyhedra (6.0×10^8 polyhedra inclusion bodies per ml (PIB/ml)) suspended in 1 ml of phosphate-buffered saline (PBS) was emulsified with 1 ml of Freund's complete adjuvant and injected intradermally at multiple sites to rabbits. Similar injections were given four times at one-week intervals and the rabbits were bled after seven days of the last injection for the collection of antiserum.

Slide agglutination test was performed using NPV and the antiserum. Different amounts of NPV suspended in PBS, containing 10–10^9 polyhedra/ml, were allowed to react with varying dilutions of antiserum (Table 1). In each case 50 µl of NPV suspension was mixed with 50 µl of antiserum on a microscopic glass slide for agglutination reaction. A control where 50 µl of NPV suspension was mixed with 50 µl of PBS was included in each case. The slides were tilted to and fro and agglutinations were visualized (with naked eye) in the form of curdlings within 2–3 min when the reactions were strongly positive (Figure 1). The curdlings could not be seen in the case of weakly positive reactions but were visualized under a microscope (150×).

The agglutination reactions were detected visually by using antiserum diluted up to 1:2000 for 10^9 and 10^8 polyhedra/ml of antigenic concentrations (Table 1). To employ this technique for disease detection, the total